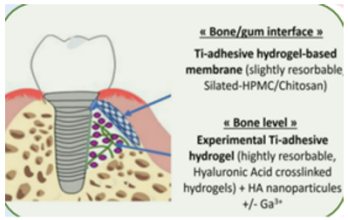
Subject 10: MATOS : Translational research in calcified tissue regeneration

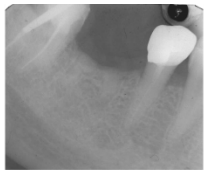
Injectable Bone substitute, Maxillo-facial and dental Bone regeneration, Ischemic bone regeneration

Pierre Weiss (PU-PH)

Program 1: Peri Implantitis treatment using active Biomaterials

- *Hydrogel barriers*
- *Injectable Bone Substitutes*
- *Anti bacterial devices*

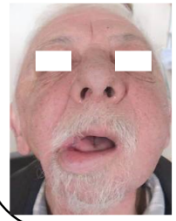


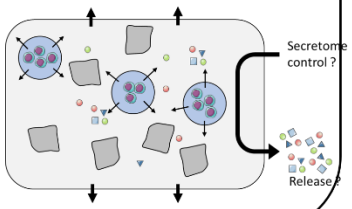


From animal models to clinical trials

Program 2: *Ischemic Bone regeneration*

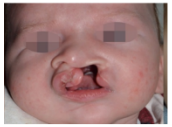
- *Assisted cell Therapies*
- *RNA delivery systems*
- *MGP antagonist strategy*

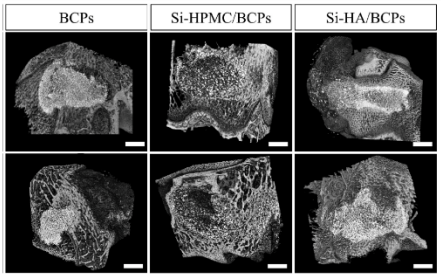




Program 3: Maxillo-facial complex reconstructions

- *Injectable Bone substitute and Foam cements*
- *Additive manufacturing*
- *BMP2 drug delivery systems*





Chichiricco et al., Bioengineering 2023
Demarquay, C. et al. Regen Biomat 2022
Flegeau, K. et al., Biomater Sci 2021

- **Perspectives:**
  - Translate fundamental knowledge of developmental biology and morphogenesis to properly address the biological causes of disc degeneration and to inspire future regenerative strategies
  - Identify new molecules that target chondrocytes homeostasis and mitochondrial metabolism
  - Bring the in vivo proof of concept that targeting specific synovial cells or pathways may be of therapeutic potential
  - Validate innovative tools to efficiently modulate innate immune cells for personalized treatment of OA
  - Development of innovative biomaterial concepts for innovative therapies and modeling approaches.
  - Restoring bone mass and balance formation/resorption during aging, and (ii) proposing new therapeutic strategies of bone repair in combination with biomaterials, through the mobilization and activation of the healing potential of the host tissue.
  - Develop new mimetics based on gut hormones and myokines for the prevention and treatment of bone fragility
  - Development of innovative materials stimulating the formation of a new vascularized tissue in compromised environment and of dedicated additive manufacturing devices for the robust and rapid and sterile production of human-sized scaffolds.
  - Develop formulation containing specific composites of silanized-HA with calcium phosphate ceramics or nanoparticles to perform new injectable self-crosslinking bone substitutes

- **Unique selling points**

  - Interdisciplinary approach
  - Use of stem cells, calcium phosphate ceramics and hydrogels for the development of reconstructive therapeutics
  - Strong collaboration with clinicians
  - Development of innovative therapies and modeling approaches.
  - Development of bioinks, organoids, and organ on a chip technologies by bioprinting to model human diseases

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Advancing CD8+ Treg cell therapies to the clinic

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Keywords
Immune tolerance
Regulatory T cells
Immunotherapy
Transplantation
Autoimmune diseases
Cell therapy, Biomarker
Chimeric Antigen Receptor (CAR)
Genetic engineering
Induced pluripotent stem cells (IPSc)

Abstract
Immune-mediated diseases are currently treated with non-specific drugs that induce serious side effects. Cell therapy using regulatory T cells (Tregs) has been shown to be effective in treating many of these diseases in preclinical studies and is currently in phase I and II clinical trials. Usually, the Tregs used in cell therapy are autologous and of CD4+ type. However, autologous therapy requires enough functional Tregs, is expensive and time-consuming and CD4+ Tregs represents a rare cell population in blood (<1% after sorting). Over the past few years, we have deeply characterized CD8+ Tregs in rodents and human and translated our finding from bench to bedside with the set-up of a GMP protocol and a phase 1 safety clinical trial of cell therapy using autologous polyclonal CD8+ Tregs to treat kidney transplant patients which will start in 2024. Our next step is to explore the therapeutic potential of universally compatible genetically modified allogeneic human CAR-CD8+ Tregs. Our ultimate goal is to treat precociously immune-mediated disorders to wean patients of IS.
With this project, we anticipate that we will be able to set up a platform of production to provide the next generation of cell therapy.

Research area
Our research interest are the analysis of immune responses and tolerance induction in transplantation and autoimmune diseases, both basic and translational research, with the development of new therapeutic strategies including Treg cell therapy and application of genetic engineering and stem cell approaches.
We work since many years on CD8+ Tregs both on basic (phenotype, function, TCR-peptide interactions) and clinical aspects (in pathological situations and as cellular therapy product like CAR-Tregs) and are part of a European consortium RESHAPE to perform the world first trial using polyclonal CD8+ Tregs in kidney transplant patients in 2024.

Synopsis
Advancing CD8+ Regulatory T cells to the clinic for cell therapy of immune-mediated disorder

Interests
Cell Therapy;Chimeric Antigen Receptor (CAR)-T cells;Genetic engineering;Stem cells;Immunology/Immunotherapies;Autoimmune diseases;In vivo models;Clinical research

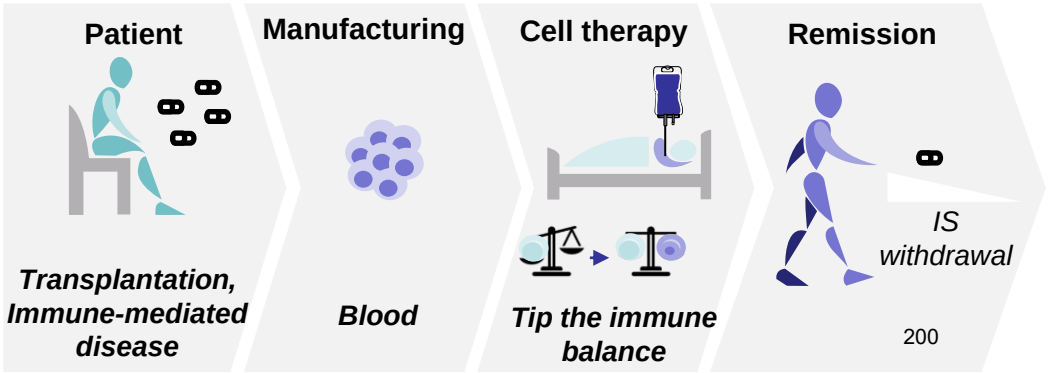
Advancing CD8$^+$ Treg cell therapies to the clinic for the treatment of immune-mediated disorders

Carole GUILLONNEAU

CR2TI - Nantes University, INSERM, Nantes

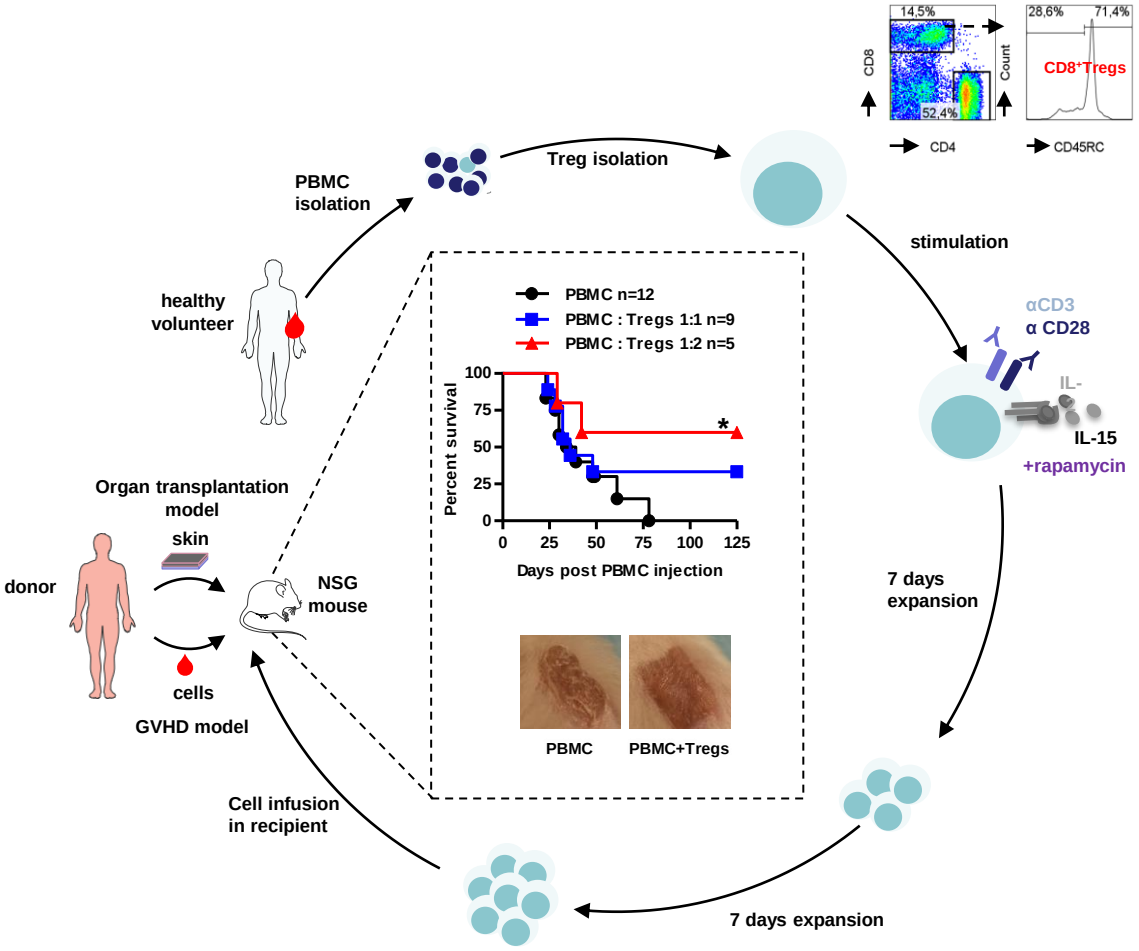
- **Objectives:**
  - To explore the diversity, identity and potential for biomarker of $CD8^+$ Tregs in blood sample and in biopsies from patients on standard (immunosuppressants) IS treatment using the latest single-cell resolution multiomics technologies
  - To evaluate, in a first phase I/IIa clinical trial in humans, on clinical criteria, the safety and the indices of efficacy of an IV infusion of autologous expanded $CD8^+$ Treg cells by replacement of standard induction IS
  - To generate the proof of concept that off-the-shelf $CD8^+$ Tregs can be genetically engineered and demonstrate their suppressive capacity and therapeutic potential in humanized mice model

- **Tools:**
  - GMP process for Treg cell preparation and expansion
  - Genetic engineering tool
  - Functional in vitro and in vivo animal models to assess the cell therapy potential
  - Discovery and generation of new inflammation related- or tissue-targeting CARs
  - Strong collaboration with several partners including the Nantes Hospital and the GMP manufacturing facility, an experienced platform for the immunomonitoring of patients, a nephrology department internationally renowned for kidney Tx and blood and biopsy biocollection, an iPSc platform, a bioinformatic R&D platform, and easy access to healthy human blood (EFS)
  - Experience/advices from an European consortium
  - Patents



Polyclonal CD8⁺ Treg cell therapy: proof of concept in transplantation and GVHD

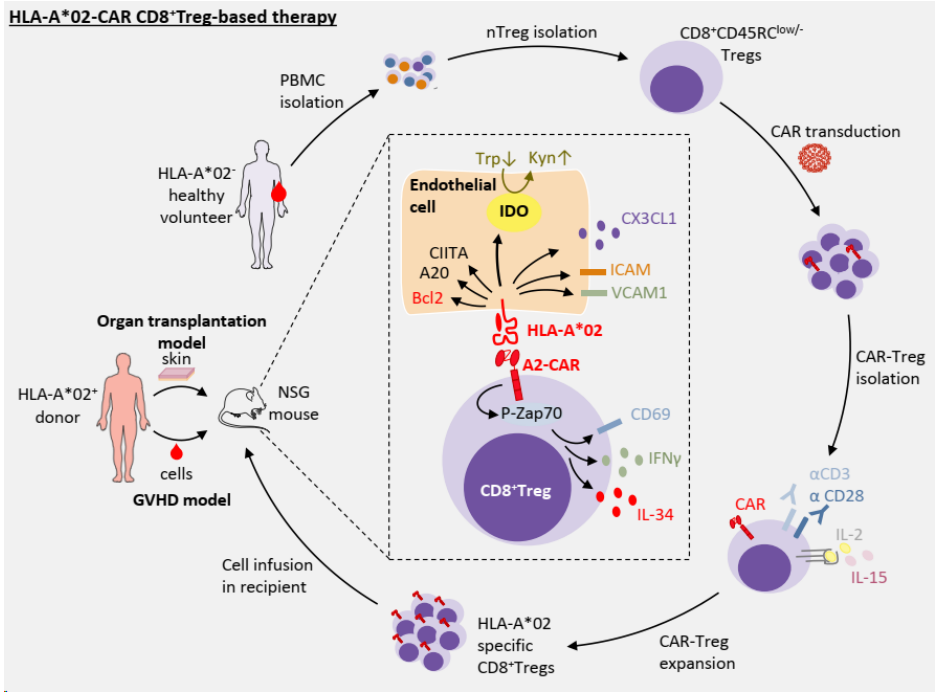
- **Results:**
  - Foxp3⁺CD45RC$^{low/-}$CD8⁺ T cells are highly potent regulatory cells present in human blood
  - CD8⁺ Tregs act through secretion of regulatory cytokines
  - CD8⁺ Tregs are non cytotoxic
  - CD8⁺ Tregs can be efficiently expanded in vitro
  - Expanded CD8⁺ Tregs control human skin allograft rejection and xenoGVHD in vivo in humanized NSG mice



Bézie et al, Frontiers Immunol, 2018

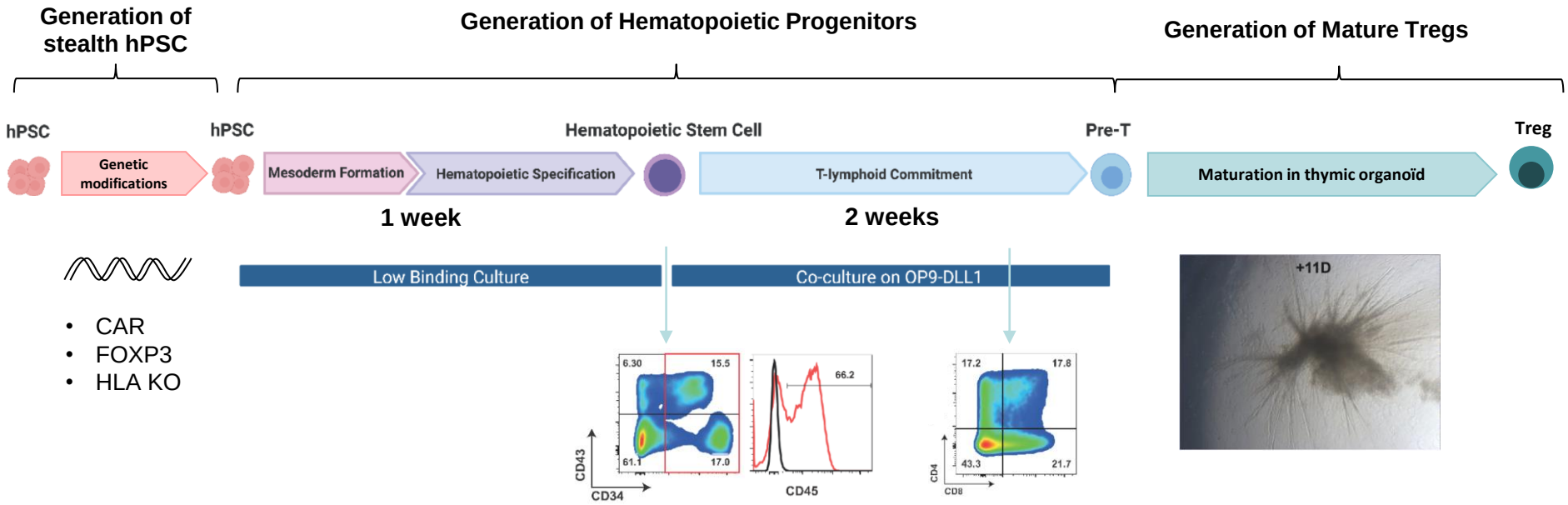
Development of a CAR-Treg-based cellular immunotherapy

- **Results:**
  - Establishment of a protocol of CAR-CD8$^+$ Tregs expansion
  - HLA-A*02-CAR CD8$^+$ Tregs maintain a Treg-like phenotype after expansion
  - CAR-CD8$^+$ Tregs suppress more efficiently in vitro than polyclonal CD8+ Tregs
  - HLA-A*02-CAR CD8$^+$ Tregs are not cytotoxic and induce tolerogenic gene expression in HLA-A2$^+$ donor kidney graft endothelial cells
  - HLA-A*02-CAR CD8$^+$ Tregs delay HLA-A*02$^+$ skin graft rejection
  - HLA-A*02-CAR CD8$^+$Tregs are still detectable in blood and spleen >80 days following transplantation
  - HLA-A*02-CAR CD8$^+$ Tregs are superior to polyclonal CD8$^+$ Tregs in preventing GVHD in HLA-A*02-recipients



Bézie et al., Blood Advances, 2019; Vimond, Mol. Ther., 2021

- **Perspectives:**
  - Advance research in both organ or cell transplantation and autoimmunity with unmet medical needs → Exploring CD8⁺ Tregs diversity in blood and infiltrating the graft, and their partners → discovery of new biomarkers → new treatments
  - Demonstrate the potential of CD8⁺ Tregs to control unwanted immunities/inflammations and wean off IS in the long term
  - Establish an active donor-specific tolerance using CARs CD8⁺ Tregs
  - Set up a universally compatible off-the-shelf cell therapy to treat all patients



Flippe et al., Cells, 2022
Flippe et al., Front Cell Dev Biol., 2020
Flippe et al, Immunological reviews, 2019
Provin et al., Front Immunol, 2022
Besnard et al., Dis Model Mech, 2021

- **Unique selling points**
  - $CD8^+$ Treg cells therapies to restore the immune-tolerance balance for long-term
  - Autologous or allogeneic $CD8^+$ Treg
  - PoC *in vivo* in rat in organ transplantation (single agent, preventive), in mice in EAE (single agent, preventive) and in immune humanized mice models of human skin rejection and human xenoGVHD
  - ≥3 times more efficient with a Chimeric Antigen Receptors (A2-CAR)
  - Good safety profile: no cytotoxicity *in vitro* against allogeneic blood and kidney endothelial cells, no cytotoxicity post 3 months treatment in NSG mice, stability in inflammatory conditions
  - Phase I/IIa ready to be launched in 2024 in solid organ transplantation
  - Developable for a broad range of inflammatory and auto-immune diseases
  - Potential for long-term tolerance and weaning of conventional immunosuppressants
  - 6 patents

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- Besnard et al, Dis Model Mech, 2021
- Benallegue et al., Neurol Neuroimmunol Neuroinflamm, 2022

Translational Neuroscience In Neurodegenerative diseases

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Keywords
- Parkinson's
- Huntington's
- Alzheimer's
- Animal models
- Non-human primate
- Brain imaging
- Behavioural analysis
- Viral vector production
- Cell therapies
- Gene therapies
- Positron emission tomography
- Magnetic resonance spectroscopy
- CEST imaging

Abstract
The Molecular Imaging Research Center (MIRCen; 8500m2) is an integrated structure dedicated to translationnal studies in gene-, cell- and drug-based therapies for neurodegenerative diseases. Based in the CEA center of Fontenay-aux-Roses, it brings together multidisciplinary teams of physicians, physicists, neurobiologists, virologists and imaging specialists, in close association with the Neurological Departments of various Parisian hospitals (Pitié-Salpétrière, Henri Mondor, Bicêtre). MIRCen ensures the coordination of research, the networking of skills and the optimization of resources in the area of translationnal neuroscience and therapeutics. The center offers a unique combination of complementary methodologies and state of the art platforms, including 7T and 11.4T MRI, microPET, radiochemistry laboratories, BSL2/3 animal housing for rodents and non-human primates, and laboratories specialized in viral vector development & production, neurosurgical, behavioural and anatomical studies, that allow the development of original models of brain diseases and the pre-clinical validation of innovative therapies. MIRCen is dedicated to hosting national and international projects and offers the opportunity to selected academic or industrial teams to temporarily access the 2000m2 of equipped platforms as well as 200m2 of dedicated laboratory space. MIRCen is part of NeurATRIS and MEDICEN Paris Region and has developed strong industrial partnerships (SANOFI-AVENTIS, Servier, Ipsen Beaufour, Oxford Biomedica Ltd, UCB) for the evaluation of new drug/gene therapy approaches, playing a pioneering role in pre-clinical and clinical development of cell- and gene-based therapies for Huntington's and Parkinson's diseases

Research area
Translational Neuroscience

Synopsis
Propose, develop and validate new therapeutic strategies for neurodegenerative diseases based on data generated using innovative animal models (including non-human primates), state-of-the-art imaging modalities and behavioural analyses

Interests
Gene therapy;Cell Therapy;Viral vectors;Neurology;Aging;In vivo models;Imaging;Translational research;Clinical research

Translational Neuroscience In Neurodegenerative diseases

Philippe HANTRAYE

MIRCen-CEA & NeurATRIS
Fontenay aux Roses

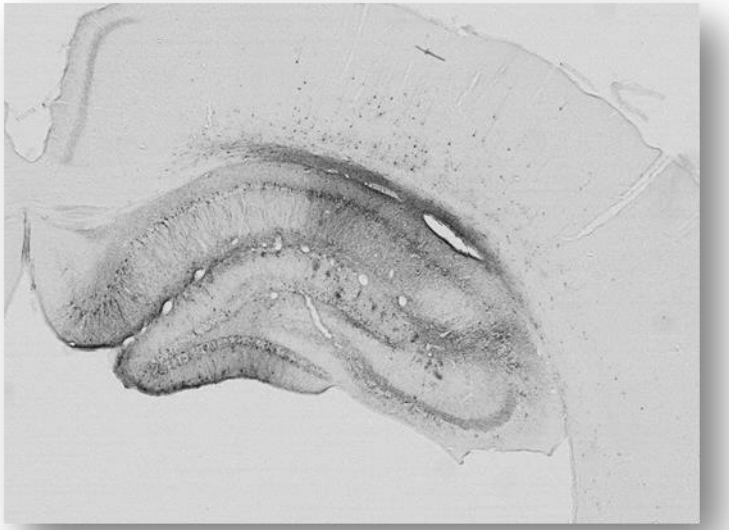
- **Objectives:**

  Modelling Parkinson's, Huntington's and Alzheimer's Disease in animals to :

  - mimic and study pathophysiological mechanisms
  - test new drug-, cell- or gene-based therapeutic strategies
  - develop & validate imaging biomarkers

- **Tools:**

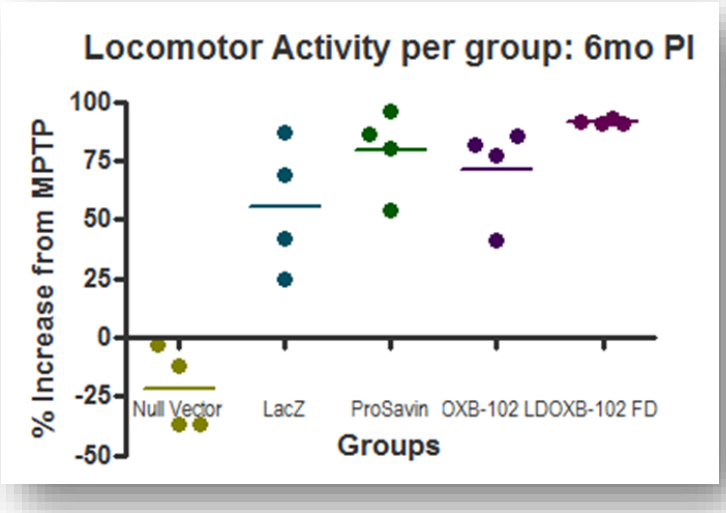
  - Neurotoxin or genetic (viral-vector) animal models including rodent (mouse, rat) & non-human primates
  - Behavioral analysis including cognitive/motor task in both species
  - MRI/neuronavigation assisted neurosurgery
  - Intracerebral cell or gene transfer
  - In vivo characterization of lesion and functional recovery using imaging biomarkers
  - PET, MRI/MRS and CEST



Rat model of tauopathy induced by local overexpression (intra-hippocampal) of a viral-vector encoding for the human wild-type tau protein.
I(section mmunostained for AT8, 2months post-injection)

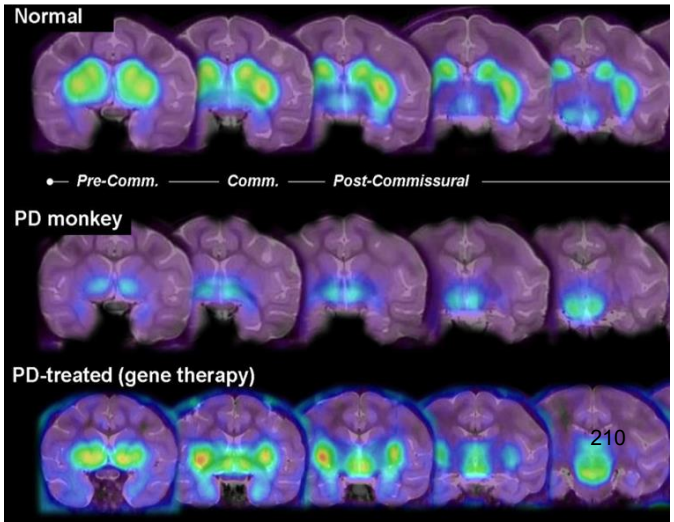
Topic 1 : In vivo gene Therapy for Parkinson's disease

- **Objectives :**
  - Assess the efficacy and safety profile of two viral vectors capable of restoring in situ dopamine synthesis following intrastriatal gene transfer
  - Study motor recovery using objective measures of locomotor activity (video-based tracking analysis) and clinical rating scales
  - In vivo PET imaging of transgene expression using a PET radiotracer (18F-FMT) selective of one of the transfected enzymes (AADC)
  - Assess potential side effects and adverse reactions through monitoring of antibodies against vector components in CSF and blood
  - Detailed post-mortem immunohistochemical analysis of gene expression and potential inflammatory/adverse reaction



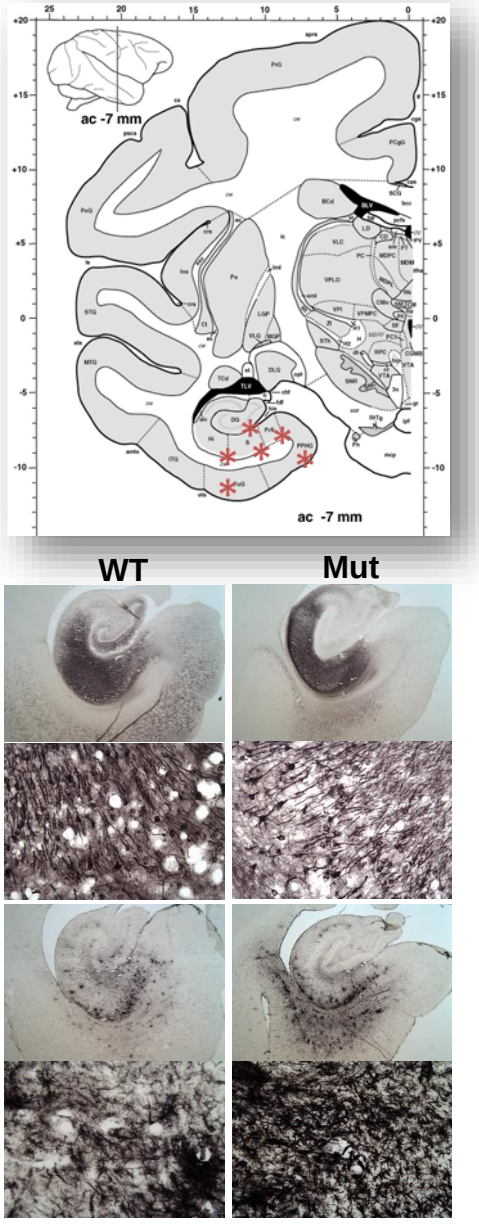
Ethovision® quantification of the total distance moved (TDM) at 6 months post vector administration for all NHP involved in the study. The percentages were calculated on the last 3 TDM values obtained before euthanasia and the last 3 values obtained at the MPTP stage prior to surgery.

PET Imaging - ^{18}F-FMT



Topic 2 : modelling tauopathies in rodents and non-human primates

- **Objectives:**
  - Develop two large animal models of tauopathy induced by local overexpression of wild-type & mutated tau species
  - Analyze longitudinally the progression of cognitive symptoms using tactile screens following intra-hippocampal viral vector delivery
  - Use PET imaging to characterize the metabolic/inflammatory consequences of these experimental tauopathies
  - Use anatomical MRI to detect adverse effects after vector delivery and potential hippocampal atrophy over time
  - Validate new biochemical and imaging biomarkers for these two types of tauopathy
  - Detailed post-mortem immunohistochemical analysis to characterize the two models of tauopathy



Immunohistochemical detection of AT8 and HLA-DR in the NHP hippocampus following AAV-mediated gene transfer in CA1

Perspectives:

- Develop and characterize new NHP models of proteinopathies using synthetic aggregate species of tau and alpha-synuclein (collab Melki & Bezard).
- Assess new (immuno) therapies for Alzheimer/tauopathies in pre-clinical NHP settings (collab Buée)
- Validate new imaging (PET) biomarkers for improved (more selective) beta-amyloid imaging, tau-imaging and alpha-synuclein imaging through well established collaborations with industry and academics
- Establish a fully reversible controlled blood brain barrier opening using ultra-sound guided magnetic resonance imaging (collab with NeuroSpin-CEA)
- Develop new gene-based therapies for Huntington's disease and other neurodegenerative disorders (collab with D. Kirik)

- **Unique selling points**

  - Pioneer in PET imaging – in vivo pharmacological characterization of the benzodiazepine receptor site in NHP and humans

  - Unique & strong expertise in the development and characterization of non-human primate models of neurodegenerative diseases

  - Pioneering work in cell transplantation for Parkinson's and Huntington's diseases

  - Pioneering work in gene therapy for Huntington's disease

  - Director of MIRCen, one of the three imaging platforms of the Fundamental Research Division of the CEA which can operate in close association with the SHFJ and NeuroSpin (pasrel-imagerie)

  - Director and scientific manager of NeurATRIS, a national infrastructure in translational neuroscience giving access to a large continuum of drug discovery platforms in the field of Parkinson, Huntington, Alzheimer and Multiple Sclerosis

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Innovative cell based therapies, Immunotherapies, auto-immunity

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Keywords
• cellular therapies, • immunotherapies, • RNA, • regenerative medicine, • reprogramming

Abstract
The IRMB Institute of Regenerative Medicine focuses on reprogramming, adult stem cells and immunotherapy. Our institute is a leader in these fields at national and European levels. The objective of our Inserm center within IHU IMMUN4CURE is the development of innovative therapies in the areas of autoimmune diseases. Currently, our research projects within UMR 1183 are based on in vivo models of humanized mice and organoids:
- The first axis is dedicated to better understanding the deregulation of the immune system leading to the development of experimental arthritis, with a particular emphasis on monocyte subsets and T lymphocytes. We have identified microRNAs specific to inflammatory monocytes, as well as genes associated with chronic inflammatory diseases with joint tropism and we studied their role in pathophysiological conditions. We develop NK based depletion immunotherapies targeting specific B cells expressing autoantibodies as well as CAR-Treg strategies.
- The second axis focuses on mesenchymal stem cells (MSC) and reprogramming with two main objectives. The first concerns the identification of the molecular mechanisms involved in cartilage regeneration and the second aims to determine the mechanisms responsible for the immunosuppressive effects of MSC. We also studied the biodistribution and migration of MSCs after their administration in vivo in different murine models and we identified their localization sites. We develop MSC differentiated from iPS et NCC cells and reprogrammed for cartilage regeneration. We also develop EV based therapies. Understanding inflammation, autoimmunity and regeneration is at the heart of our approach and allows the development of precision biomedicines derived from biotechnologies for the curative treatment of autoimmune diseases supported by the IHU IMMUN4CURE.

Research area
Stem cells and immunotherapy

Synopsis
Development of cell therapy and regenerative medicine as well as therapeutic RNA in the fields of autoimmune diseases, oncology and osteoarticular diseases.

Interests

Cell Therapy;mRNA;Stem cells;Extracellular vesicles;Oncology;Immunology/Immunotherapies;Autoimmune diseases;In vitro models/ Organ-on-chip;In vivo models

No results presentation available

Extracellular Vesicle-based vector for therapeutics delivery

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City PARIS

Keywords
- Vectorization,
- Extracellular Vesicle
- Cell/gene therapy
- Cancer
- Brain disease.

Abstract
We are actively advancing a cutting-edge bioprocess designed to deliver precise therapeutics to specific cells and tissues. This innovative approach harnesses the power of Extracellular Vesicles (EVs), which serve as natural vehicles in intercellular communication. Notably, EVs are devoid of viral components, eliminating a significant challenge in vector manufacturing and safety issues.
Our engineered EVs possess advanced capabilities, enabling them to:
1. Load a wide range of peptide-based cargos of interest.
2. Target specific cells with precision.
3. Efficiently deliver cargos and therapeutics into target cells using a human fusogen.
As a proof of concept, we have successfully developed "Killer EVs" for the targeted elimination of cancer cells and "Editing EVs" for the reprogramming of CD8+ target cells. Our current focus is to validate these applications in vivo and move toward industrializing the process. This entails internal development of the two aforementioned products and forging partnerships with biopharmaceutical companies for new or existing translational programs.

Research area
Membrane trafficking, Extracellular Vesicle

Synopsis
Our Research aims at establishing Extracellular Vesicles-based vector for targeted therapeutics delivery.

Interests
Gene therapy;Cell Therapy;Non viral delivery systems;Extracellular vesicles;Oncology;Immunology/Immunotherapies;Neurology;Specific targeting;Translational research

Extracellular Vesicle-based vector for therapeutics delivery

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- **Objectives:**
  - Establishing Virus-free/EV-based vectors as a new standard for targeted therapeutics delivery
  - Integrate “Killer EVs” and “Editing EVs” in new or existing gene/cell therapy programs.

- **Tools:**
  - EV bio-Engineering Platform
  - Qualitative and Quantitative EV Uptake and Delivery assays
  - Cas9/CRIPSR Surfaceome library

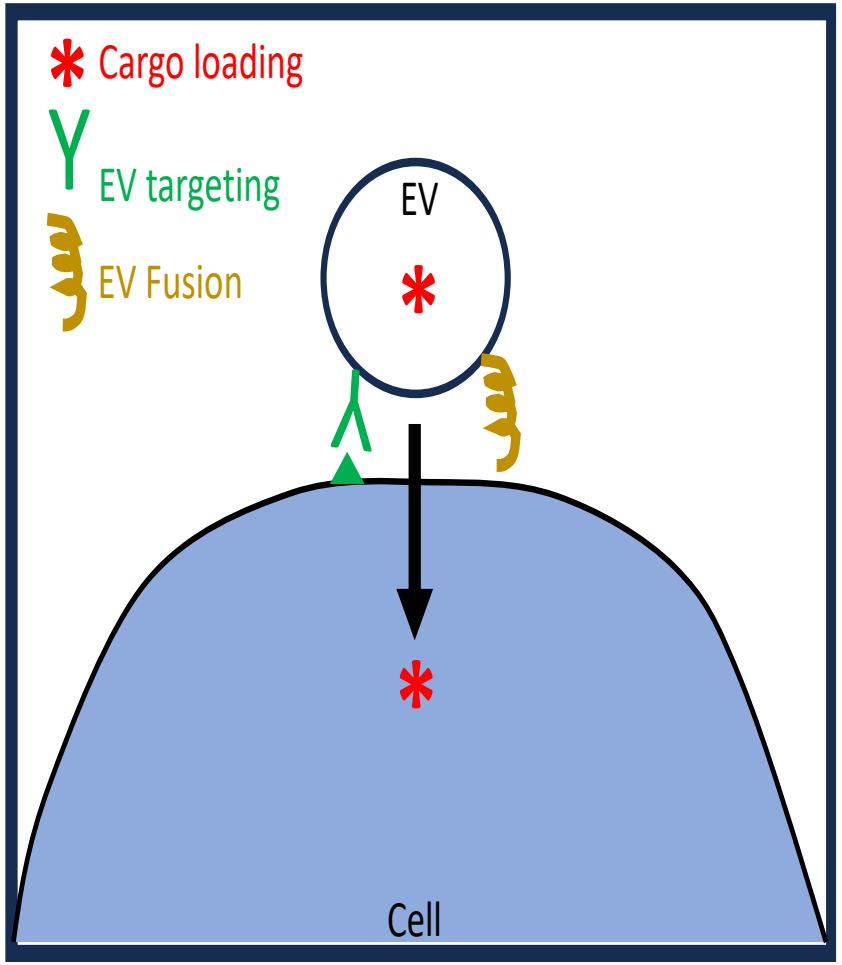
3 steps process for EV-based vector bioenginnering

- **Results:**
  - Modular system to enhance EV delivery properties

  1) inducible and reversible EV-Cargo Loading system

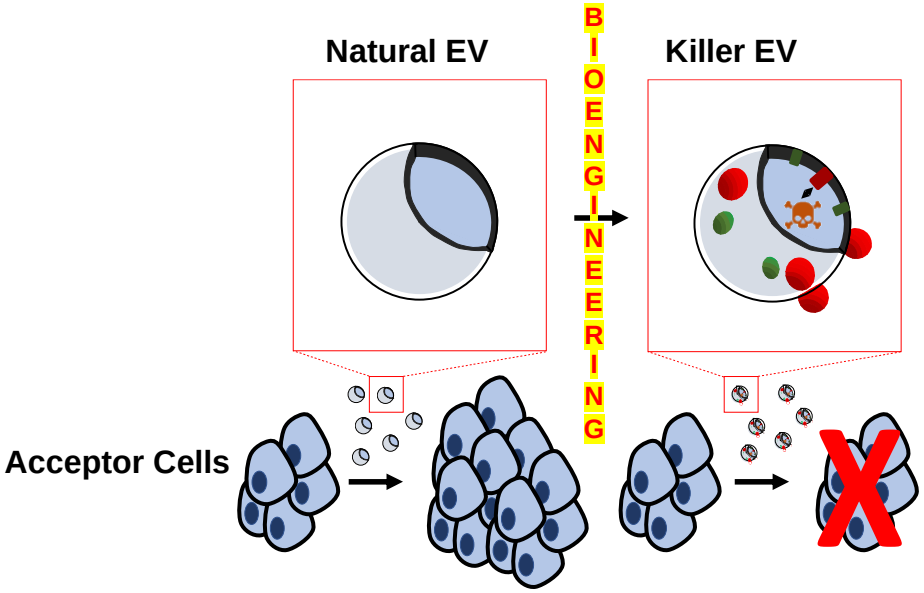
  2) Versatile Targeting system using R&D grade of clinically validated Antibodies

  3) Enhanced delivery property through syncitin1, a human fusogene.
  - Killer EVs contain DTA, a lethal toxin , and eliminate cancer cells
  - Editing EVs contain Cas9/rRNA and reprogram target cell of interest.



POC: KILLER EVS

- **Results:**
  - Killer EVs are loaded with DTA, a potent letal toxin,
  - Killer EVs fuse with target cancer cell via Synctitn1, a human fusogene
  - Killer EVs eliminate cancer cells



Bui S, Dancourt J, **Lavieu G***. *Virus Free-Method to Control EV-Cargo Loading and Delivery*. ACS A Bio Mat. 2023.
Dancourt J, Piovesana E, **Lavieu G***. *Efficient cell death mediated by bioengineered killer extracellular vesicles*. Sci Rep. 2023.

- **Perspectives:**
  - New EV-based vectors for cell/gene therapies
  - Killer EVs for tumor ablation, *in vivo*
  - Editing EVs to enhanced Tumor infiltrating lymphocyte efficacy
  - EV based vectors applied to brain-related disease

- **Unique selling points**

  - VIRUS FREE method → safer and less immunogenic
  - Modules (loading, targeting and fusion) are independent but synergistic
  - Implementation of quantitative quality controls at each step of the process

Selected bibliography

- Bui S, Dancourt J, **Lavieu G***. *Virus Free-Method to Control EV-Cargo Loading and Delivery*. ACS A Bio Mat. 2023.
- Dancourt J, Piovesana E, **Lavieu G***. *Efficient cell death mediated by bioengineered killer extracellular vesicles*. Sci Rep. 2023.
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From lipid nanoparticles to advanced sensors and microfluidic : empowering biotherapies with micro/nano-technologies

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Keywords
- Lipid Nanoparticles
- Drug delivery
- RNA delivery
- Holographic imaging
- In situ monitoring
- Quality control
- Electrochemical (bio)sensors
- Mac
- AI,suspended micro/nano-channel resonator, Microfluidic, Organ-on-chip
- single cell manipulation

Abstract
The Biology and Health division of CEA-Leti aims at playing a key role in the development of technologies for health with a “One health” strategic positioning, which takes into account human in interaction with its environment as a key issue. Its Core R&D competencies are the development, design, integration and qualification of micro- and nanotechnologies in application to health (in the broad sense as defined by WHO) and life sciences. These include sensors and actuators, imaging technologies, microfluidics, chemistry, biochemistry and electrochemistry, biology and instrumentation, including mechanics, software, information processing and electronics.
Our teams have acquired expertise in developing product prototypes with a system-development perspective for four applicative axes (in vitro diagnostics, implanted and wearable medical devices, systems for the exposome monitoring and tools for pharmaceutic and biotechnology processes) according to the user needs.
Our facilities cover the whole value chain devoted to health technologies from the technological platform dedicated to medical device development (constituted of the “Microfluidic Integration” and “Numerical Medical Devices” platforms) to preclinical and clinical investigations.
In the field of biotechnologies, we bring three complementary scientific and technologic visions. First, ware developing new lipid nanovectors dedicated to RNA delivery (lipidots). Secondly, owing to the support of Leti’s technology platform we are involved in the design of advanced sensors / systems dedicated to the closed-loop control or the quality control of bioprocesses. Finally, we investigate disruptive microfluidic technologies that could be game changers for the future of drug screening and delocalized biotherapies production in small batches.

Research area
Leti – Health division

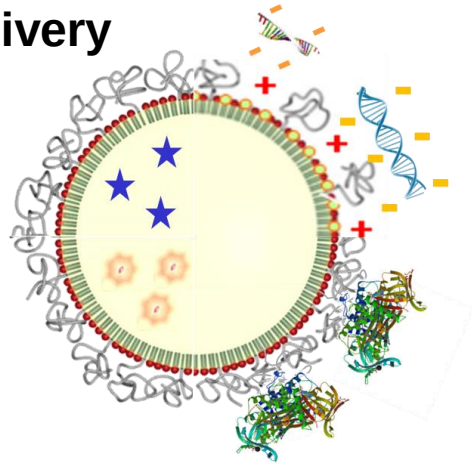
Synopsis
From lipid nanoparticles to advanced sensors and microfluidic : empowering biotherapies with micro/nano-technologies

Interests
Non viral delivery systems;mRNA;In vitro models/ Organ-on-chip;Sensors and biosensor;Artificial Intelligence (AI);Single cell manipulation;Imaging;Nanotechnology;Translational research

From lipid nanoparticles to advanced sensors and microfluidic : empowering biotherapies with micro/nano-technologies

Pascal MAILLEY, *PhD*

Cationic lipid nanoparticules for RNA delivery



- **Objectives:**
  - Vectorisation
  - Delivery
  - Gene therapy

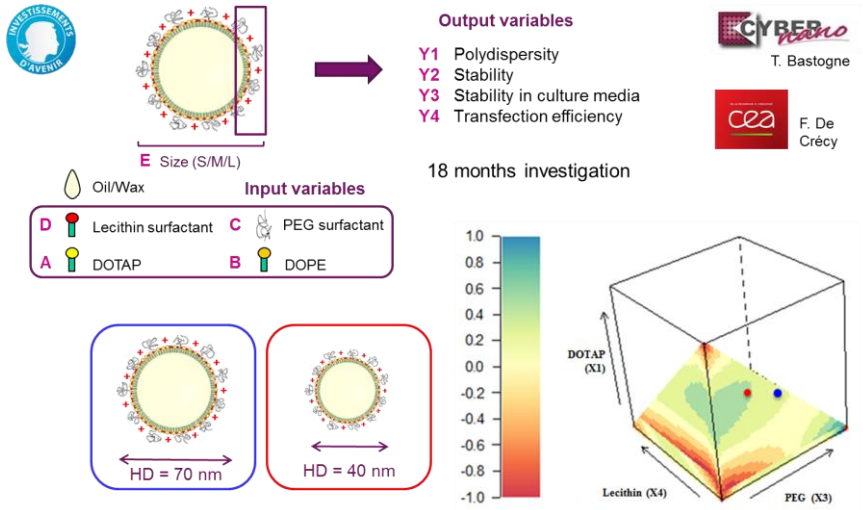
- **Tools:**
  - KNPs: Patented Lipidot® technology
  - Dispersion of an oily phase in an aqueous phase (O/W nanoemulsion)
  - Highly stable nanoparticles exhibiting an oily core surrounding by a “PEGilated” shell with tailored diameters
  - Great flexibility: same vector batch can be used for several medicines
  - Validated scalability (CEA NanO’Up platfor and GT Bioways Toulouse)
  - **Easy (bio)chemical post-functionalization of the shell by** :
    - Cationic moieties
    - Proteins such as Ab (targeting)
    - Chemical ligands
  - **Electrostatic immobilization of RNA/DNA**
    - siRNA
    - mRNA
  - **Encapsulation of lipophilic chemicals in the oil phase**
    - Fluorescent probes
    - Drugs

GTP Bioways CDMO
Continuum for biologics

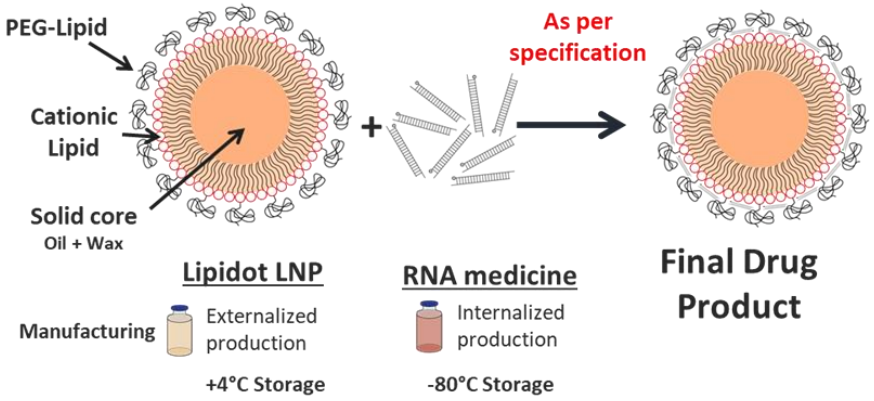
Ceva Santé Animale

Cationic lipid nanoparticules for RNA delivery

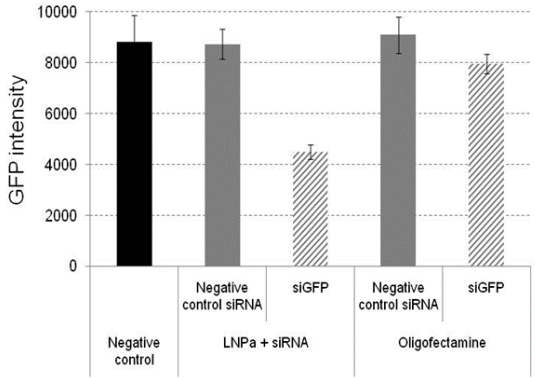
- **Results:**
  - Cationic-LNP formulation through a design of experiments (DoE) approach. Screening and selection of 2 formulations for delivering RNA while preserving the stability
  - Simple, scalable and stable RNA loading through electrostatic interactions
  - mRNA vaccines with equivalent activity than classical RNA-lipid nanoparticles (presenting lower stability and more complex formulation protocols)
  - *In vitro* siRNA delivery with high level of down regulation efficiency even on cell spheroids



Cationic LNP formulation using DoE approach



RNA loading on cationic LNPs



Comparison of the down regulation efficiency of LNPs and Oligofectamine on cells spheroid models

Tools for process and quality control in biotherapies

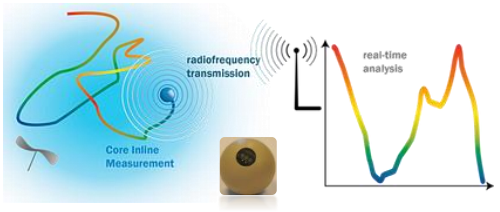
- **Objectives:** Sensors for USP and DSP monitoring
  - *In situ* and on line physicochemical sensors
  - At line cell imaging
  - Integration of the characterization tools
  - AI, data treatment and analysis
  - Automation and closed-loop control of bioprocesses

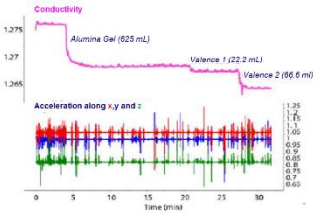
- **Tools:**
  - **Bioreactor – USP**
    - Electrochemical sensing platform (O_2, CO_2, pH, conductivity…) for *in situ* localized monitoring of cell culture parameters
    - Lensfree microscopy (Holographic microscopy) for at line imaging of cells

  - **Extraction &purification – DSP**
    - Photonic biosensors (silicon based Mach Zehnder interferometers) for the detection of contaminants
    - Suspended Nanochannel Resonators for vaccine nanoparticles quality control

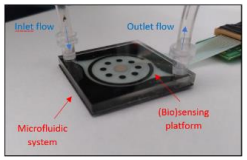
Tools for process and quality control in biotherapies

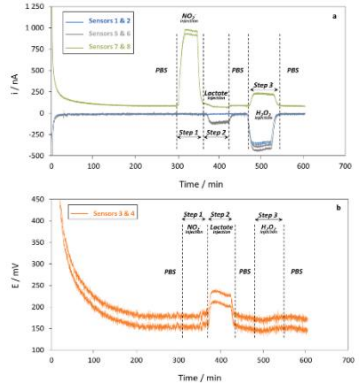
- **Results:**
  - Multiparametric electrochemical platforms (from sensors to integration and data transfer and analysis) enabling in situ monitoring of cell culture bioreactors
  - Contamination biosensors based on Mach Zehnder interferometers chemically modified by specific probes. (other less matured technologies such as SiNW FET, photoacoustic resonators or acoustic resonators are also available)
  - Lensfree imaging for label-free cell-viability assessment in the bioreactor. AI reconstruction of the cell images from diffracted images and calculation of the viability physical descriptor.
  - Suspended Nanochannel Resonators for the quality control of vaccine nanoparticles (for example RNA loading in adenovirus vectors)



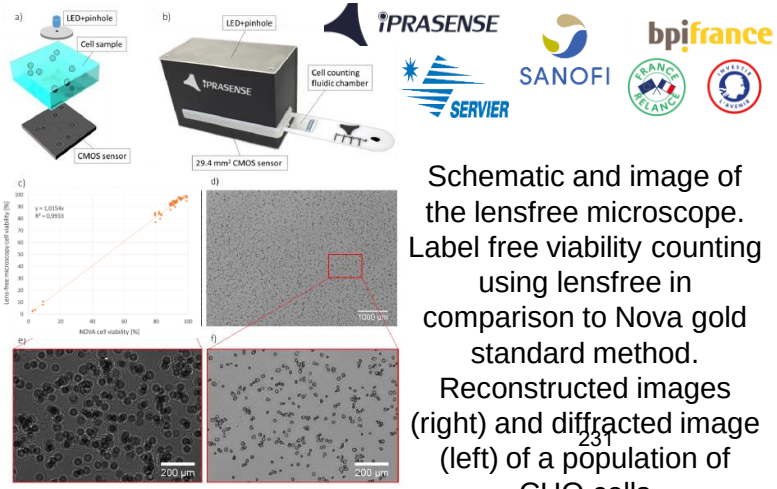


Conductimetry tracker for in situ monitoring of vaccinal formulation homogeneity

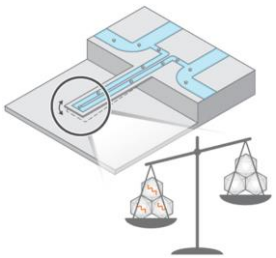


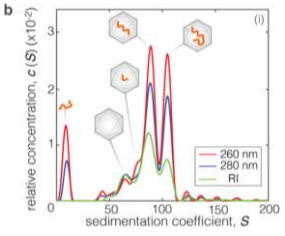


Multiparametric EC platform for cell culture monitoring lactate (H_2O_2, NO_x, pH)



Schematic and image of the lensfree microscope. Label free viability counting using lensfree in comparison to Nova gold standard method. Reconstructed images (right) and diffracted image (left) of a population of CHO cells





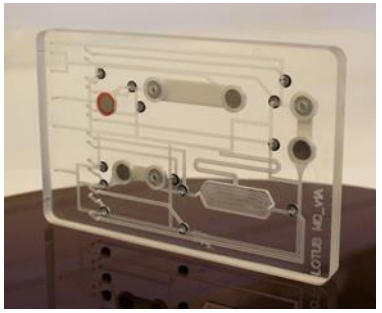
Principle of SNR function and discrimination by weight of DNA loading in adenovirus vectors

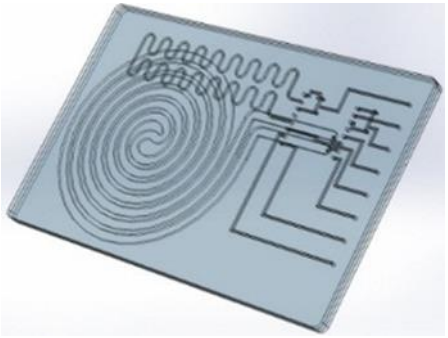
Microfluidics for cell therapies

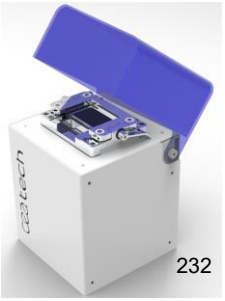
- **Objectives:**
  - Single biological object operation (OoC, cell)
  - Sorting, encapsulation, counting, transfection…

- **Tools:**
  - **Integrated microfluidic automatons**
    - Cell and fluids manipulation
    - Cell transfection (EC or US)
    - Full automation including actuators, imaging and sensing
    - Adapted to small batch processing (cell therapies), µfactory

  - **Toward Organ-on-Chip systems**
    - Screening of therapeutic drugs/biomolecules
    - Sensors and imaging integration

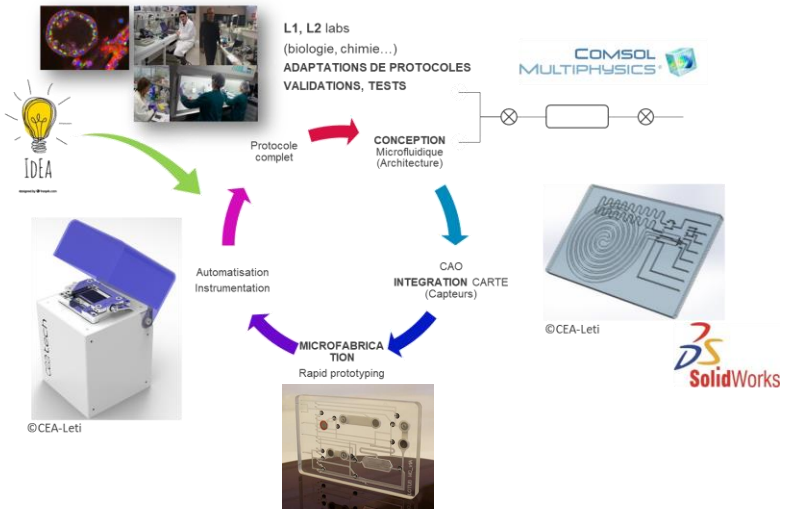




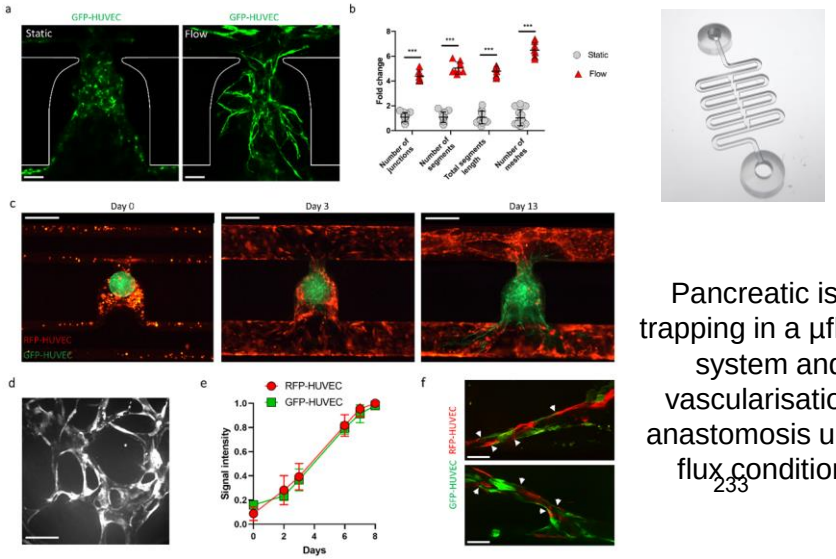


Microfluidics for cell therapies

- **Results:**
  - Single biological object manipulation in complex fluids using different microfluidic technologies (digital, EWOD, capillarity, pressurized flux) microfabricated on different materials (Silicon, COC, PDMS) according to the size of the biological entity
  - Existing technologies for single biological object (cells like T-Cars, organoid) manipulation (encapsulation, trapping) and/or induction (chemical or physical)
  - Vascularized pancreatic islets in instrumented OoC platform (O_2 detection, holographic imaging, TEER, Insulin secretion)
  - µfluidic integration of sensors for on chip cell or organoid survey (drug screening, biochemical induction) and/or actuators for transfection (US, electrical)



Design of microfluidic lab-on-chip systems with examples of cartriges dedicated to pancreatic islets encapsulation or radiotracor µfactory and automaton for proteic biomarkers profiling in whole blood



Pancreatic islet trapping in a µfluidic system and vascularisation / anastomosis under flux conditions

Perspectives:

- **Lipidiots®**
  - **First in man tests on Lipidots nanovectors**
  - **Implementation of Lipdots in RNA therapies**

- **Biotherapies process control**
  - **Integration of sensing capabilities (USP and DSP)**
  - **Toward small batches manufacturing using milli / micro-fluidic with monitoring and actuation integration**

- **Microfluidics**
  - **Toward integration of technological bricks for single cell processing**
  - **Implementation of OoC technology for drug / therapy screening**

Unique selling points

- **Lipidots®**
  - **New simple to manufacture (premix) and easy to functionalized versatile nano-vectors exhibiting extending lifetime and stability in room temperature conditions**
  - **Possibility to implement simple or Multiple biological activities (delivery, targeting, imaging)**

- **Biotherapies process control**
  - **Unique one-stop-shop providing sensor design, manufacturing and integration**
  - **Multimodal survey of biotechnology processes (imaging and EC, optical or MEMS sensors)**

- **Microfluidics**
  - **Unique one-stop-shop providing µfluidic cartridges for single biological objects manipulation with sensing/actuation capabilities**

Selected bibliography

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  - Bussooa et al, *Biosens. & Bioelec*., 2022
  - Parent et al, *Anal, Chem.,* 2021

Investigation of therapeutics using human pluripotent stem cells

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Keywords
- Drug screening
- 2D and 3D cell models
- Rare diseases
- Human pluripotent stem cells
- Automation
- Bioproduction

Abstract

Research area
The I-Stem laboratory is a structure dedicated to the exploration of all the potential therapeutic applications offered by human pluripotent stem cells to treat monogenic diseases. It is therefore presented in the scientific-medical continuum as an R&D center carrying out translational research. Therapeutic innovation receives constant inputs from fundamental research to refine, modify or redesign treatment strategies and this constant preoccupation with the therapeutic target is reflected in the organization of the teams around projects and schedules.

Synopsis
Understanding the mechanisms by which a genetic variation contributes to diseases is a central aim of human genetics and should greatly facilitate the development of preventive strategies and treatments. Implementing this approach is particularly challenging for diseases for which access to affected cell types from patients is challenging. Despite the wealth of existing cellular and animal models, progresses towards identification of new treatments have been hampered by the incomplete understanding of the pathogenic mechanisms involved in these diseases as well as the availability of relevant screening tools. The development of relevant Human models that even more closely replicate the disease will undoubtedly improve pathological modeling of neuromuscular disorders as well as more adapted therapeutics.
In this context, our research interests focus on developing in vitro human "tool box" to establish specific pathological models of rare diseases based on the use of human pluripotent stem cells. While developing these models, our proposal has several objectives: (1) deciphering the pathophysiological mechanisms (2) to use biomarkers revealed by the mechanistic approach as readouts for drug screening and (3) validate these developments by a proof-of-concept high-throughput screening (4) to convert these models into therapeutic products

Interests

Cell Therapy;Stem cells;Rare diseases;In vitro models/ Organ-on-chip;Automation;Translational research;Clinical research;Bioproduction

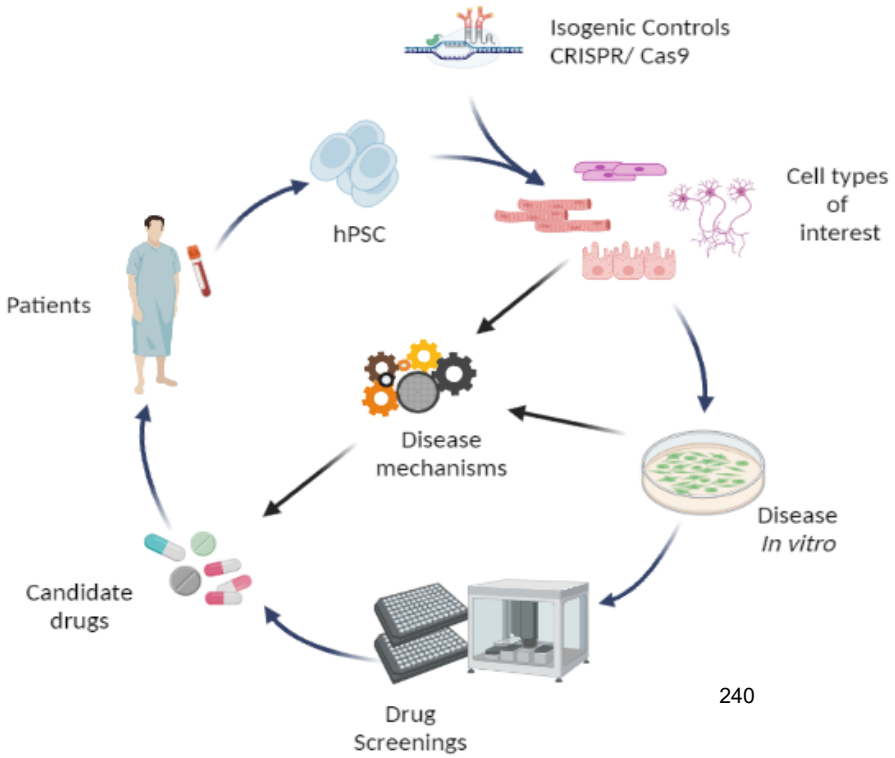
Investigation of therapeutics using human pluripotent stem cells

Cécile MARTINAT

UMR861 INSERM / UEVE / I-Stem

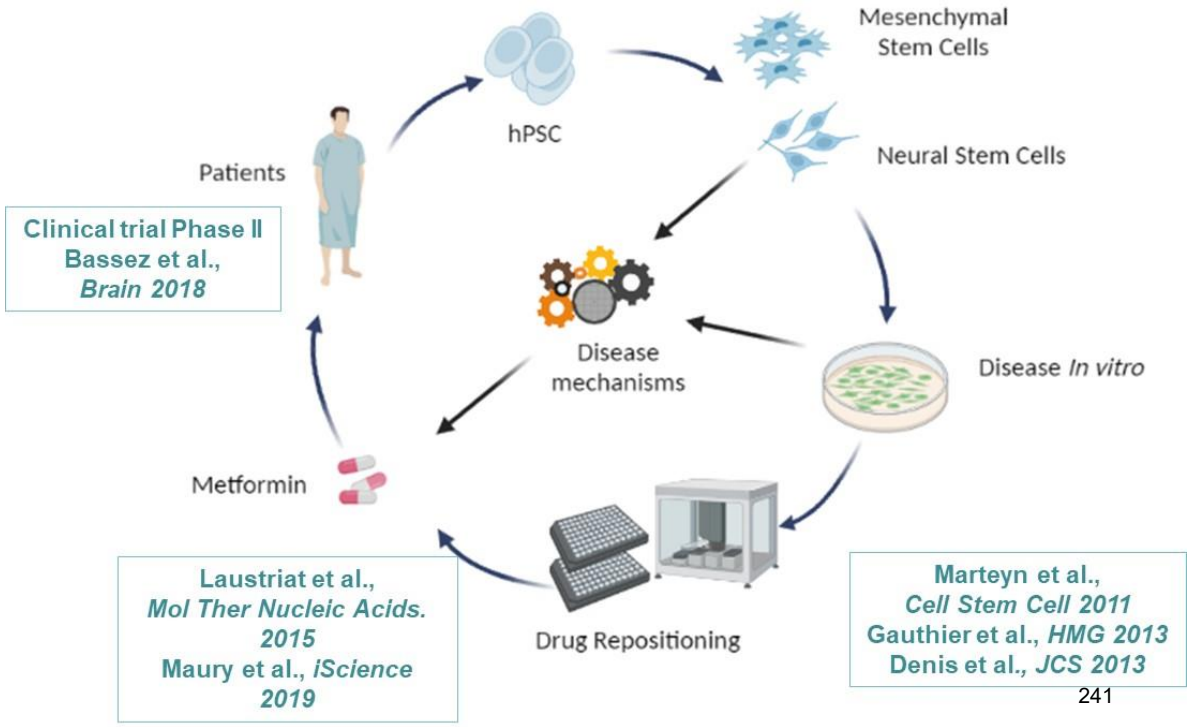
- **Objectives:**
  - To provide a platform for new disease specific humancell models with a focus on rare diseases
  - To apply comprehensive genomic profiling
  - To identify new therapeutic targets
  - New drug development for rare diseases

- **Tools:**
  - Disease specific human induced pluripotent stem cells
  - CRISPR/ Cas9 engineered hiPSC lines
  - Differentiation into cell types of interest
  - High throughput drug screening platforr



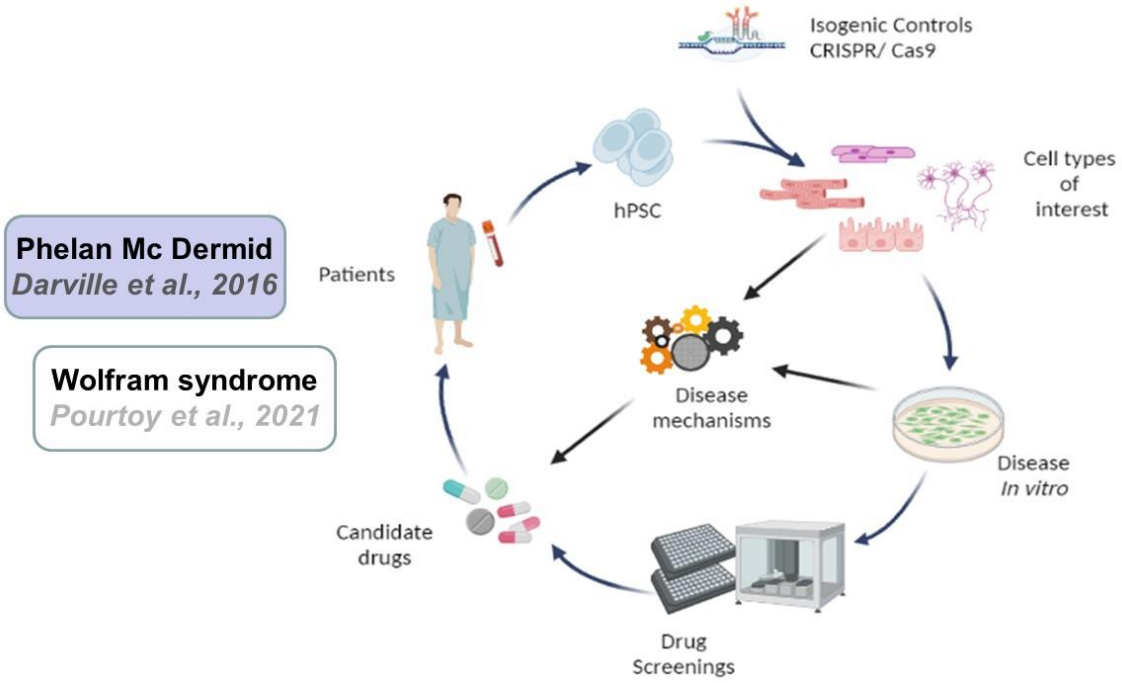
Demonstration of our experimental paradigm with Myotonic Dystrophy type 1

- **Results:**
  - Myotonic Dystrophy Type 1: the most common inherited form of dystrophy in adult
  - Generation of disease specific human pluripotent stem cell based models
  - Identification of druggable targets
  - Metformin identified as repositionable for this disease
  - Promising results obtained during a Phase II clinical trial
  - Two phase III recently launched

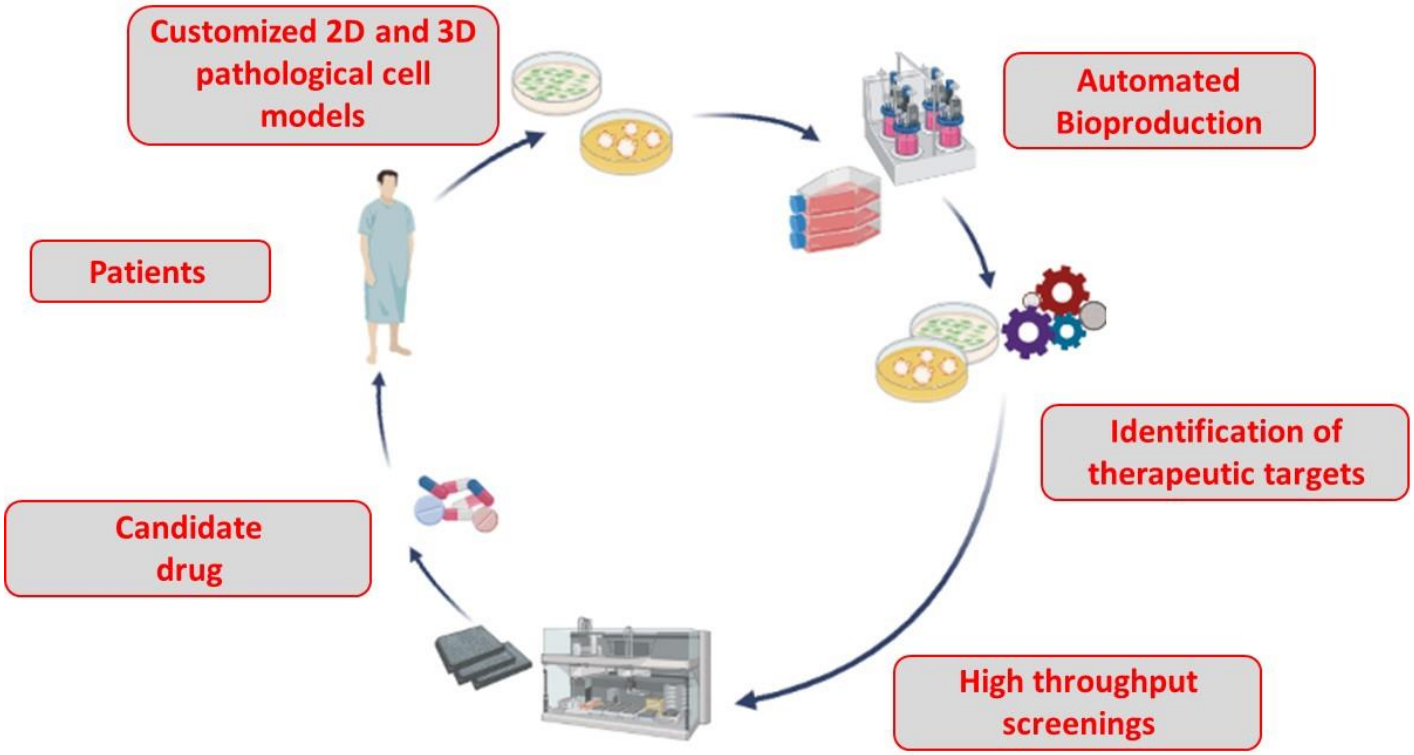


Validation of our experimental paradigm on two other rare diseases

- **Results:**
  - Identification of a potential therapeutic compound for the neurodevelopmental Phelan McDermit syndrome
  - Identification of a potential therapeutic compound for the complex Wolfram Syndrome



- **Perspectives:**
  - Providing well characterized disease specific human 2D and 3D cell models for rare diseases
  - Applying gene expression profiling to identify new therapeutic targets
  - Identifying and/ or validating new drugs candidate



- **Unique selling points**
  - Development of an integrated technologic 2D and 3D cellular platform for rare diseases
  - Integrative disease specific gene expression atlas for new therapeutic target identification
  - High-throughput screening platform based on nanodelivery of compounds
  - Reduce the developmental costs of pharmaceutical products

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Treatment of heart failure by a cardiac cell-derived secretome

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Keywords
- Heart failure
- Cell therapy
- Stem cells
- Secretome
- Extracellular vesicles
- Exosomes
- Cardiac fibrosis
- CAR-T cells
- Cell delivery

Abstract
Our group has a long-standing interest in stem cells for the treatment of chronic heart failure with a definite commitment towards clinical applications. While the initial research has focused on the transplantation of skeletal myoblasts (first-in-man implantation in 2000), it then moved towards the combination of cardiac progenitors derived from human embryonic stem cells with a tissue engineering-based construct. The first-in-man trial testing this cell-loaded patch has now been successfully completed. In parallel, mechanistic studies have unravelled the predominant role of paracrine signalling and, consequently, the group has shifted its research towards a-cellular cell therapy based on the exclusive use of the secretome with the objective of further streamlining the clinical translatability of this myocardial repair strategy and offering a user-friendly, non immunogenic, off-the-shelf and economically sustainable biological medication. The first clinical trial testing this a-cellular secretome has just started while, in parallel, preclinical studies continue with the objectives of better understanding the mechanisms of action of this secretome and optimizing its cardiac delivery. In parallel, the recognition that myocardial fibrosis is a key contributor to chronic heart failure has led the group to also initiate a collaborative program for assessing the effects of CAR-T cells against a fibrosis-associated protein in Duchenne Muscular Dystrophy.

Research area
Cardiovascular diseases

Synopsis
Treatment of heart failure by the secretome of cardiovascular cells, from basic research to the first clinical application

Interests

Cell Therapy;Chimeric Antigen Receptor (CAR)-T cells;Stem cells;Extracellular vesicles;Neuromuscular disorders;Rare diseases;Cardiovascular diseases;Translational research;Clinical research

Treatment of heart failure by a cardiac cell-derived secretome

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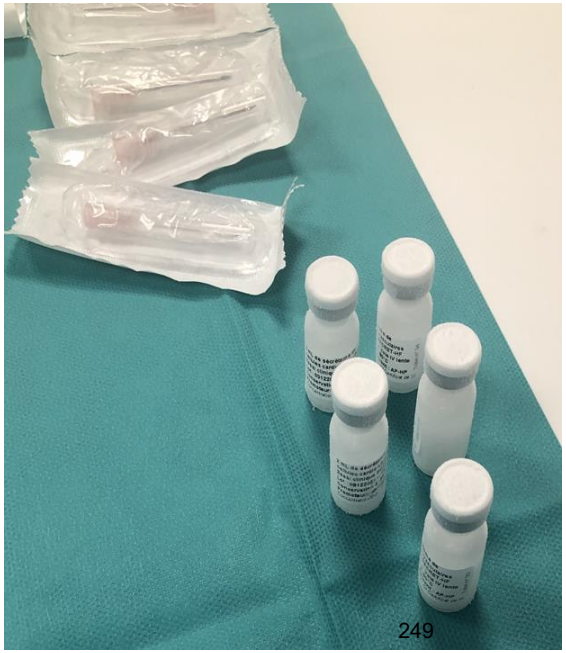
- **Objectives:**
  - Developing a clinical-grade biological medication made of the secretome of cardiovascular cells for treating advanced-stage heart failure
  - Defining the optimal route for the secretome delivery
  - Repositioning the use of Chimeric Antigen Receptor (CAR)-T cells for alleviating myocardial fibrosis

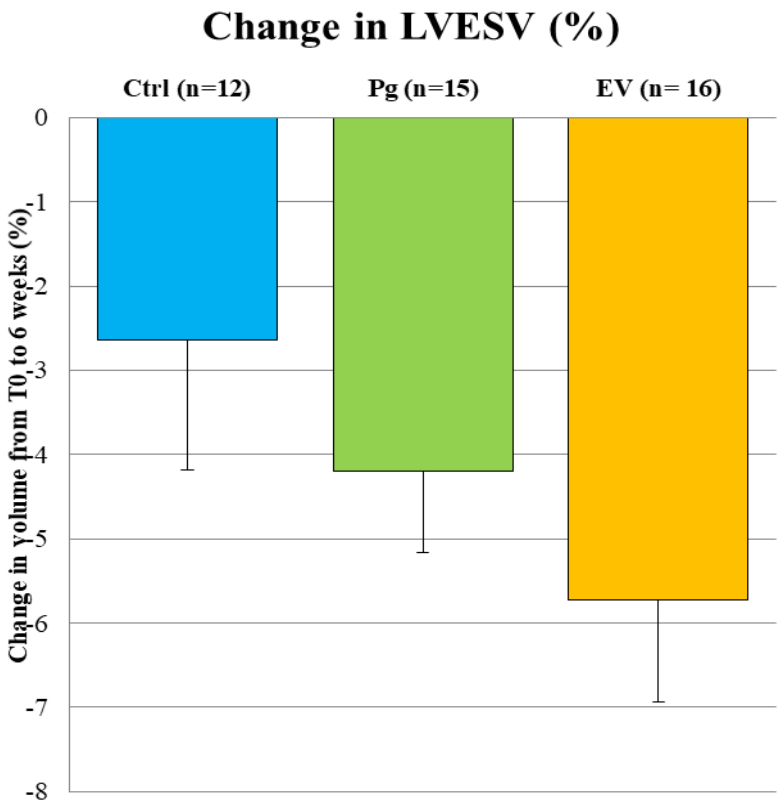
- **Tools:**
  - Preclinical models of myocardial ischemia and Duchenne Muscular Dystrophy
  - GMP platform for the production of ATMPs
  - Access to the recently labeled Genother biocluster

Vials of the recently manufactured off-the-shelf and non immunogenic cellular secretome currently tested in a phase 1 trial



Assessment of a cardiac cell-derived secretome

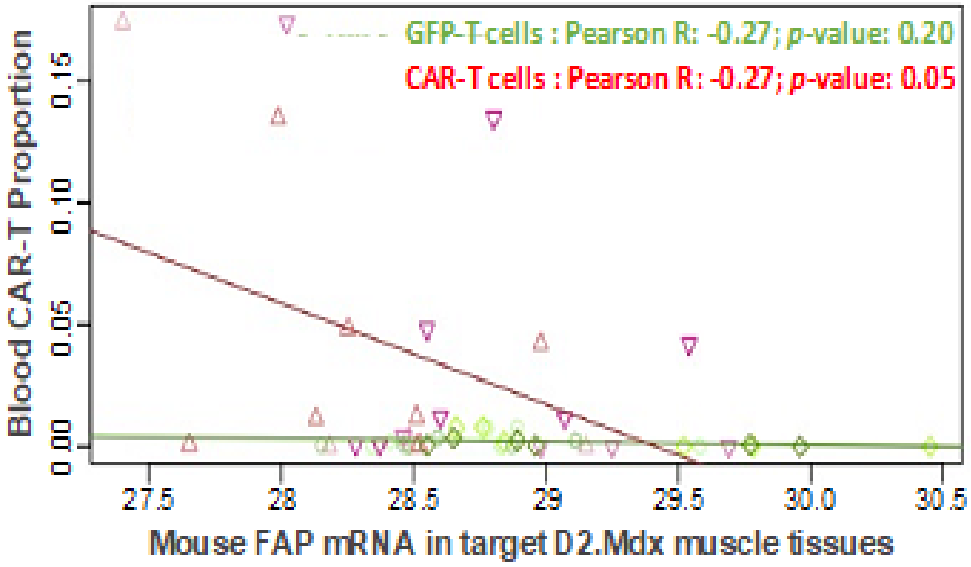
- **Results:**
  - Demonstration, in pre-clinical models of cardiac ischemia, of the functional equivalence between the transplantation of pluripotent stem cell-derived cardiovascular cells and administration of their sole secretome (Figure)
  - Demonstration of two major advantages of the secretome over cellular products: its non immunogenic nature and its stability under cryo-storage
  - Demonstration that this secretome still retains its cardio-reparative properties when given intravenously, thereby enabling convenient repeated administrations
  - Production of a cell-derived secretome under cGMP conditions and at Phase I clinical manufacturing scale which has allowed to launch a phase 1 safety trial



In this mouse model of chronic heart failure, mice treated with cardiovascular cell progenitors (Pg) or the secretome ot those same cells enriched in extracellular vesicles (EV) have similarly and significantly improved cardiac function compared with controls; LVESV: left ventricular end-systolic volume; the lower the volume, the better the prognosis (*Kervadec et al. J Heart LungTransplant 2016;35:795–807*)

Assessment of CAR-T cells for mitigating cardiac fibrosis

- **Results:**
  - Successful retroviral transduction of CAR-T cells targeted at Fibroblast Activation Protein (FAP), one of the most upregulated proteins in human cardiac fibrosis
  - Characterization of a mouse model of Duchenne Muscular Dystrophy selected for the first proof-of-concept because of the presence of fibrosis in both cardiac and skeletal muscles
  - Establishment of an effective and well tolerated protocol of lympho-depletion to enable *in vivo* CAR expansion
  - Demonstration of the first proof-of-concept manifest as an *in vivo* CAR expansion associated with a down-regulation of FAP expression in cardiac and skeletal muscle target tissues (Figure)
  - First evidence for a concomitant improvement in heart function



Negative correlation between *in vivo* CAR-T detection in blood and FAP expression (in heart and Tibialis Anterior). Lymphocytes expressing Green Fluorescent Protein and used as controls are in green, anti-FAP CAR-T cells are in red

- **Perspectives:**
  - Secretome project: To better understand the mechanism of action by identifying the cardiac targets of the main components of the cargo and to optimize its cardiac delivery with a focus on the intravenous route for assessing to what extent it may trigger a rewiring of the endogenous immune system with remote benefits on heart tissue.
  - CAR-T cell project: Further confirmation of the benefits of targeting FAP for mitigating cardiac and skeletal muscle fibrosis and concomitantly improving functional outcomes; subsequent validation in the more clinically relevant GRMD model of genetically dystrophic dogs before considering phase 1 trials in combination with gene therapy.

- **Unique selling points**
  - Production of a clinical-grade cardiac cell-derived secretome for the treatment of heart failure
  - Development of a GMP-compliant platform which can be leveraged to produce the secretome of any cell type for non cardiac therapeutic applications
  - Repositioning of CAR-T cell-based therapy outside from oncology to mitigate cardiac fibrosis

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Therapeutic strategies for hematopoietic disorders

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Keywords
- Gene therapy
- genome editing
- epigenome editing
- beta-hemoglobinopathies
- transcription
- epigenetics
- red blood cells
- hematopoietic stem cells
- hematopoietic disorders, sickle cell disease.

Abstract
Dr. Miccio's main interests are the transcriptional control of hematopoiesis, and the development of therapeutic approaches to -hemoglobinopathies (-thalassemia and sickle cell disease, SCD), which are caused by mutations affecting adult hemoglobin -chain production in red blood cells. Transplantation of autologous, genetically modified hematopoietic stem cells is an attractive therapeutic option to produce normal red blood cells in the patients. As a PhD student, Dr. Miccio generated a lentiviral vector (LV) successfully used in an early clinical trial for -thalassemia. As a post-doc and later as an assistant professor, she gained experience in gene regulation during erythroid development and in evaluating the efficacy of gene therapy approaches for hematopoietic disorders. As a Lab Director at Imagine, she pursued her studies on transcriptional regulation in normal and diseased stem cells, and their progeny. These basic research studies are instrumental in developing novel strategies for -hemoglobinopathies. In particular, she optimized the design of LVs currently employed in a clinical trial for sickle cell disease and developed CRISPR/Cas9 strategies for -hemoglobinopathies. However, current gene therapy strategies based on the use of LVS or double strand break (DSB)-inducing CRISPR/Cas9 nuclease are not equally effective in all the patients and/or raise safety concerns. Base, prime and epigenome editing are novel, promising CRISPR/Cas9-based genome editing technologies that allow the DNA manipulation without generating dangerous DSBs. Dr. Miccio is using these editing strategies either to correct the genetic defect or to target disease modifiers and provide a safe and effective treatment for -hemoglobinopathies and other hematopoietic disorders.

Research area
Gene therapy, editing strategies for hematopoietic disorders, beta-hemoglobinopathies, transcription, epigenetics.

Synopsis

Combination of basic and applied research studies to develop innovative therapies for hematopoietic disorders.

Interests

Gene therapy;Gene editing;Viral vectors;Stem cells;Haematology;Epigenetics;Translational research;Clinical research

Therapeutic strategies for hematopoietic disorders

Miccio ANNARITA

Imagine Institute of genetic diseases,
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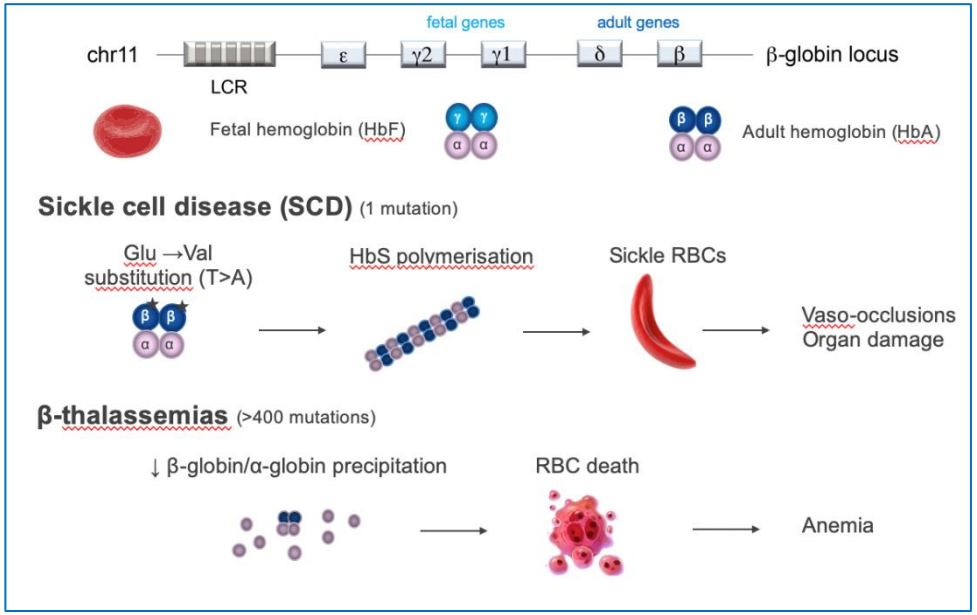
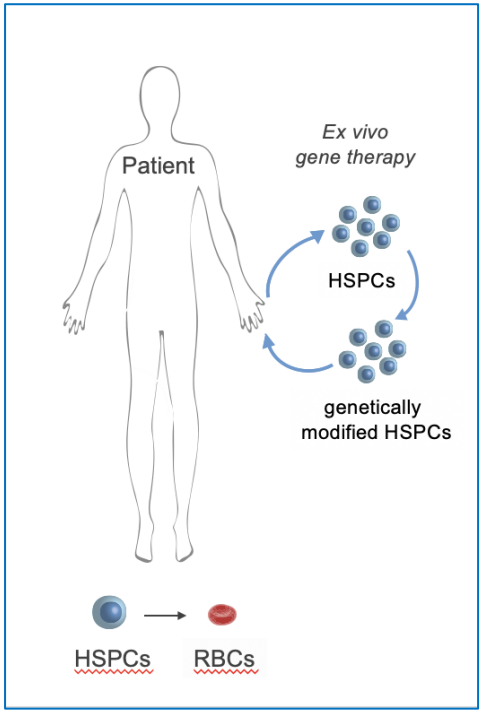
- **Objectives:**
  - Development of lentiviral vectors for gene therapy of beta-hemoglobinopathies
  - Development of editing strategies for beta-hemoglobinopathies

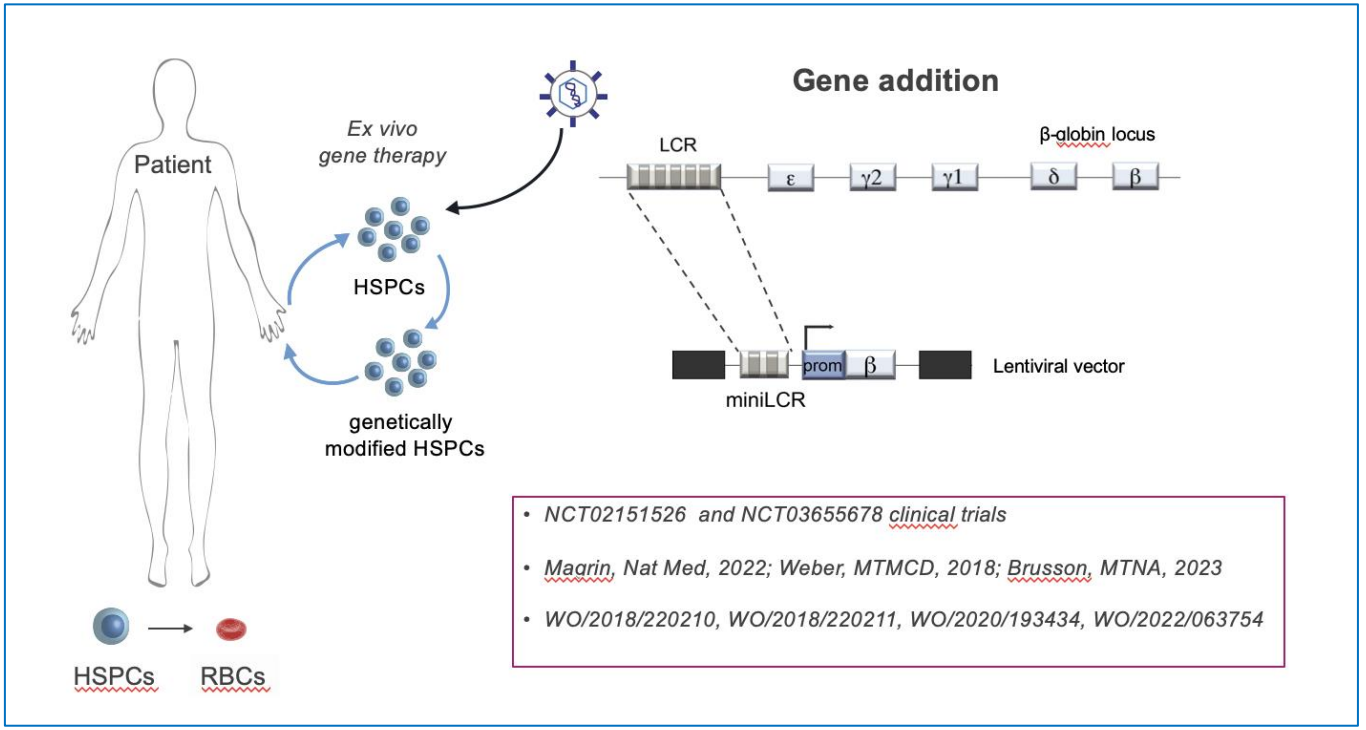




- **Tools:**
  - Lentiviral vectors, CRISPR/Cas9
  - *In vitro* and *in vivo* models of human hematopoiesis
  - Genomic, transcriptomic and epigenetic analyses

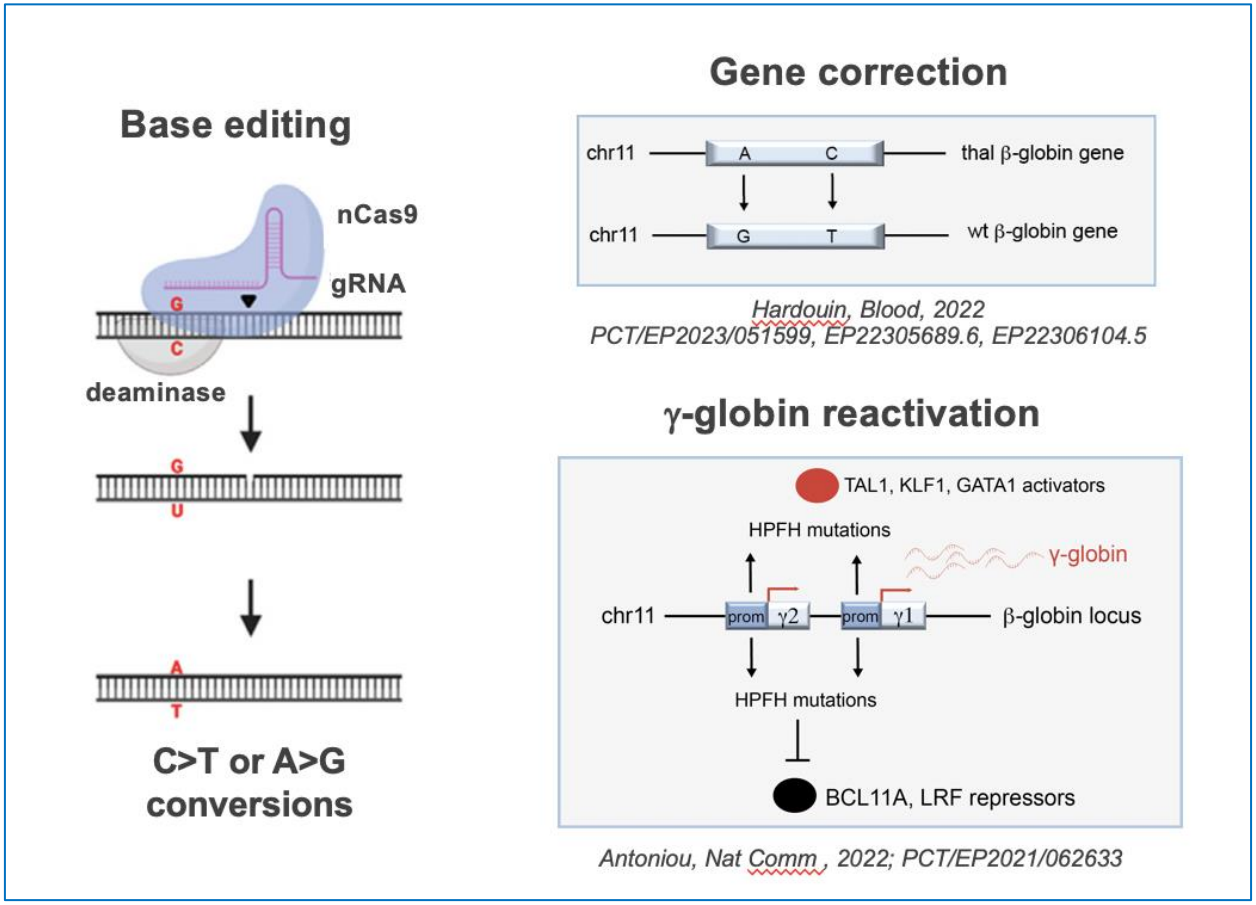
Subject 1: Lentiviral vectors carrying a beta-like globin transgene for gene therapy of beta-hemoglobinopathies

- **Results:**
  - Generation of novel lentiviral vectors carrying a beta-globin transgene
  - Preclinical studies demonstrating the safety of these lentiviral vectors
  - Two clinical trial demonstrating amelioration of the clinical phenotype in 6 out of 9 patients (a third clinical trial expected to start in 2025)

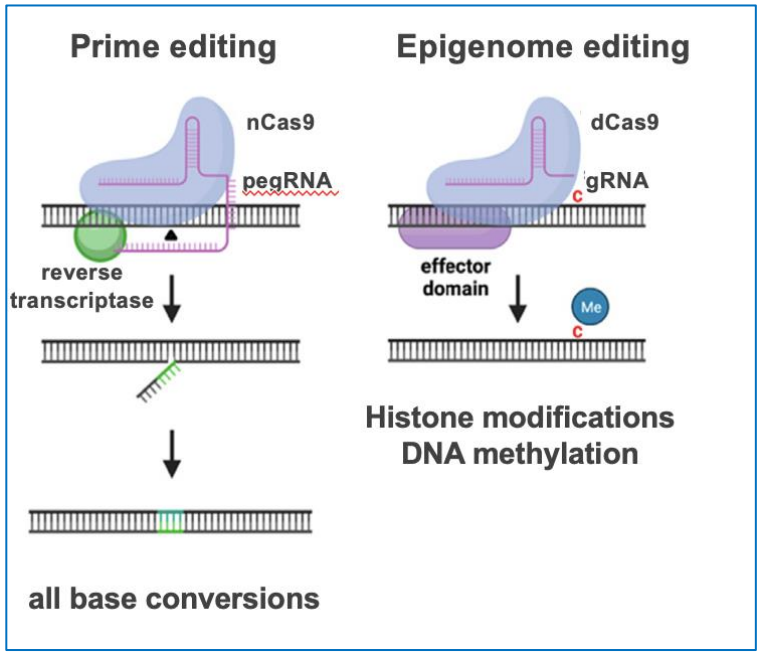


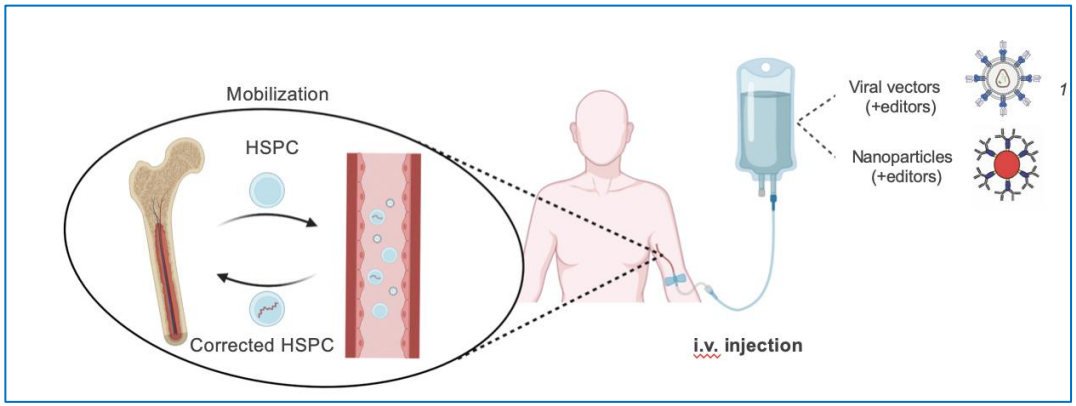
Subject 2: CRISPR/Cas9-based strategies for beta-hemoglobinopathies

- **Results:**
  - Correction of prevalent beta-thalassemic mutations using base editing
  - Reactivation of fetal hemoglobin by recruiting activator or disrupting repressors from the fetal gamma-globin promoters using base editing



- **Perspectives:**
  - Clinical trials for beta-hemoglobinopathies using *ex vivo* base editing approaches
  - Development of novel therapeutic approaches for beta-hemoglobinopathies and other hematopoietic disorders using the editing toolbox *ex vivo* or *in vivo*





  - Investigating the mechanisms underlying pathophysiology of β-hemoglobinopathies to identify novel therapeutic targets
  - Development of novel therapeutic approaches for other hematopoietic disorders using the editing toolbox *ex vivo* or *in vivo*

Unique selling points

- Combination of basic and applied research studies to develop innovative therapies for hematopoietic disorders
- Study of disease pathophysiology to ameliorate the outcome of gene therapy approaches
- Therapeutic approaches developed in the lab can be readily implemented for the clinical application at the Necker Hospital
- The presence in the campus of patient reference centers and unique patient's cohorts
- Collaboration with a multidisciplinary mix of internationally recognized scientists and doctors

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Non-viral gene delivery for gene and cell therapies

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Keywords
- Nanomedicine
- Lipid nanoparticles
- Gene delivery
- Formulations
- Imaging agents
- Biotherapies
- Immunotherapy

Abstract
The members of the UTCBS are interested in innovative technologies for Health, in particular the development of new pharmaceutical formulations of biotherapeutics, such as siRNA, DNA, antibodies, cytokines, but also the research of new diagnostic agents and biomarkers in cancer and hepatic fibrosis. These approaches are treated in a multidisciplinary way with the development of innovative approaches for the synthesis, the analytical and physicochemical characterization of these complexed molecules. As their main expertise lies in lipid nanoparticles, they have set-up a microfluidic set-up for their production in a continuous flow which aims to be implemented with on line separation and measurement. UTCBS researchers are evaluating how these nano- and biotherapies can modulate gene expression or activate certain immune pathways to propose new therapeutic approaches. They also aim to generate CAR-NK cells via these non-viral lipidic nanovectors using their own designed lipids and plasmid.

Research area
Vectors for Targeted Imaging and Therapy

Synopsis
Interdisciplinary skills dedicated to innovation for medical applications.

Interests
Chimeric Antigen Receptor (CAR)-T cells;Non viral delivery systems;Genetic engineering;Oncology;Immunology/Immunotherapies;Nanotechnology;Translational research

Non-viral gene delivery for gene and cell therapies

Nathalie MIGNET

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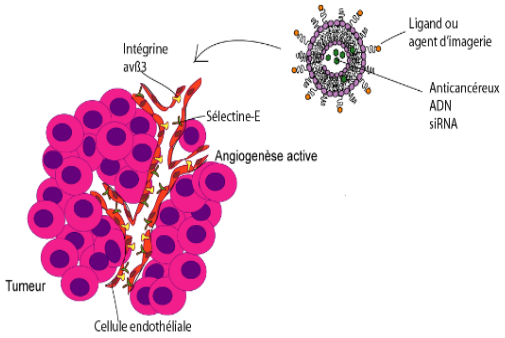
de Pharmacie, Paris

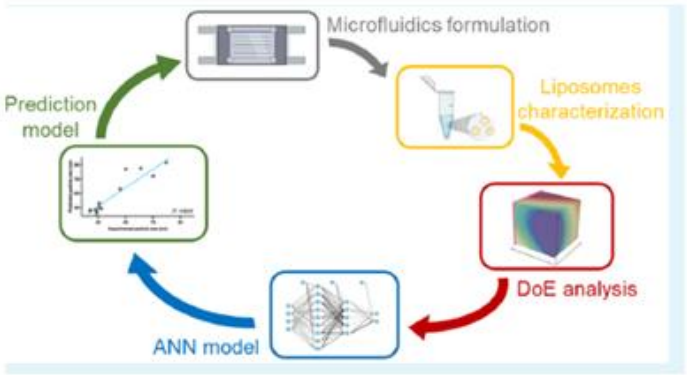
- **Objectives:**
  - To develop novel lipid nanoparticles
  - To improve plasmid DNA production
  - To evaluate novel combination therapies
  - To develop non-viral transfection tools for CAR-NK cells

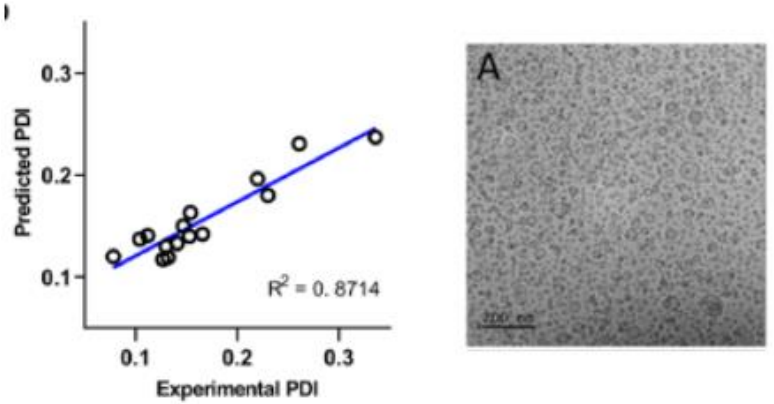
- **Tools:**
  - Microfluidic method affiliated to artificial intelligence to develop lipid nanoparticles
  - Formulations methodologies
  - Methods to characterize nanoparticles
  - Methods to characterize antibodies
  - pFAR4 plasmid
  - in vitro facilities
  - In vivo optical imaging platform



Conception, production and purification of lipid nanoparticles

- **Results:**
  - Set-up microfluidic coupled with on line DLS measurement for the production of liposomes
  - Affiliated to design of experiment and neuron network to promote quantitative results
  - Demonstration of a positive correlation between prediction and experimental results

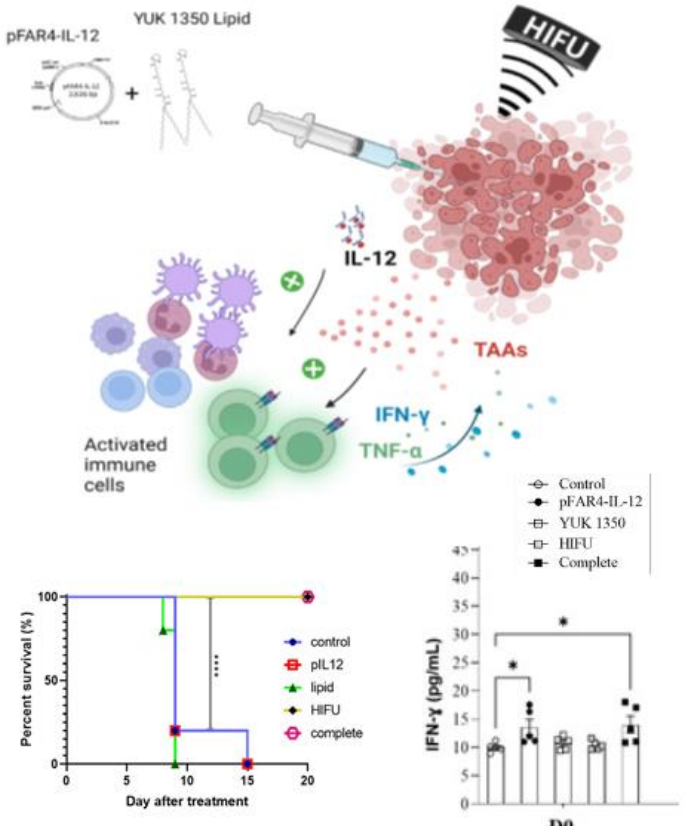




In line production of lipid nanoparticles and correlation with the prediction.

Conception of lipid nanoparticles for non-viral transfection of NK cells to obtain CAR-NK

- **Results:**
  - Design of pFAR4 plasmid devoid of antibiotic resistance for a higher safety
  - Integration of sleeping beauty to prolong transgene expression
  - pFAR4 plasmid production at large scale for phase I clinical trial
  - Example of application: combination of gene therapy and ultrasound provides a systemic response against metastases
  - Strong expertise in gene therapy and NK cells thanks to the team leader Salima-Hacein-Bey-Abina, performing 1st in man clinical trial in gene therapy at Necker Hospital.



Strategy to enhance the systemic immunne response of ultrasounds therapy, in vivo demonstration4.

- **Perspectives:**
  - Hire a chemist for the synthesis of novel lipids
  - Search for collaborations with chemists in the lipid field
  - Finalize the microfluidic production with on line DLS measurement
  - Implement a microfluidic separation method within the on line production
  - Enlarge the applications of lipid nanoparticles to cell therapy
  - Enlarge the applications of lipid nanoparticles to novel immunotherapy

- **Unique selling points**

  - Multidisciplinary expertises going from analytical chemistry, formulations to cell therapy and immunology
  - Conception of novel non-viral lipidic formulations for gene delivery
  - Physico-chemical methods of nano-characterization (thermal analyses, capillary electrophoresis, DLS, TRPS)
  - Optical imaging platform for small animals (fluorescence, bioluminescence kinetic of gene expression, biodistribution of vectors)

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Genetic and protein engineering to decode and restore cytokine communication between immune cells

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Keywords
- Cytokine biology,
- Immunotherapy,
- Cytokine therapy,
- Cellular immunotherapy,
- protein engineering.

Abstract
Scientific Expertise: As an expert in immunology and bioengineering, my focus is on cytokine signaling pathways, employing molecular biology, genetic manipulation, and protein engineering to advance our understanding of immune cell communication.
Research Strategy: My team's work on replicating extracellular conditions, like tumor acidity, to study cytokine-receptor interactions aims to precisely control immune responses. Our strategy involves the targeted engineering of cytokines to work effectively within these environments, potentially revolutionizing immunotherapy by reducing systemic toxicity.
Major Data: We've engineered IL-2 and IL-6 partial agonists to fine-tune cellular activity, with IL-2 variants heading toward clinical application. In partnership with Ignacio Moraga's lab, we've developed a superior IL-10 monomer. Our groundbreaking 'Switch-2' IL-2 variant, which thrives in acidic conditions, showcases the untapped potential of pH-sensitive cytokine design—a transformative step in cytokine biology and engineering.
Perspectives: The goal is to harness this pH-responsive cytokine technology to forge new paths in the treatment of immune-mediated diseases, marking a paradigm shift in cytokine-based therapies.

Research area
Molecular and cellular immunotherapy

Synopsis
Our research pioneers innovative genetic and protein engineering to decode and restore cytokine communication between immune cells, offering groundbreaking treatments for various diseases.

Interests
Genetic engineering;Immunology/Immunotherapies;Autoimmune diseases;Translational research

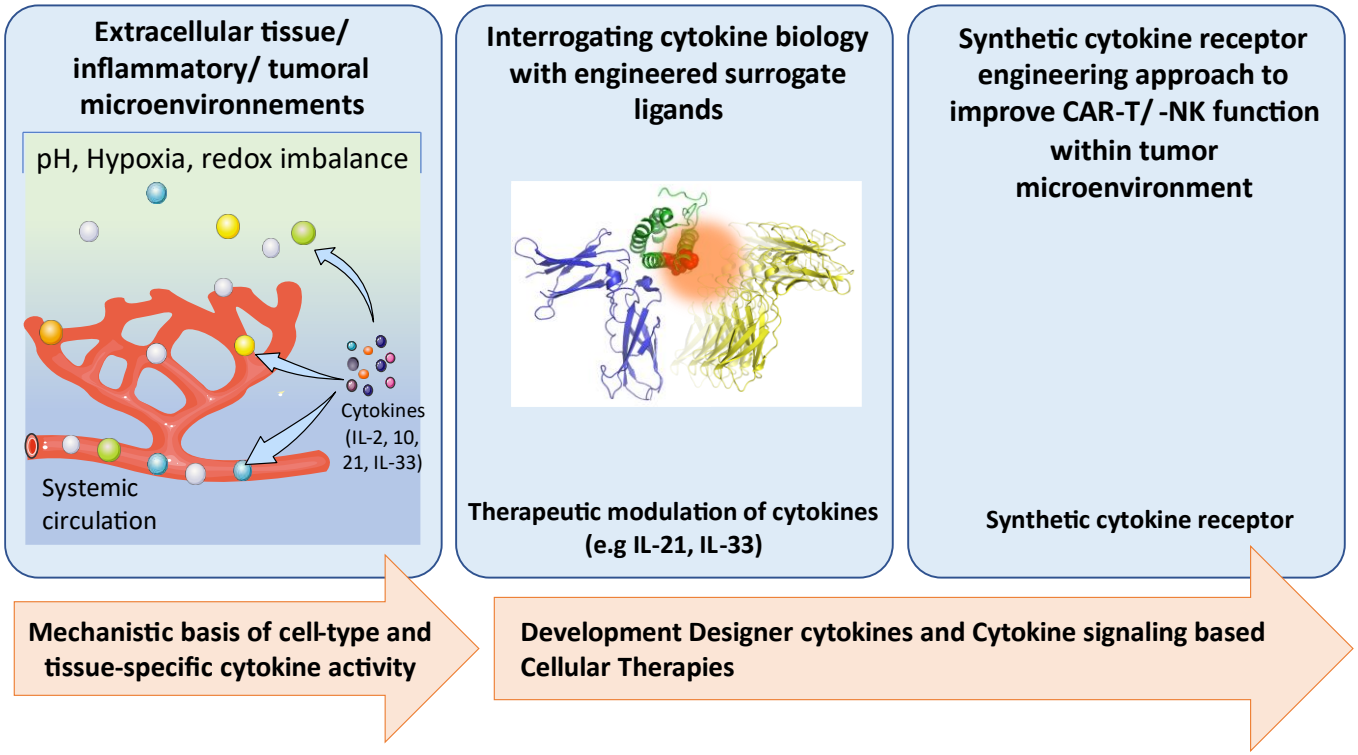
Genetic and protein engineering to decode and restore cytokine communication between immune cells

MITRA Suman, Ph.D

INSERM UMR 1277, Lille

- **Objectives:**

  - Explore extracellular pH and matrix tension effects on cell/cytokine activity in disease.
  - Create engineered cells/cytokines to boost immune response to tumors/inflammation.
  - Clarify natural vs. synthetic immunity interplay in cancer treatment.



Tools:

- Design T cells via genetic manipulation.
- Advance cytokine engineering for targeted functions.
- Utilize single-cell technologies for detailed analysis.
- Employ syngeneic/xenogeneic models and organoids for disease modeling.

Subject 1: Understanding and Exploiting Cytokine Activity in tissue environments using protein engineering

Background:

Hypoxic and acidic conditions in the tumor microenvironment challenge immune cell efficacy. Cytokines including, IL-2's activity is compromised under acidic conditions, affecting T cell and NK cell functions.

Results:

Directed evolution utilized to create IL-2 variants with enhanced receptor binding in low pH. Development of "Switch-2" variant: robust binding at pH 6, reduced interaction at neutral pH. Switch-2 exhibits strong antitumor activity in acidic environments, minimal activity at physiological pH. Significant in vivo tumor reduction in mice models.

Implications:

SwitchKine Engineering Platform allows for localized immune modulation. Potent therapeutic action in acidic tumor environments. Minimized cytokine activity in circulation to reduce therapy-related toxicity.

Conclusion:

The "Switch-2" IL-2 variant demonstrates a dual advantage: enhanced efficacy in tumor environments and an improved safety profile.

Prospective:

Exploring the frontier of immunotherapy with precision-engineered cytokines.

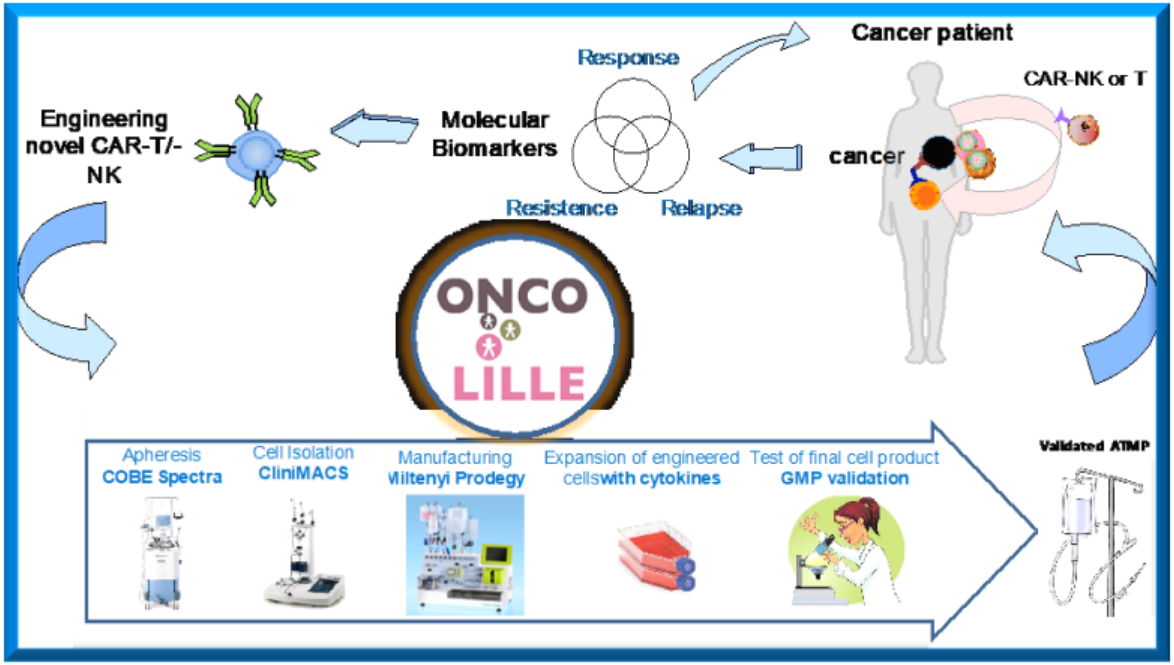
Advancements in IL-6 Cytokine Engineering:

The IL-6 breakthrough involves a partial agonist engineered to signal through the gp130 receptor independently of IL-6Ra. This decouples its immunomodulatory functions from inflammatory processes, offering targeted therapy even when IL-6Ra is inhibited. It's a significant step towards fine-tuning immune responses without triggering inflammation.

Subject 2: Optimizing Synthetic Immunotherapies: Enhancing CAR-T and BiTE Responses in the Tumor Microenvironment (TME)

Results:

- Utilized single-cell and spatial technologies to profile CAR-T and BiTE responses in tumor biopsies alongside blood samples, providing a comparative insight into therapeutic engagement in situ versus peripheral circulation.
- Elucidated the modulatory role of the extracellular matrix on T cell responses, revealing potential targets for enhancing T cell infiltration and activity within the tumor microenvironment.
- Engineered novel cytokine receptors capable of modulating T cell activity independent of exogenous cytokine administration, aiming for self-sustained and regulated T cell responses.
- Developed nanobody-cytokine conjugates that augment BiTE therapy, leading to improved targeting and efficacy in T cell-mediated tumor cell killing.



- **Perspectives:**

Our research advances synthetic immunotherapy by mapping CAR-T and BiTE responses in tumors and blood, uncovering how the extracellular physio-chemical environment affects T cell function. Innovations include autonomous cytokine receptors and nanobody-cytokine conjugates, enhancing therapy precision and potency in the tumor microenvironment, promising improved cancer treatments.

- **Unique selling points:**

  - **Designer Cytokines:** Custom-engineered cytokine variants that activate T cells within the acidic tumor microenvironment, dramatically improving targeted efficacy.
  - **Designer Cells**: Tailor-made immune cells with enhanced infiltration and persistence in solid tumors, designed to thrive in hostile tumor conditions.
  - **Advanced Profiling:** Cutting-edge single-cell and spatial analyses to track and optimize the performance of synthetic immunotherapies in real-time. Importantly, access to clinically relevant samples in a direct collaborations with CHU de Lille.
  - **Self-Sustaining Therapies:** Innovative cytokine receptors that independently stimulate T cells, reducing the need for exogenous cytokine administration and mitigating systemic side effects.
  - **Precision Targeting**: Nanobody-cytokine conjugates that refine BiTE therapy, ensuring direct and potent anti-tumor activity while minimizing off-target effects.

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- Martinez F. J*, Wang L*, Pohler E, Lokau J, Garbers C, Cozzani A, Kazemian M#+, **Mitra S#+,** Moraga I #+. CDK8 fine-tunes IL-6 transcriptional activities by limiting STAT3 resident time at the gene loci, Cell Reports, (2020). * # These authors share senior authorships, +corresponding authors.
- Martinez F. J, Wilmes S, Wang L, Hafer M, Pohler E, Lokau J, Garbers C, Cozzani A, Piehler J, Kazeman M#*, **Mitra S#*,** Moraga I#*. Kinetics of cytokine receptor trafficking determine signaling and functional selectivity. eLife, (2019). #*co-senior and corresponding authors

PATENTS AND OTHER SCIENTIFIC PRODUCTION:

- SUPERAGONISTS, PARTIAL AGONISTS AND ANTAGONISTS OF INTERLEUKIN-2 (US10150802B2)
- Engineering novel IL-10 agonists for application in immunotherapy, including cell therapy. (Patent application number: PE960351GB). Patent with Dundee University and INSERM
- Modified IL-2 molecules with improved activity at acidic pH for use in the treatment of a range of diseases and/or conditions. Patent with Dundee University and INSERM

Fine-tuning of nanoparticles for gene transfer using nanoparticles

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Laboratory UMR INSERM 1078 “Genetique, Génomique fonctionnelle et Biotechnologies” – “Gene Transfer and Combined therapeutic Approaches” Team
City BREST

Keywords
- Nanoparticles
- Cystic Fibrosis
- Duchenne muscular dystrophy
- Aerosol
- Mucus
- High pressure Limb Vein injection
- New drug development
- Optimised transgen

Abstract
Our research focuses on the development of multimodular nanoparticles (NPs) adapted to local administration constraints (aerosol or HLV). This includes both the design of the new synthetic vectors as well as their formulations and the optimization of the transgenes delivered.
NPs were designed and were showed to be more effective than GL67A after in vivo aerosolization. These NPs may also possess some antibacterial and mucolytic properties to facilitate their progression into the CF pulmonary environment.
Other formulations have been shown to be highly effective after HLV or Intra-Muscular injections, leading to a transgene expression much more important than the gold-standard naked DNA transfection. In these inflammatory contexts, transgene optimization is an important point to enable the progression of nucleic acid construction from the cytoplasm to the nucleus.

Research area
From understanding genetic diseases to developing innovative therapeutic approaches.

Synopsis
Development and fine-tuning of multimodular systems for efficient gene delivery

Interests
Gene therapy;Non viral delivery systems;Neuromuscular disorders;Rare diseases;In vivo models;Translational research;Clinical research

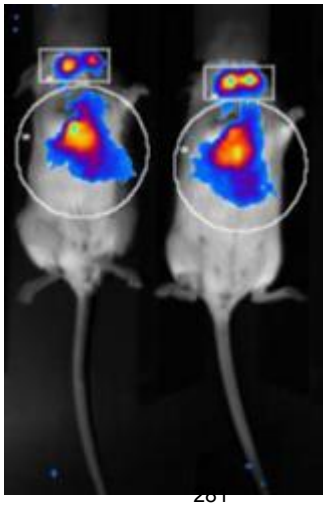
Fine-tuning of nanoparticles for gene transfer using nanoparticles

Pr Tristan Montier (MD-PhD)

UMR INSERM 1078 "Génétique, Génomique fonctionnelle et Biotechnologies"
Gene Transfer and Combined therapeutic Approaches Team
Faculté de médecine et des sciences de la santé
Université de Bretagne Occidentale
Brest - France

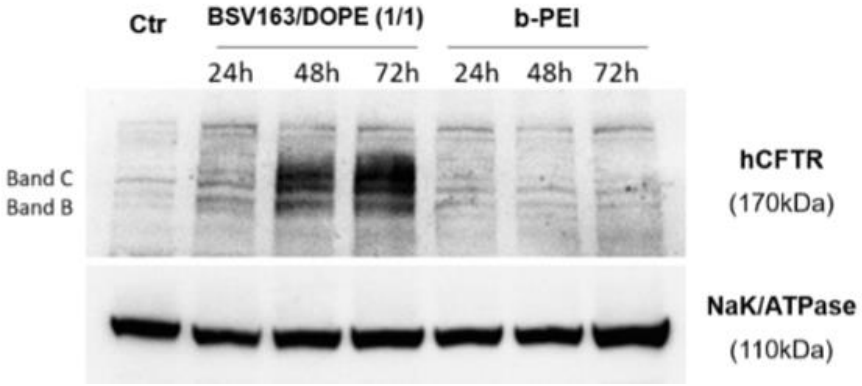
- **Objectives:**
  - To develop new formulations for gene delivery
  - To adapt the formulations to the pathway of administration
  - To optimize nucleic acids constructs
  - To decipher the transfection process
  - To overcome extracellular barriers

- **Tools:**
  - Relevant loco-regional delivery procedures : HLV and Aerosol
  - Functional in vitro (Air-Liquid Interface ALI) and in vivo models
  - Molecular biology to adapt plasmids
  - In vivo bioluminescence and biofluorescence
  - Blood parameters to evaluate the safety and the inflammatory response



Gene transfection using branched cationic amphiphilic compounds for an aerosol administration in cystic fibrosis context

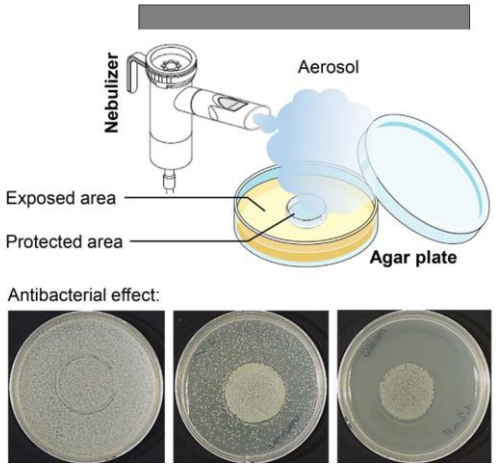
- **Results:**
  - Air-Liquid Interface (ALI) cell-culture and mice are expressing Luc and hCFTR after aerosol delivery with NPs based on BSV163/DOPE/DMPE-PEG500
  - more efficient than the use of b-PEI-based formulation
  - Scale up production and repeated administration



Western blot of hCFTR performed on CFBE41o- 48h after transfection

Antibacterial activities in Cystic Fibrosis context

- **Results:**
  - Previously, we have reported arsonium-containing lipophosphoramides as poly-functional nanocarriers capable of simultaneous antibacterial action against Gram-positive bacteria and gene transfer into eukaryotic cells
  - Nanoparticles combined with an N-heterocyclic carbene-silver complex in order to extend the spectrum of antibacterial activity, including towards the Gram-negative Pseudomonas aeruginosa.
  - Antibacterial effects are compatible with efficient and safe gene delivery into human bronchial epithelial cells



Antibacterial effects of DNA formulations incorporating KLN47, DDYSA, or KLN47/DDYSA (mixed at equimolar ratio) following nebulization over agar plates inoculated with various bacteria

- **Perspectives:**

  - Extensive library of chemical compounds adapted to biological environment
  - Smart multifunctional and adapted formulations to overcome the different barriers faced by non-viral nanocomplexes
  - Improvement of the efficiency while maintaining the high tolerance
  - Combination of therapeutic transgenes – optimized nucleic acids constructions

- **Unique selling points**
  - Formulations adaptable to the conditions of use
  - Transfection capacities and antibacterial properties
  - Loco regional administration methods
  - Evaluation of efficacy and safety in cellular and animal models

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- Ghanem R, Roquefort P, Ramel S, Laurent V, Haute T, Le Gall T, Aubry T, Montier T. Apparent Yield Stress of Sputum as a Relevant Biomarker in Cystic Fibrosis. **Cells** (2021)
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A STEM CELL THERAPY PIPELINE WITH A FOCUS ON RETINITIS PIGMENTOSA AND OTHER RARE INDICATIONS WITH HIGH UNMET MEDICAL NEEDS

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Keywords
- Tissue Engineering
- Cell therapy
- retinal disorders
- biomaterials
- human pluripotent stem cells
- automation
- bioproducti

Abstract
In developed countries, blindness and visual impairment are caused mainly by diseases affecting the retina. These retinal degenerative diseases, including Aged-Macular Dystrophy (AMD) and inherited retinal diseases such as Retinitis Pigmentosa (RP), are the predominant causes of human blindness worldwide and are responsible for more than 1.5 million cases in France and more than 30 million cases worldwide. The diseases often remain incurable and lead more or less rapidly to blindness in patients. Since these last fifteen years, the team has acquired a unique experience in the development of hESC-derived cell therapies for retinal rare disorders. The team has developed protocols for guided differentiation of pluripotent cells towards different cell populations of the retina. In particular, the scientific coordinator has coordinated during 5 years proof of concept studies to demonstrate efficacy/safety of a RPE bio-engineered product, which has now entered in a clinical trial. Her team has in particular developed innovative methods for the preparation of bio-engineered tissue (patent pending: Ben M'Barek et al., N°18/52114 (12/03/2018)) and transplantation strategies in rodents (Ben M'Barek et al., 2017) and non-human primates (Ben M'Barek et al., 2020). I-Stem has also acquired an increasing level of automation (Regent et al., 2019; Frank et al., 2023) and continues to optimize and develop innovative methods as reflected by advanced methodologies, cutting edge equipment and adequate know-how in large-scale culture of pluripotent stem cells and their differentiation into various GMP compliant cell types. Moreover, in collaboration with Dr Hamouda (Université Paris-Saclay) and Pr Letourneur (Inserm U1148), we have developed cell engineering products that combine matrices/scaffolds and cells recapitulating the cellular organization of the retina.

Research area
The I-Stem laboratory is a structure dedicated to the exploration of the potential therapeutic applications of human pluripotent stem cells to treat monogenic diseases. It is therefore presented in the scientific-medical continuum as an R&D center carrying out translational research. Therapeutic innovation receives constant inputs from fundamental research to

refine, modify or redesign treatment strategies and this constant preoccupation with the therapeutic target is reflected in the organization of the teams around projects and schedules.

Synopsis

The program of our team is the development for ready-to-use engineered products for clinical application in retinal diseases that will allow the treatment of a large number of patients with minimising the cost of production.

Interests

Cell Therapy;Stem cells;Ophtalmology;Rare diseases;In vivo models;Automation;Translational research;Bioproduction

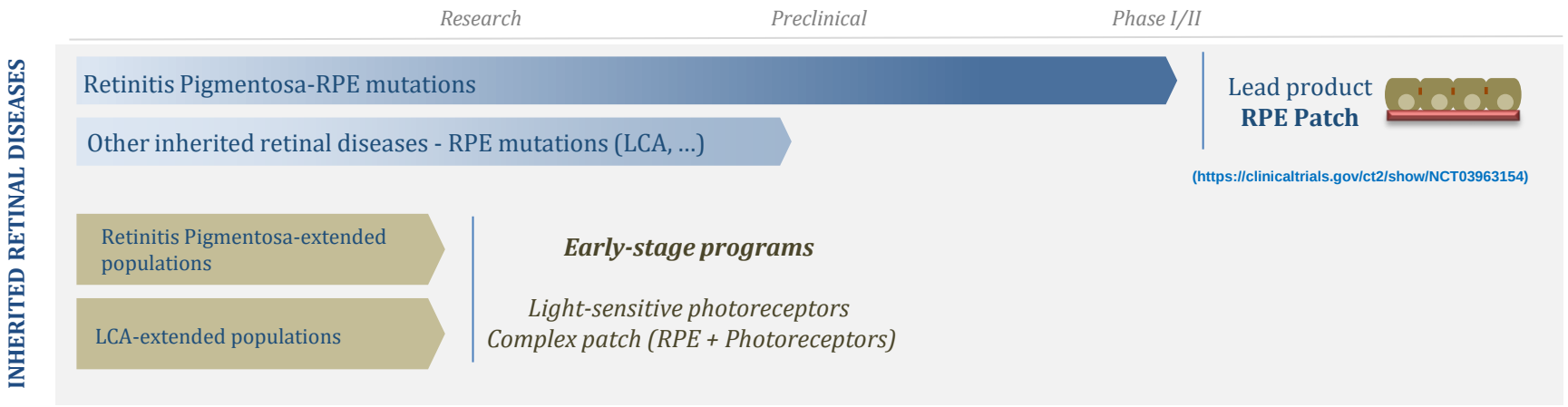
A STEM CELL THERAPY PIPELINE WITH A FOCUS ON RETINITIS PIGMENTOSA AND OTHER RARE INDICATIONS WITH HIGH UNMET MEDICAL NEEDS

Christelle Monville

UMR861 INSERM / UEVE / I-Stem

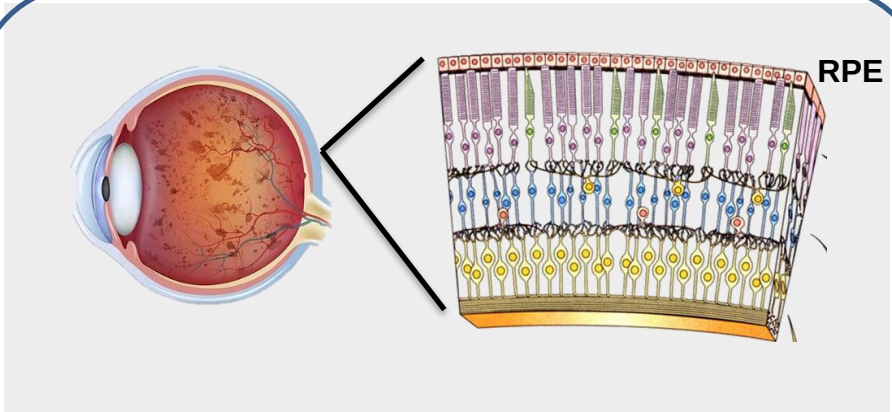
- **Objectives:**
  - To develop cell therapy products by tissue engineering for monogenic retinopathies (Retinitis Pigmentosa) and Age-related Macular Degeneration (AMD).

- **Tools:**
  - Automation bioproduction platforms
  - Cellular models from human pluripotent stem cells
  - Functional in vitro assays
  - CRISPR-Cas9 platforms

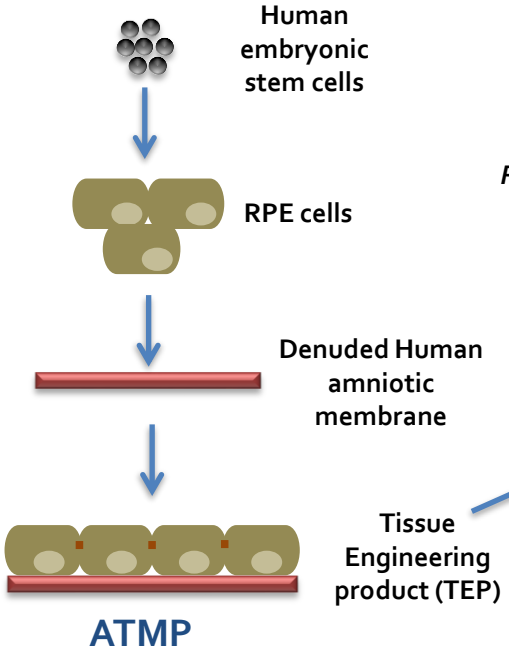


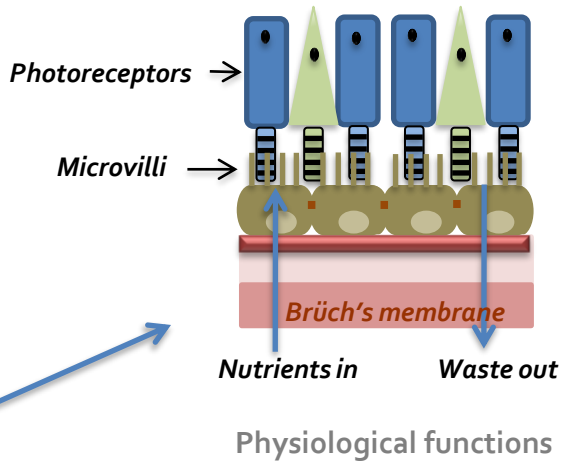
Subject 1: Replacement of the malfunctioning RPE layer with an RPE layer generated from human embryonic stem cells (hESCs) (https://clinicaltrials.gov/ct2/show/NCT03963154)

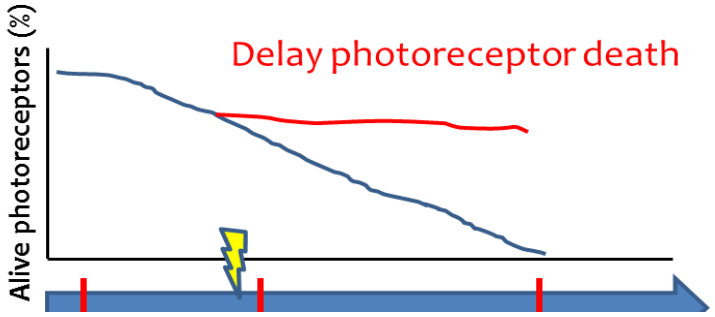
- **Results:**
  - Preclinical safety experiments in mice
  - Functional proof of concept in rats
  - Safety surgical evaluation in non-human primates
  - Specific medical device development
  - Phase I/II clinical trial on going



The RPE layer has multiple critical roles in the health function of photoreceptors and the retina as a whole







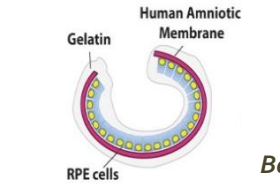
Program: STREAM (Stem cells Therapy for Retinal Epithelium Assay in Monogenic retinopathies)

Consortium with clinicians and legal experts

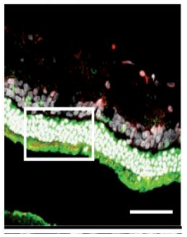
hES → Progenies of interest/TEP conception ✓ → Proof of concept ✓ → GMP facilities ✓ → Safety studies (pre-clinical) ✓ → IMPD application ✓ → Phase I/II

agence de la biomédecine
NOR : AFSB1610277S

Scientific Advice (EMA) ✓



Lustremant et al. 2017
Reichman et al. 2014
Ben M'Barek et al. 2018

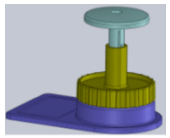


Vision preservation in dystrophic rat

Science Translational Medicine AAAS

Ben M'Barek et al. 2017

2.10⁸ RPE In 200 vials



Device
Patent: Ben M'Barek et al. N°18/52114

- Teratoma / biodistribution in rodents (NUDE mice/rats)
- Surgery safety in non-human primates

Ben M'Barek et al. 2020

Submission: 26th of July 2018 (ANSM: 2018-000457-53)

2019 Clinical trial for RP patients 12 patients (LRAT, MERTK)

AUTHORIZED

I-Stem · Institut de la Vision · Assistance Publique Hôpitaux de Paris

EFS · Atlantic Bio GMP · Medical Device Engineering

MircCen · CERB

Biotherapies Institute · Quinze-Vingts

ICTA · Quinze-Vingts