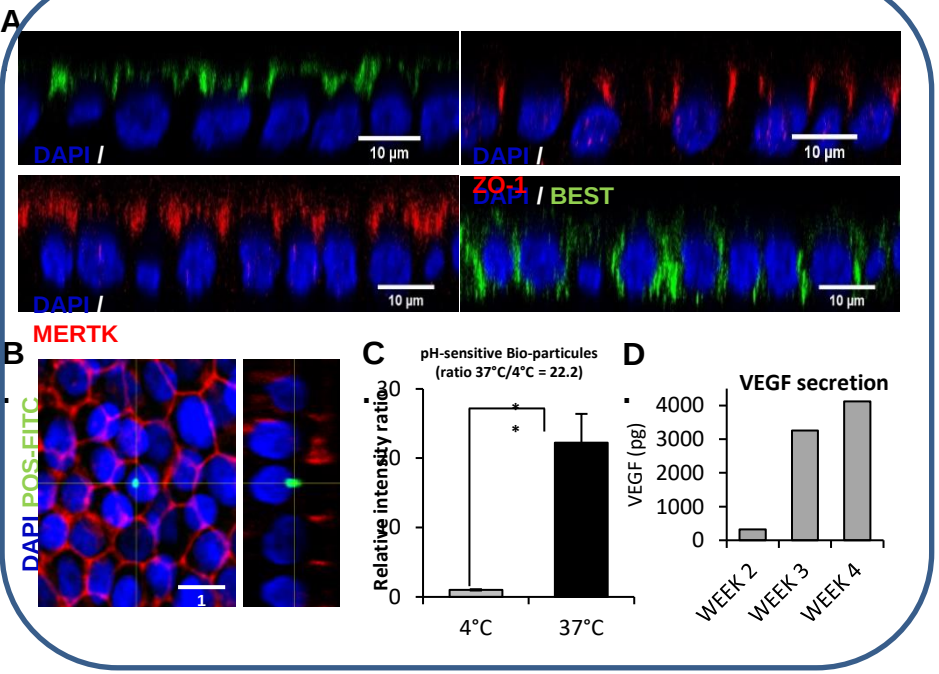
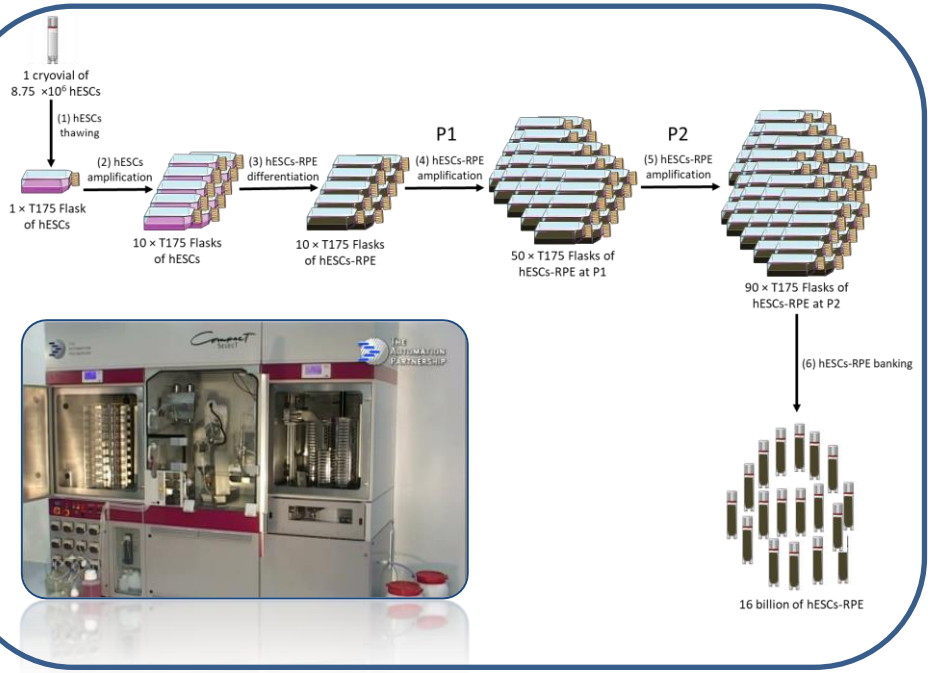
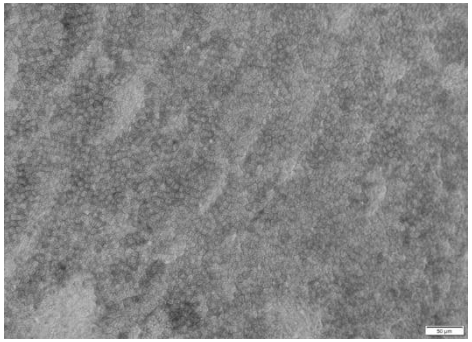
Subject 2: Scale up and cryopreservation of the cell therapy product

- **Results:**
  - Automated scale-up of RPE production
  - Production of billions of *bona fide* RPE
  - Full quality control
  - Cryopreservation of the patch

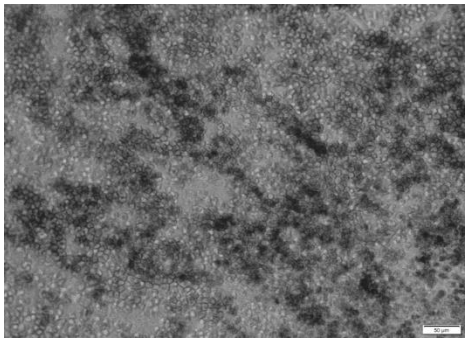




Before freezing

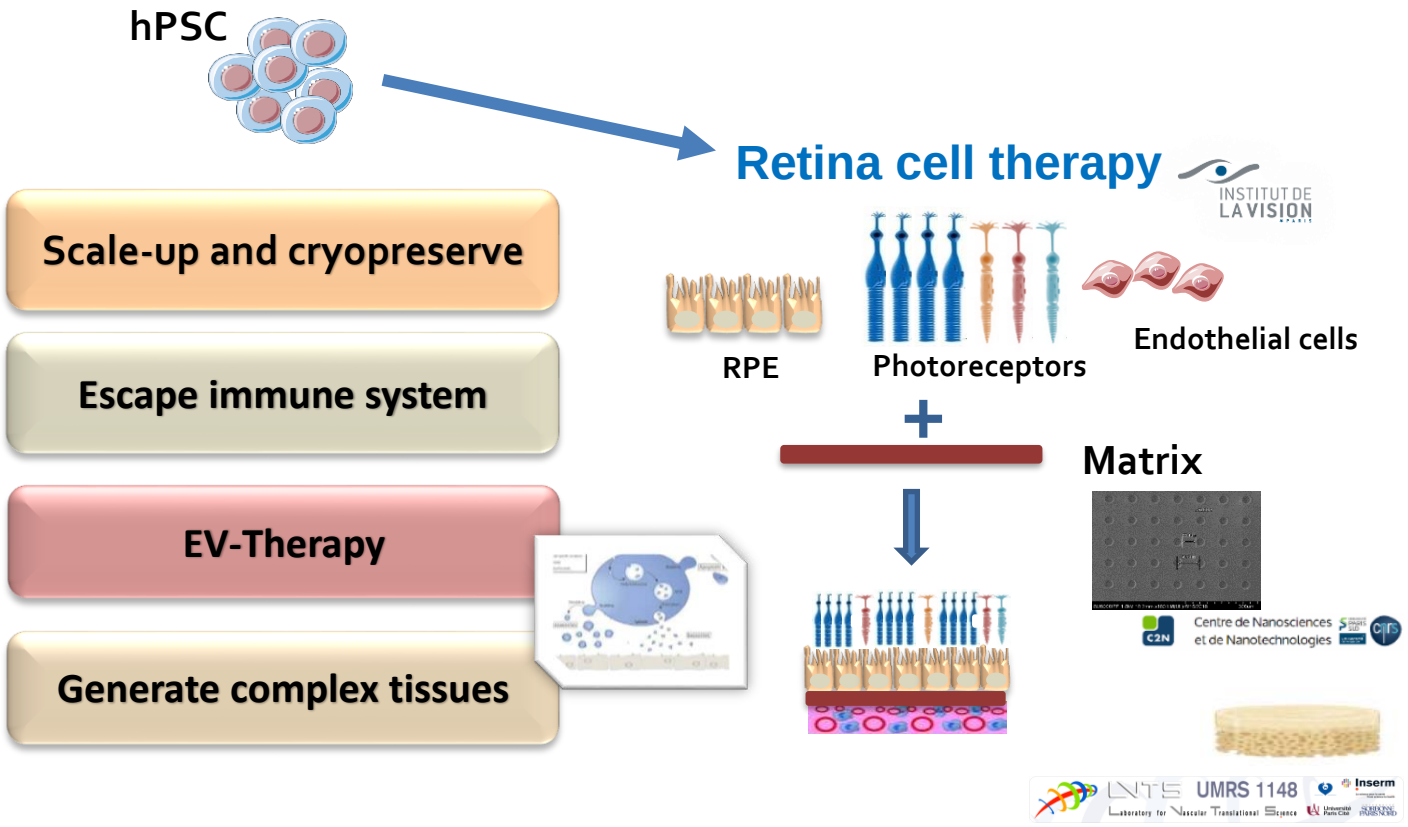


Day 15 post-thawing



Régent et al., 2019; Patent EP n°19 305 807

- **Perspectives**
  - Engineering complex tissues containing different retinal cells (photoreceptors, RPE, endothelial cells,…) to address different types/stages of retinal diseases
  - Generate hypoimmune cells in order to reduce the need of immnunosuppressors
  - Address the use of extracellular vesicles (EV) for acellular neuroprotective therapy or in combination with cell therapy product engraftment
  - Use Artificial Intelligence in combination with bioproduction to decrease cost production



- **Unique selling points**
  - Leading the one of the two French clinical trial using hPSC
  - Capacities to develop translational clinical programs from basic research to clinical trials
  - Unique technical and R&D platforms for bioproduction, genetic engineering, and genomic analysis

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Next-generation virotherapy by combining disruptive rational design and directed evolution for vector development

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Keywords
- Lentiviral vectors
- oncolytic viruses
- gene therapy
- cancer treatment
- specific targeting
- directed evolution

Abstract
Our laboratory has a sound expertise in molecular virology and evolution applied to virotherapy. We aim at conceiving surface viral proteins that drive transduction by lentiviral vectors or infection by oncolytic viruses selectively toward cancer cells. The lack of vectors or viruses endowed with sufficiently marked selectivity for targeting cancer cells in vivo is the main obstacle for an extensive use of anticancer virotherapy in clinics. At present, in fact, only the administration of the treatment directly inside the cancer lesion has been carried out in clinical trials. This constraint, though, limits the applications of virotherapy to cancers with a unique lesion, while most forms encountered in the clinics, as those presenting more than one lesion, diffuse forms and metastatic cancers, cannot be treated. Furthermore, administration in situ, also demands complex medical infrastructures, more specialised medical agents and is more expensive than a systemic administration. Our approach is to combine disruptive rational design of recombinant proteins and a novel method of directed evolution that exploits some properties of lentiviral replication for producing and marketing lentiviral vectors/oncolytic viruses that can be administered systemically to patients. In our approach, specificity is conferred by surface markers present on cancer cells. Hence, besides cancer treatment, our approach also applies to the production of viral vectors useful for all cases of gene therapy aimed at targeting a specific subset of cells in the human body, provided that specific surface markers are known.

Research area
Production of innovative tools for gene therapy

Synopsis
We develop anticancer virotherapies, focusing on lentiviral vectors and oncolytic viruses, by innovative directed evolution approaches and disruptive rational design of glycoprotein envelopes for in vivo specific cell targeting with unpreceded ability for specific targeting.

Interests

Gene therapy;Oncolytic viruses;Viral vectors;Oncology;Specific targeting;Translational research

Next-generation virotherapy by combining disruptive rational design and directed evolution for vector development

Matteo NEGRONI

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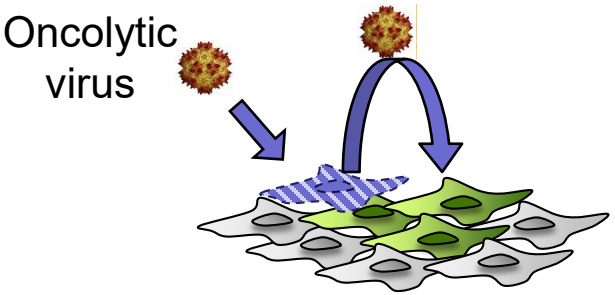
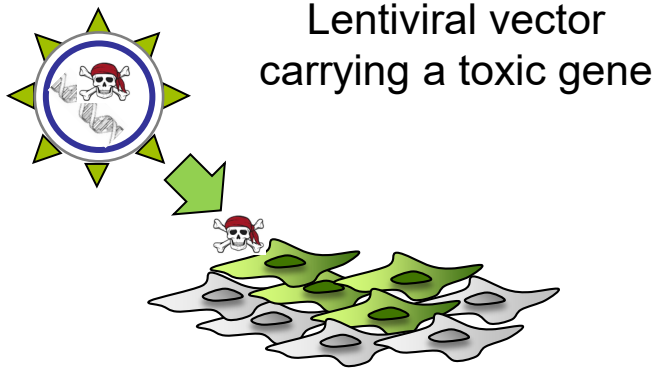
Strasbourg

- **Objectives:**
  - Cell-specific delivery of transgenes based on the expression of specific surface markers
  - Create new viral envelope proteins by disruptive approaches
  - Reach levels of selectivity suitable for systemic administration in vivo

- **Tools:**
  - Rational protein design based on structural studies
  - Genetic engineering of glycoproteins
  - Directed evolution in cell culture using lentiviral vectors
  - Lentiviral vectors
  - Oncolytic viruses



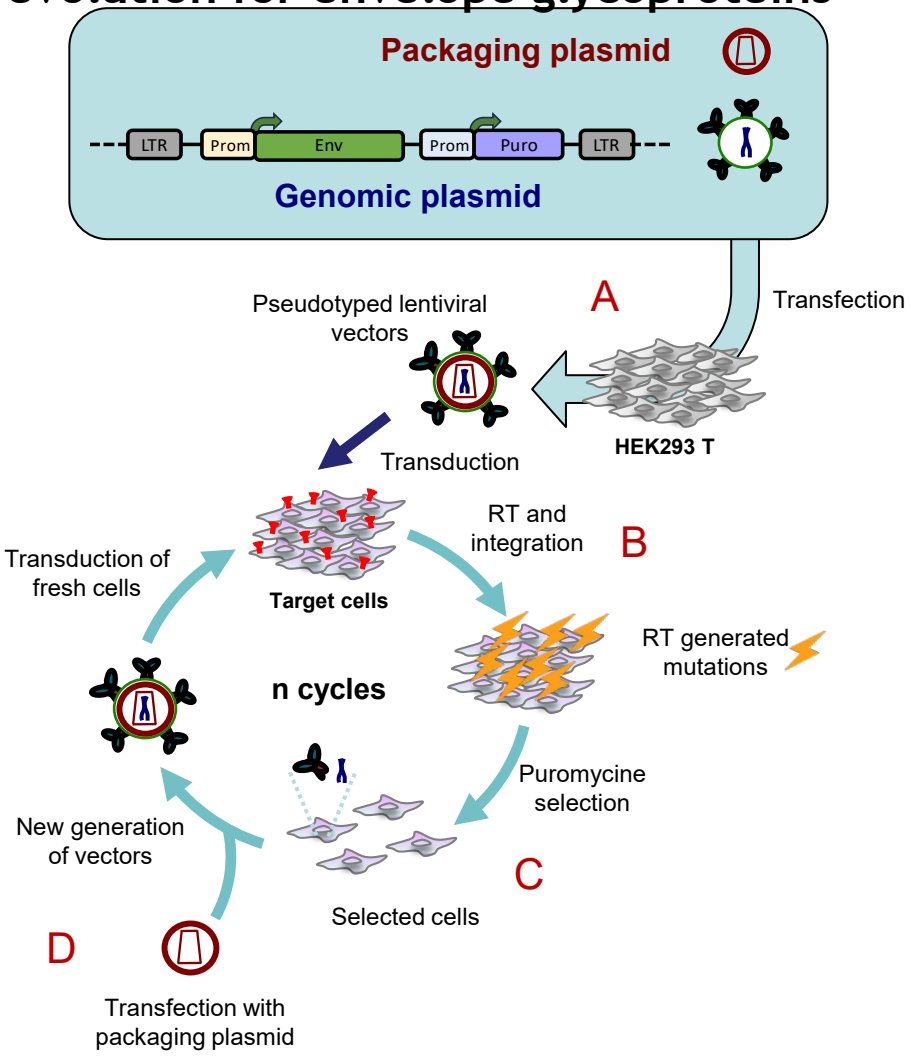
Grey cells: heathy cells.
Green cells: cancer cells.
Blue cell: cancer cell lysed by infecton.



Grey cells: heathy cells.
Green cells: cancer cells.

Subject 1
Retrovolution : an innovative directed evolution for envelope glycoproteins

- **Result:**
  - Successful development of an innovative method of directed evolution relying on lentiviral vectors (LV)



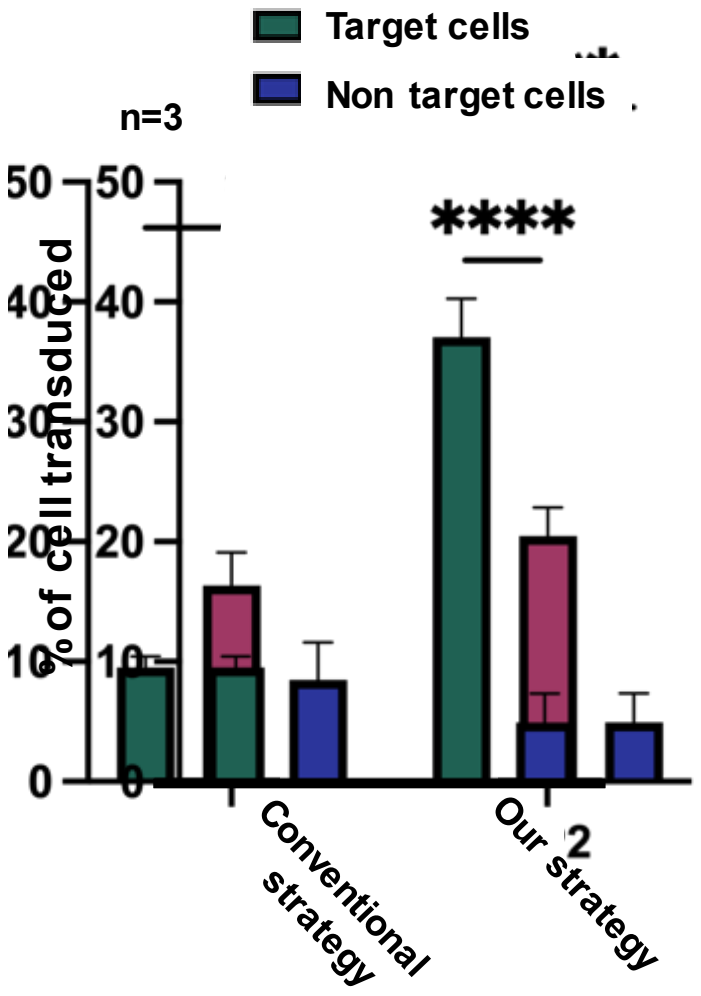
A: production of LV by co-transfection with two plasmids and transduction of of the target cells. B: generation of genetic diversity by error-prone reverse transcription and integration of the reverse transcription product. C: antibiotic selection for cells harbouring a provirus. D: generation of a viral progeny by transfection only with the packaging plasmid and transduction of fresh target cells. The cycles are repeated at will. During the procedure mutants with an improved ability of transduction will be selected. The predominant variant(s) will be characterized.

This way, the proteins evolve directly in the vector that will be used in the clinics and selection is carried out directly in the complex model constituted by the cells that are the ultimate target of the procedure.

Subject 2
Disruptive design of a viral's envelope glycoprotein for specific targeting

- **Result:**

  Using a novel approach for engineering a viral envelope glycoprotein, we obtained a variant that allows transduction by viral vectors of a specific type of cancer cells with an unpreceded preference than cells not expressing it (figure "our strategy", ****P<0.0001). Targeting the same type of cancer cells using other viral envelope proteins, modified using conventional strategies had been so far unsuccessful as shown in the literature and confirmed in our laboratory (figure "conventional strategy").



- **Perspectives:**

  - Extend the validity of our results from tissue culture to animal models in sight of preclinical assays
  - Apply our approach to target other types of cancer cells
  - Follow the same approach for targeting non-cancer cells for gene therapy

- **Unique selling points**
  - Directed evolution occurring in the same vector that will be used for the final application
  - Use our disruptive approach of rational design of recombinant proteins for other proteins
  - Competences ranging from the initial design of the proteins to the functional test of the vectors or viruses in cell culture and in preclinical models

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Technologies and Gene Therapy for Deafness

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Keywords
- Genetic hearing loss
- inner ear
- deafness
- Otology
- Robotics
- AAV
- Gene therapy

Abstract
The TGTD team led by Dr. Saaïd Safieddine and Prof. Yann Nguyen focuses on innovative technologies and gene therapy for deafness and balance disorders with recent notable contributions in the context of DFNB9 auditory synaptopathy. This recessive form of deafness is linked to otoferlin, a protein encoded by the OTOF gene, essential for glutamate exocytosis in auditory sensory cells (inner hair cells). We have achieved a significant breakthrough by showing that AAV gene therapy with either murine or human cDNA administered to the profoundly deaf otoferlin-null mice at P30, effectively reverses deafness phenotype (Akil et al., PNAS 2019). This groundbreaking result paved the way for gene therapy in humans with the ongoing clinical trial, under the RHU Audinnove project, which actively involves our team.

Meanwhile, we develop robot-based technologies to ensure an atraumatic access and drug delivery to the inner ear. We are now bridging the gap between animal's models and patients in order to treat patients. Our technology and expertise can also be transferred to other malfunctioning genes implicated in hearing loss or balance disorders affecting the inner ear. We expect from these two Hybryd Days to meet potential industrial partners to accelerate clinical transfer of these therapies.

Research area
Gene therapy for hearing loss

Synopsis
The team's aim is to develop effective gene therapies for hearing and balance disorders by optimizing therapeutic vectors and their delivery systems. We combine fundamental scientist and ENT surgeons in the team to close a bench to clinic loop. Our team will constitute a flagship project for the IHU Reconnect

Interests

Gene therapy;Viral vectors;Neurology;Rare diseases;In vivo models;Translational research;Clinical research

Technologies and Gene Therapy for Deafness

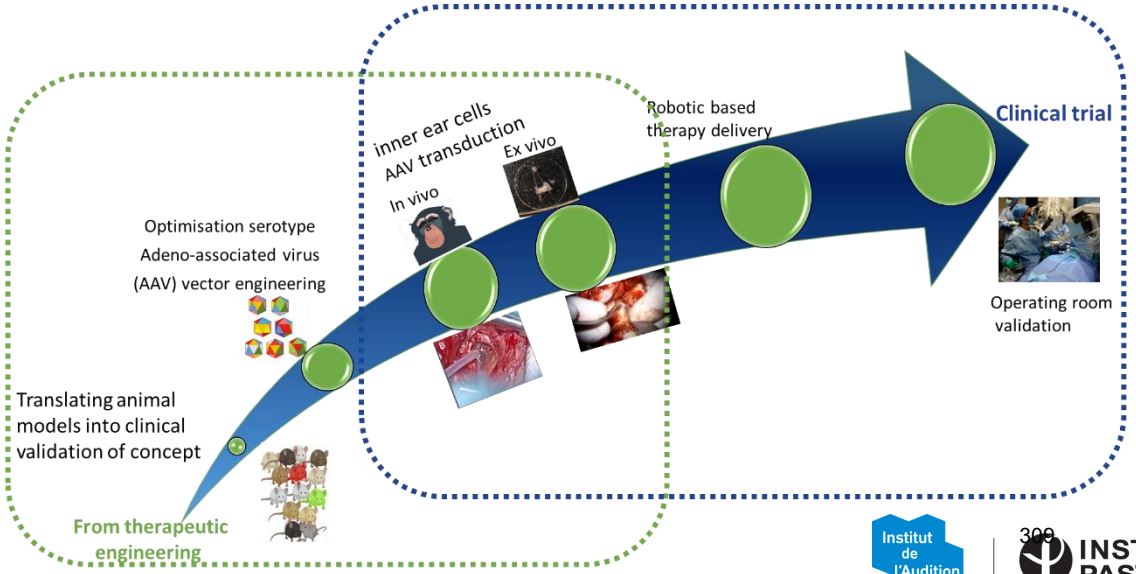
Saaid Safieddine, PhD
(Institut Pasteur)

Yann Nguyen, M.D. PhD
(Sorbonne Université/AP-HP)

Pasteur/Inserm/Université Paris Cité,
Paris 75012

- **Objectives:**
  - Design animal model to decipher hearing loss genetics mechanism
  - Build genetic impairment specific therapy
  - Optimize viral vector and surgical approach
  - Screen, diagnose, recruit and treat patients for genetic hearing loss

- **Tools:**
  - Animal models with full genotyping and phenotyping platform dedicated to hearing and balance pathologies
  - Viral vector library generation and selection adapted to genetic aetiology and intracochlear cell type targeting
  - Surgical expertise to access inner ear with worldwide leadership on robotics for hearing loss surgery

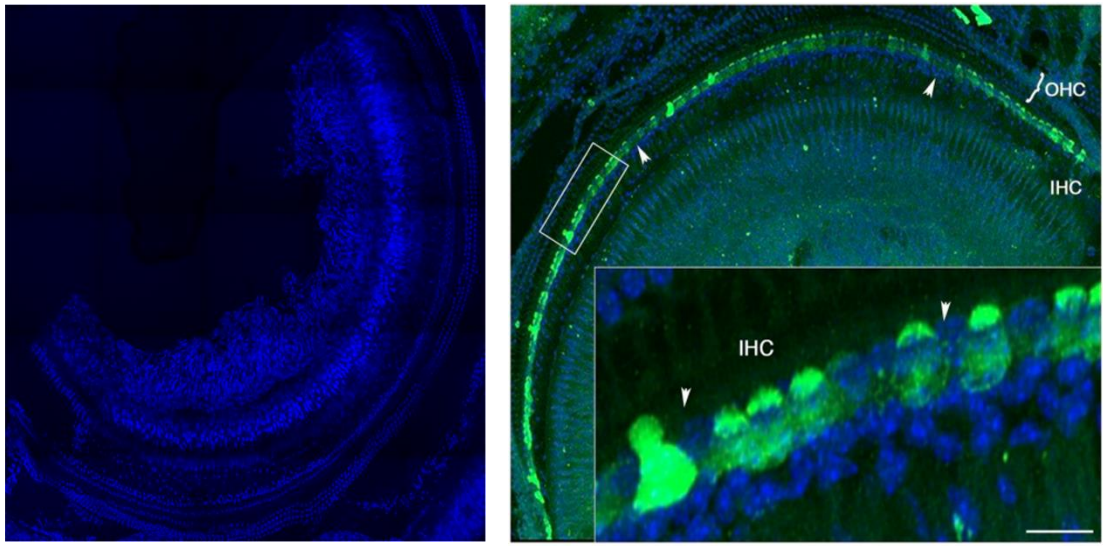


Gene therapy restores normal otoferlin expression and hearing in *DFNB9* mouse model

- **Results**

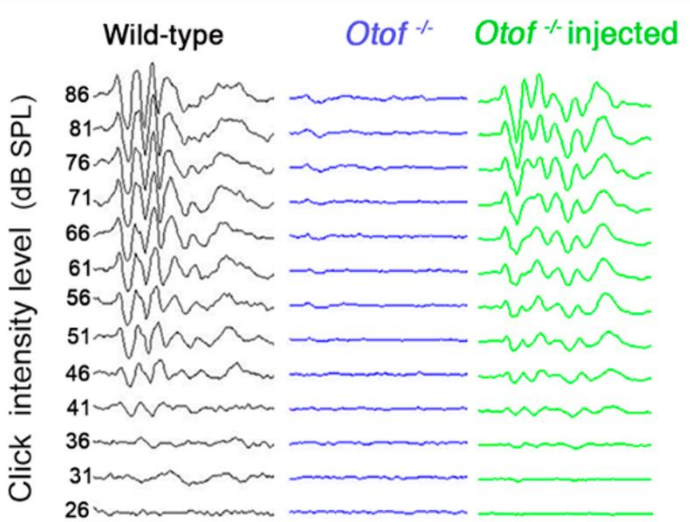
Gene therapy led to:

Otoferlin expression restored

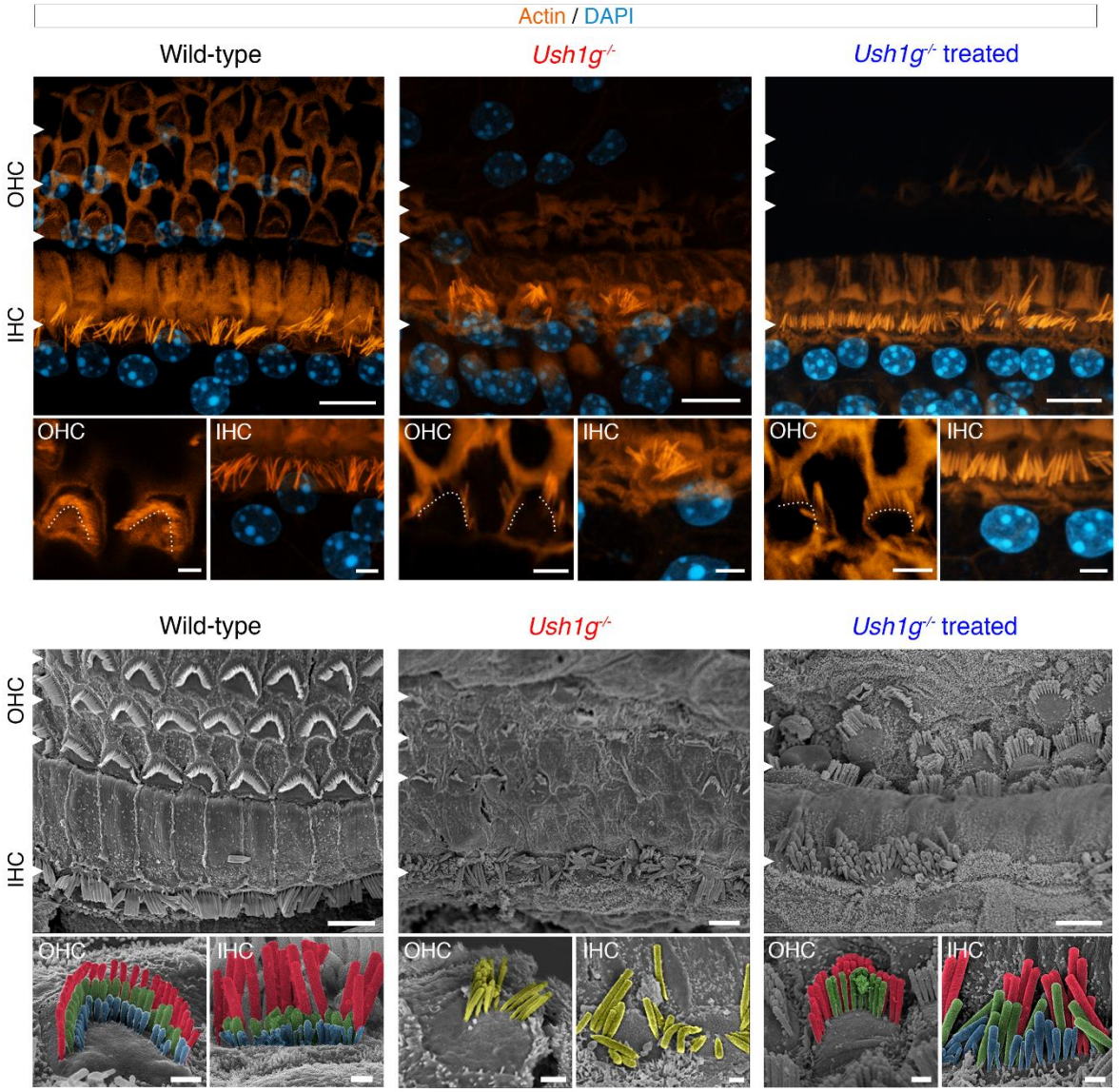


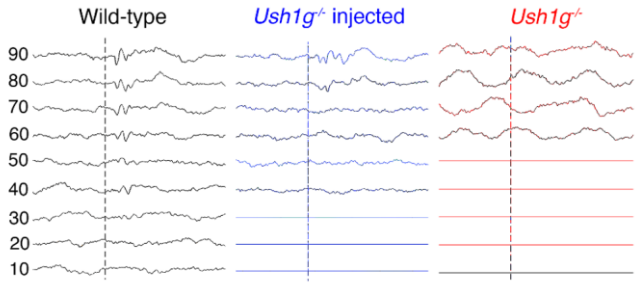
Akil et al., PNAS, 2019

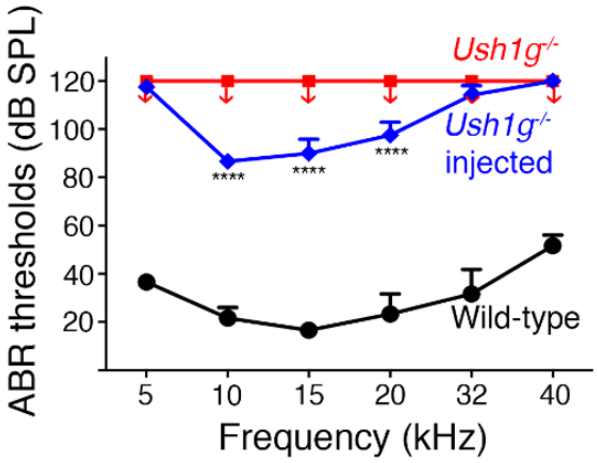
Hearing restored to normal thresholds



Extended time window for restoring inner ear function by gene therapy in a preclinical model of Usher 1G

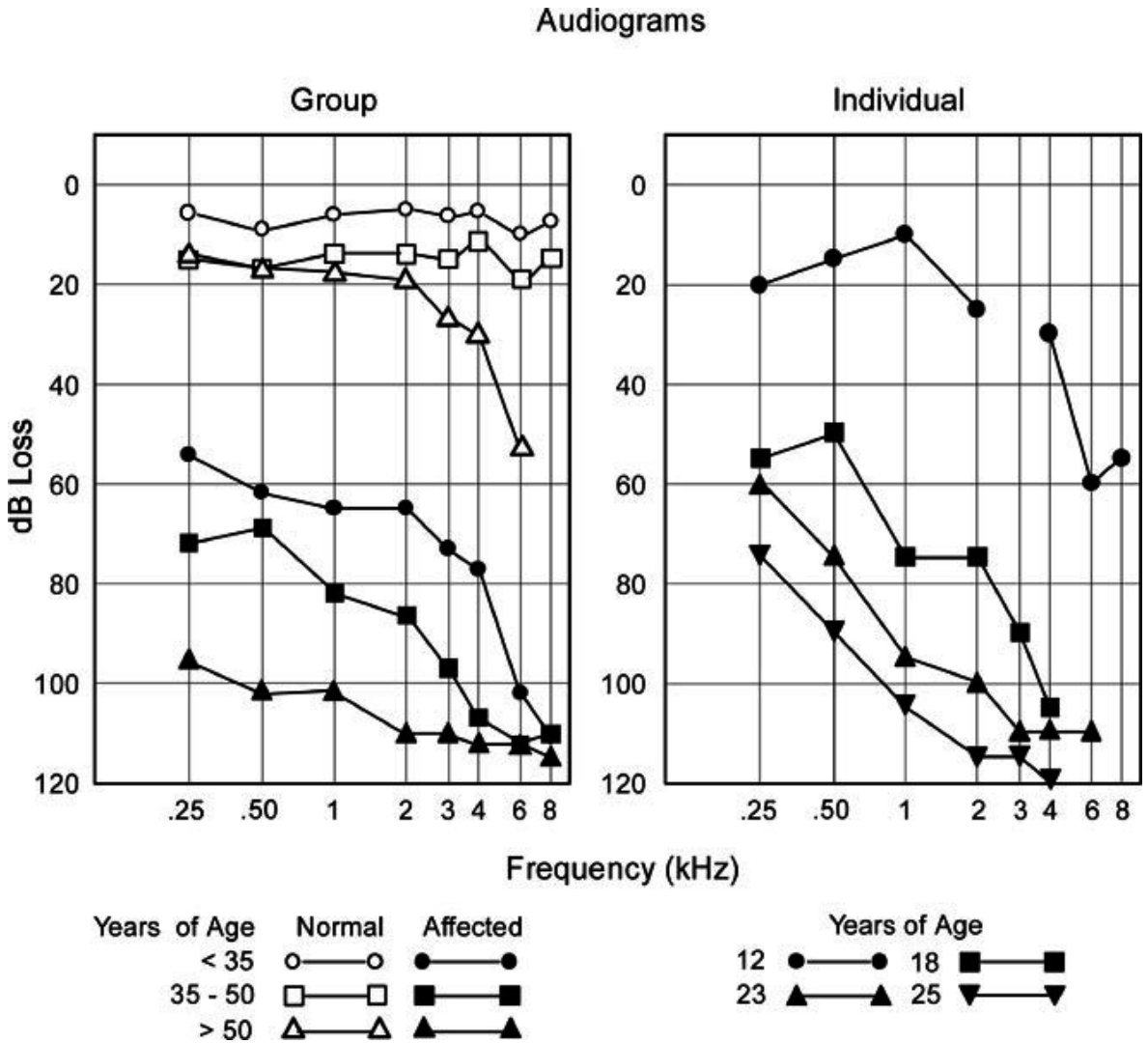






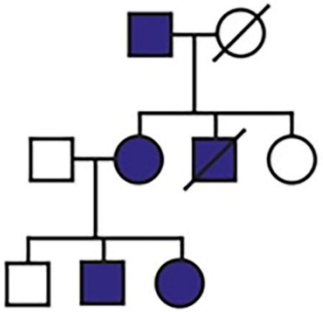
Lahlou et al., in revision

Ongoing project: Gene therapy to prevent inherited progressive deafness



Lahlou et al.

Ongoing project: Gene therapy to prevent inherited progressive deafness



Mutated gene

Mouse model for inherited progressive deafness

Gene therapy

Lahlou et al.

- **In vitro viral transduction of AAV in human inner ear cells, collected during vestibular schwannoma surgeries**

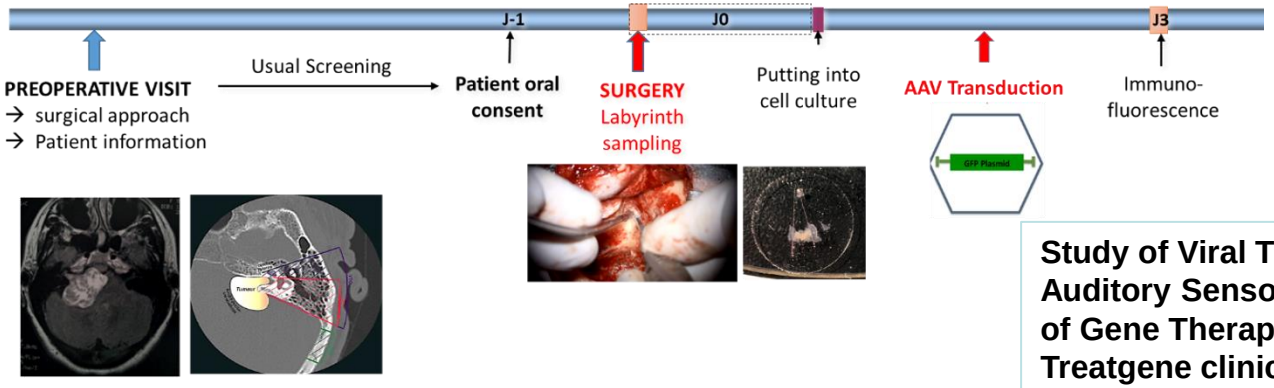
Results:

- For the first time we were able to select the best viral vector for transfection in human inner ear explant

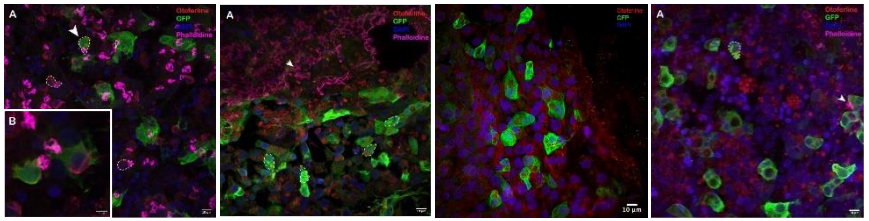
To examine the **cellular tropism** of commonly used **AAV serotypes** in preclinical animal models through human inner ear explants.

HÔPITAUX UNIVERSITAIRES PITIÉ SALPÊTRIÈRE CHARLES FOIX

- Clinicaltrial : NCT03996824
- Feb 2019 → Feb 2023
- 49 patients



Study of Viral Transduction of Human Auditory Sensory Cells for the Development of Gene Therapy
Treatgene clinical trial
Sponsors: Pasteur-APHP



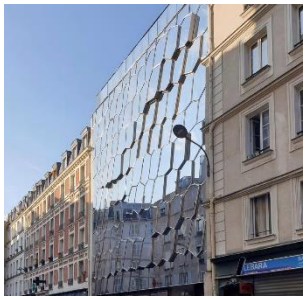
AAV2-CMV-GFP *AAV2.7m8-CMV-GFP* *Anc80-CMV-GFP* *AAV9-CMV-GFP*

Institut de l'Audition | INSTITUT PASTEUR

Perspectives:

- Clinical transfer and treatment of OTOF patients
- Design and transfer of other genes therapies
- Atraumatic vectorization of drug to the inner with robot-based technology
- Apply these treatments on the largest cohort of adult and children patients suffering from genetic hearing loss in France in Pitié Salpêtrière Hospital and Necker Hospital

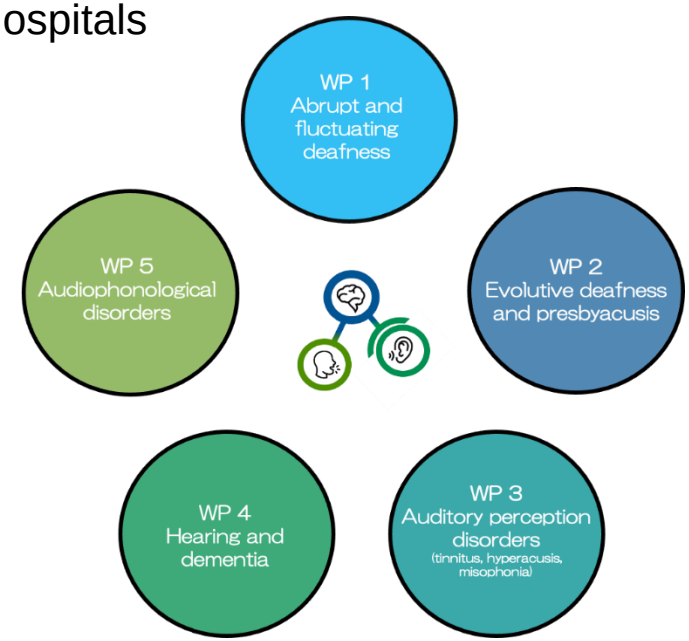
- **Unique selling points**
  - Team composed of half and half researchers and clinicians
  - Longstanding experience on animal model of hearing loss
  - Efficient results on gene therapy on animal model
  - Previous work on all models required from bench to clinical transfer (cell culture, organ explant, mice, non-human primate, adult and children patients)
  - Close partnership with largest french referral ENT departments for adult and children specialized in hearing loss technologies
  - Team part of the futur IHU Reconnect with a rich research environment at the Institut de l'audition and 3 majors academic Hospitals



IDA Team TGDG

"Technologies and Gene Therapy for Deafness"

reconnect
institut hospitalo-universitaire



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Next-generation monocyte-based cellular immunotherapies for solid cancers

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Keywords
- Therapeutic innovation
- Gene therapy
- Next-generation cellular immunotherapies
- Monocytes
- Innate immune checkpoints
- Nanotechnology
- Translational research
- Clinical research
- Biomarkers
- Hard-to-treat solid cancers

Abstract
Our research interests focus on the development of innovative biotherapies to improve cancer treatment. Deciphering the complexity of antitumor innate immune response with the support of biotechnological innovation is crucial to rapidly increase scientific knowledge in immuno-oncology, to discover novel molecular and cellular targets and biomarkers, to identify novel biotherapeutic approaches and to foster the development of innovative cancer biotherapies.

Through the development of integrative research programs in adoptive myeloid cellular therapies and nanomedicines for cancer treatment, our clinical and basic research objectives aim (i) to identify and target new innate immune checkpoints, (ii) to genetically “arm” monocyte-based adoptive cellular therapies with innovative delivery systems to better target and destroy cancer cells and (iii) to stimulate in situ vaccination against solid cancers. Our integrative research programs are expected to support the development of next-generation cellular immunotherapies for hard-to-treat solid cancers such as triple negative breast cancer, lung cancer, liver cancer, pancreatic cancer and childhood cancers.

Research area
Biotherapy and therapeutic innovation in oncology

Synopsis
Next-generation monocyte-based cellular immunotherapies for solid cancers

Interests
Gene therapy;Oncology;Immunology/Immunotherapies;Specific targeting;Biomarkers;Nanotechnology;Translational research;Clinical research

Next-generation monocyte-based cellular immunotherapies for solid cancers

Jean-Luc PERFETTINI

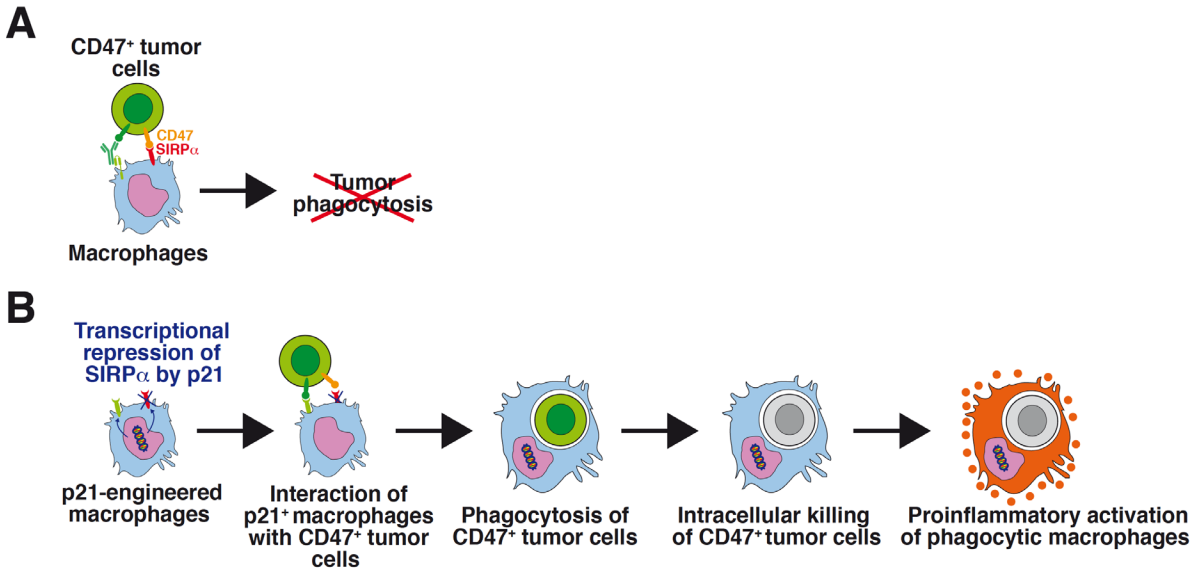
Inserm U1030, Université Paris-Saclay,
Gustave Roussy,
Villejuif

- **Objectives:**
  - To identify and target novel immune checkpoints dictating tumor phagocytosis and functional reprogramming of tumor-associated macrophages,
  - To engineer and characterize “armed” monocyte-based adoptive cellular therapies for unleashing novel innate immune checkpoints,
  - To support the development of next-generation monocyte-based cellular immunotherapies and combinatorial strategies for the treatment of hard-to-treat solid cancers.

- **Tools:**
  - High content molecular screening platform for tumor phagocytosis,
  - Lentiviral- and/or lipid nanoparticle-based gene editing for phagocytosis immune checkpoint abrogration, CAR expression and tumor-associated macrophage reprogramming,
  - Functional in vitro (cell lines and primary cells) and in vivo (immunodeficient, immunocompetent and humanized mice models) assays with monocyte-based cellular immunotherapies and combinatorial therapies for hard-to-treat solid cancers.

Topic 1: Basic and translational research on macrophage phagocytosis immune checkpoint and functional reprogramming

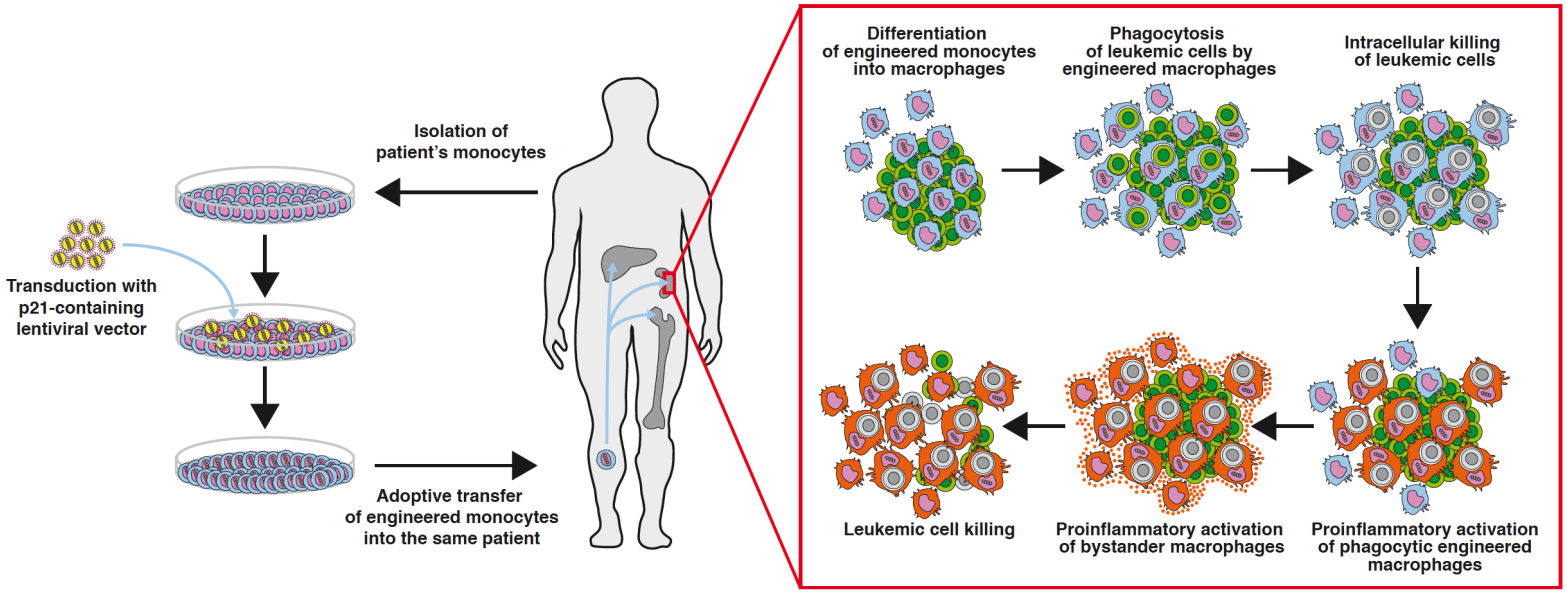
- **Results:**
  - Discovery of a non-cell autonomous modality of macrophage proinflammatory activation that starts with tumor phagocytosis,
  - Identification of cyclin-dependent kinase inhibitor CDKN1A (p21) as a master regulator of tumor cell phagocytosis through SIRPα transcriptional repression,
  - In-vitro and in-vivo characterization of the antitumoral and immunological activities of p21-engineered macrophages.



Legend: p21 overexpression overcomes the phagocytosis immune checkpoint CD47-SIRPα (A) and acts as a trigger of phagocytosis-guided proinflammatory macrophage reprogramming (B) (Allouch et al., *Nat Commun* (2022)).

Topic 2: Use of p21-engineered monocytes as «Therapeutic Trojans » for next-generation cancer cellular immunotherapies

- **Results:**
  - Identification of p21-engineered monocyte-based cellular therapy as novel cancer immunotherapy,
  - Characterization of tumor homing and in situ differentiation of adoptively transferred p21-engineered monocytes into phagocytosis proficient tumor-associated macrophages,
  - In-vivo characterization of antitumoral and immunological activities of adoptively transferred p21-engineered monocytes.



Legend: Engineering and adoptive transfer of "Therapeutic Trojans" for the treatment of acute T cell leukemia (Allouch et al., *Nat Commun* (2022)).

- **Perspectives:**
  - Development and characterization of next-generation, phagocytosis-guided “armed” monocyte-based cellular immunotherapies for hard-to-treat solid cancers,
  - Development of an innovative technological platform for the engineering of next-generation, phagocytosis-guided “armed” monocyte-based cancer cellular therapies,
  - Personalization of next-generation, phagocytosis-guided “armed” monocyte-based cancer cellular therapies,
  - Identification of cancer-specific combinatorial strategies.

- **Unique selling points**
  - Our team is pioneering the development of monocyte-based cellular immunotherapies for cancer treatment,
  - Monocyte-based cellular immunotherapies can address key challenges faced by current adoptive cellular therapies,
  - Fast gene therapy using monocytes.

Selected bibliography

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- Multi-parametric detection of non-cell-autonomous and cell-autonomous deaths reveals the heterogeneity of response to anticancer treatments. Martins et al., *Cell Death Dis.* 9(2018):716. doi: 10.1038/s41419-018-0747-y.

- The NOX2-dependent ATM kinase activation dictates pro-inflammatory macrophage phenotype and improves effectiveness to radiation therapy. Wu et al., *Cell Death Differ*. 24(2017):1632-1644. doi: 10.1038/cdd.2017.91.

- Modulating Both Tumor Cell Death and Innate Immunity Is Essential for Improving Radiation Therapy Effectiveness. Wu et al., *Front Immunol.* 8(2017):613. doi: 10.3389/fimmu.2017.00613.

Optimized mRNA formulations for immune cells and alternative cost-effective mRNA production

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Keywords
- mRNA vaccines
- mRNA therapeutics
- mRNA delivery
- Lipid-based nanoparticles
- mRNA bioproduction
- Protein replacement therapy
- Gene editing
- mRNA-based immune cells engineering

Abstract
We have a long-lasting expertise on the development of nanomedicines based on nucleic acids. The lab is carrying out fundamental investigations combined with cutting-edge technologies to build their strategies to advance delivery of nucleic acids. The first achievement concerns the substitution of polymers and lipids with histidine residues or imidazole moieties. We were amongst the first to report the feasibility of targeting splenic dendritic cells upon intravenous administration of lipid/polymer hybrid-formulated mRNA cancer vaccines nanoparticles bearing mannose ligands.
Recently, we established a highly disruptive technology, the “RNA yeast –based cell factory” that leads to accumulation and storage of heterologous mRNA in a specific compartment of the yeast. This patented breakthrough technology is quite challenging, holds a real promise, but is under-derisking for its valorization. The goal is to search for alternative mRNA synthesis that ensures large batch production with lower cost for clinical development.
Last, we succeeded to produce novel mRNA formulations based on lipid nanoparticles for immune cells that are highly efficient for NK, B and T cells engineering. The perspective for the future is quite straightforward, implement LNP and mRNA technologies for gene editing of immune cells for various purposes, for the production of mucosal vaccines and for protein replacement therapy based on mRNA in various pathologies.

Research area
Messenger RNA technologies

Synopsis
Our research is focused on both developing mRNA production and delivery strategies to accelerate their integration into a range of therapeutic applications

Interests

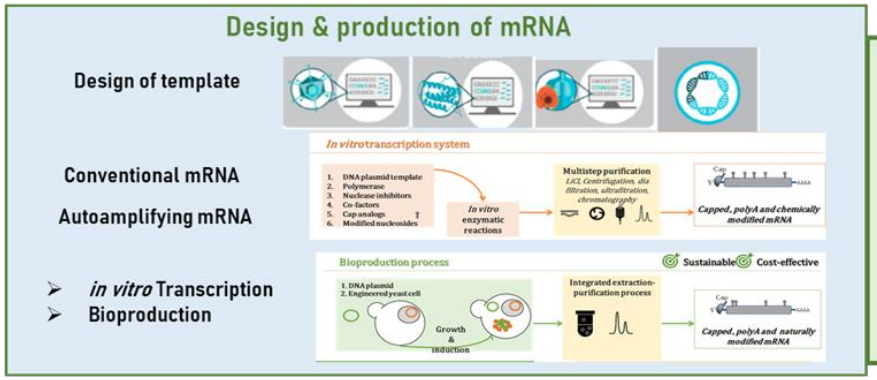
Gene editing;Non viral delivery systems;mRNA;Oncology;Vaccine;Specific targeting;Nanotechnology;Bioproduction

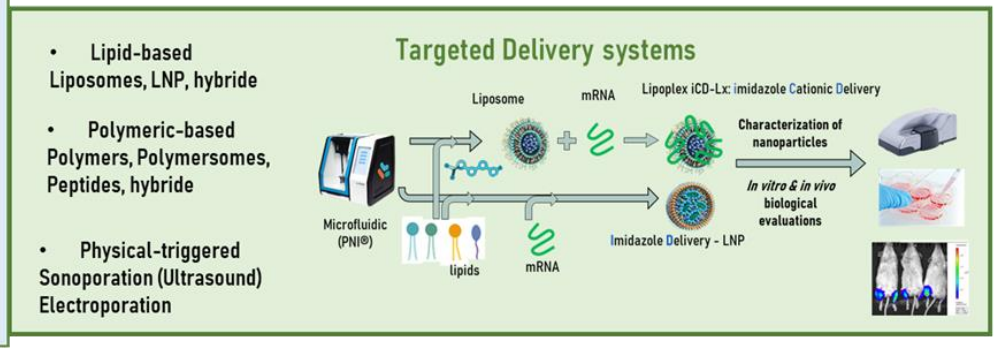
Optimized mRNA formulations for immune cells and alternative cost-effective mRNA production

Chantal PICHON

ART-ARNm INSERM, Orléans

- **Objectives:**
  - Design conventional and self amplifying mRNA for vaccination and therapeutic applications.
  - Develop cutting edge technologies for RNA delivery.
  - Accelerate the development of mRNA technologies through a coherent set of partnership projects.
- **Tools:**
  - Platform for the design and (bio) production of conventional and self amplifying mRNA including analytical workflow equipments.
  - Platform for the development of RNA formulations based on lipids (liposomes, LNP), polymers, hybrid lipid/polymers and microbubbles using microfluidic systems including microfluidic systems including analytical workflow equipments.
  - Pre-GMP and preclinical cells and *in vivo* studies to evaluate the efficiency of RNA biomedicines.

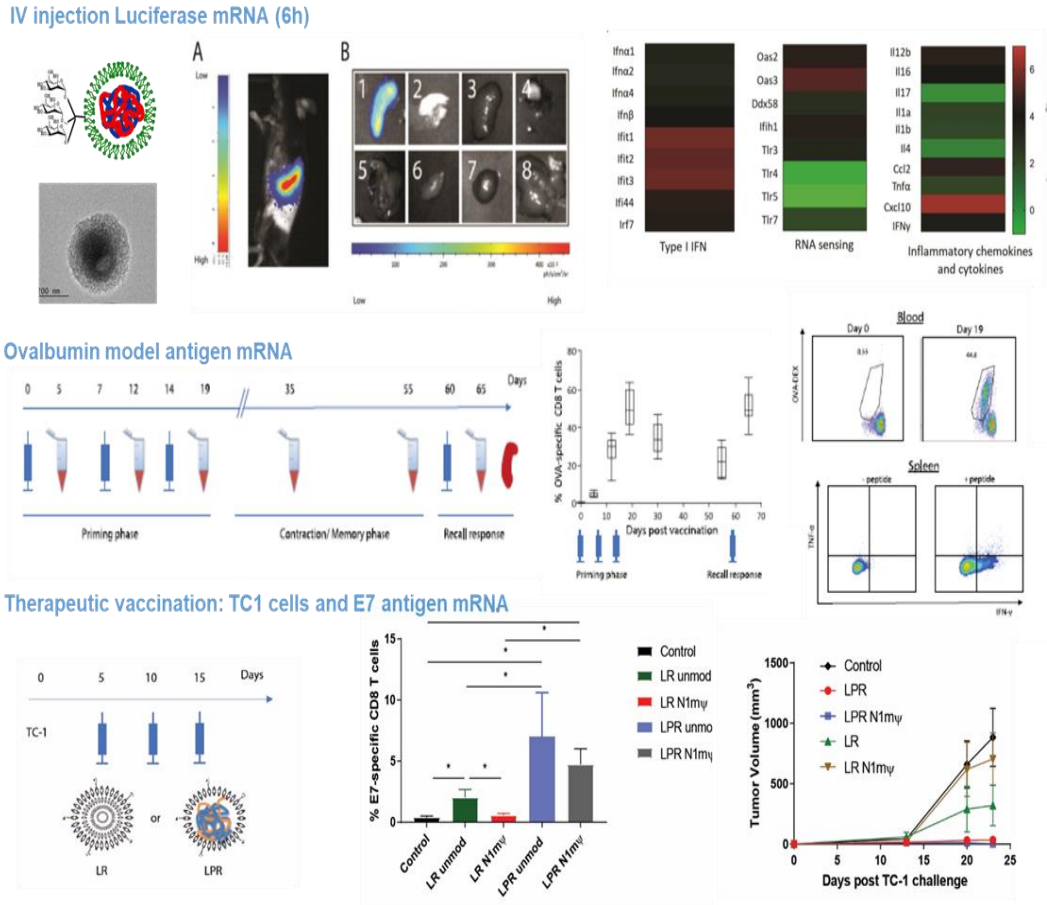




Expertise & Human resources | Manufacturing technologies and processes | Analytical equipment and related methods

Mannosylated hybrid lipid/polymer for mRNA cancer vaccines

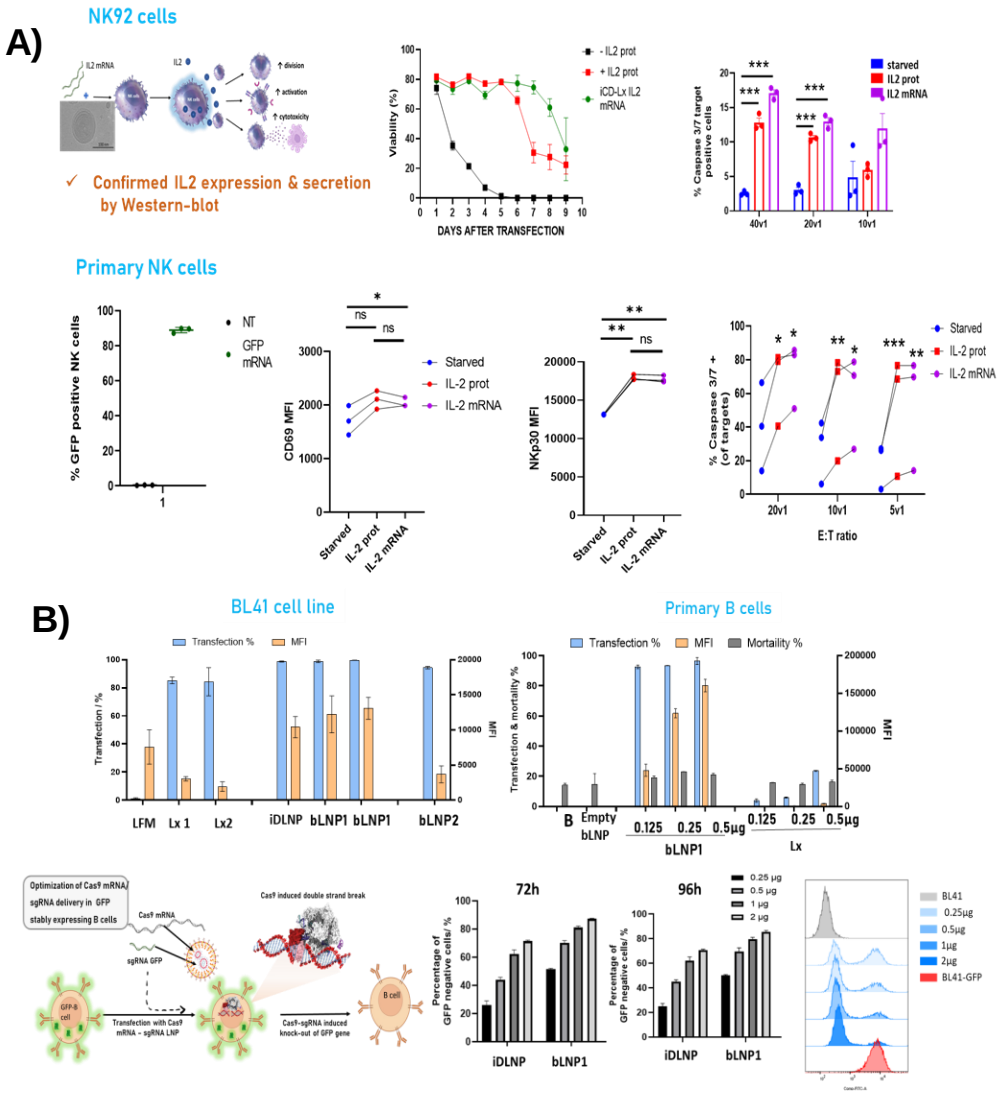
- **Results:**
  - Hybrid lipid/polymer mRNA (LPR) nanoparticle bearing trimannose motif **specifically targeted to spleen** after IV injection.
  - Transcriptome profiling of spleens 3 h after administration of vaccines shows upregulation of mRNA of **IL-12, IFN-γ and CXCL-1 with low or no increases of inflammatory and regulatory cytokines**.
  - Sytemic administration of our mRNA vaccines instigates a potent cell immunity with **an induction of specific CD8+ specific cells as well a strong immunological memory**.
  - Our vaccines **elicit profound antitumor immunity with an inhibition of tumor growth**.
  - The of **use modified mRNA is dispensable to induce the therapeutic vaccination**.



Specific spleen targeting of trimannosylated LPR upon IV injection. LPR made with antigen mRNA instigates a potent T cell immunity with a potent memory that leads to the inhibition of tumor growth.

Novel lipid-based formulations for mRNA-based immune cells engineering

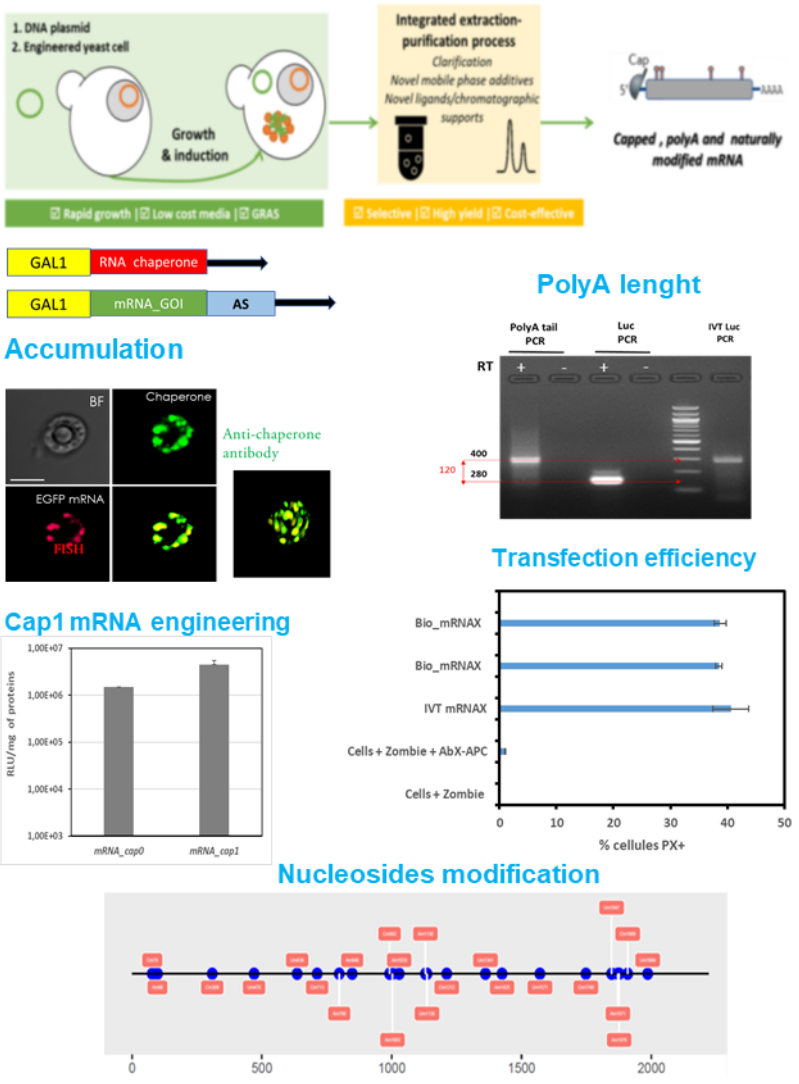
- **Results:**
  - Liposomal formulation are efficient for mRNA-based NK cells engineering (>80%) without impacting cell viability even after overnight incubation.
  - Human primary NK cells transfected with IL2 mRNA have **a sustained proliferation, conserved biomarkers activity and cytotoxicity feature** towards lymphoma cells as does IL2 recombinant protein.
  - LNP formulation are able to transfect **primary B cells and human B lymphoma (90-100%)** without impacting cell viability.
  - Our formulations made of Cas9 mRNA and gRNA were **efficient for gene editing**.
  - **Modification of mRNA is dispensable** rendering the technology **cost-effective.**



Liposomal (A) and LNP (B) formulations for efficient mRNA delivery in human natural killer cells and human B lymphocytes, respectively .

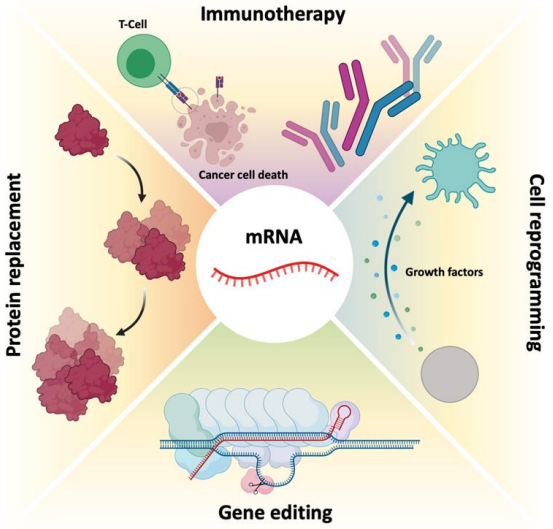
Bioproduction of mRNA:
a breakthrough cots- effective alternative technology

- Results:
  - Design **of a tailor-made yeast-based mRNA factory platform** for large scale and **cost-effective manufacturing** of mRNA therapeutics, which is crucial for their widespread applications
  - mRNAs of interest are not translated but **accumulated inside yeast** allowing their purification.
  - Bioproduced mRNA can **be tailored with a desired structures and sequences as polyA lenght and adding cap1.**
  - Bioproduced mRNA is **endogenously modified** as we have in human cells.
  - **Efficient *in cellulo* expression** of bioproduced mRNA.



Yeast-based mRNA factory platform at a glance

- **Perspectives:**
  - Matching our formulations to specific indications:
  - mucosal vaccination for cancer and viral infection (in progress *via* RNAvac PEPR project).
  - *In-vivo* cell targeting for various purposes (B and NK cell engineering, metabolic diseases, cardiac injury and rare diseases…)
  - Regeneration and reprogramming applications.
  - Demonstrate their effectiveness in cell therapy clinical trials through different networks including ERDERA (european rare diseases research alliance for ex): development of chronic life-saving therapies.
  - De-risking the bioproduction of mRNA to validate their cost-effectiveness: expanding the frontier of mRNA applications (diseases and countries).



- **Unique selling points**

  - **Capacity for innovation**: skills, expertises and state of the art experiments for mRNA technologies.
  - **A solid network with accredited national integrators and platforms**: MAGENTA: (A. Galy-Evry), CITHERA (A. Bennaceur, Evry), IVETH (F. Gazeau, Paris), NACRE (Hirtz, Montpellier, TIBH (F. Daboussi, Toulouse).
  - **A solid network with centers able to conduct clinical trials**: VRI (Y. Levy, Paris), Immun4cure (C. Jorgensenn, Montpellier), BeCAT (P. Reinke, La Charité Berlin)
  - **International recognized expertises on mRNA technologies with an involvement in different networks and societies**: French Society of Nanomedicine, Altanpole biotherapies, University hospital federation GenoMedS

    ETP nanomedicine, European society of Gene and Cell Therapy, American society of gene and cell therapy.

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Antisense oligonucleotide (ASO)-based nanomedicine targeting stress-induced proteins mRNA for personalized therapy and imaging

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Keywords
- Therapy Resistant Prostate Cancer
- Hsp27 signalling pathway
- mRNA targeting
- Antisense oligonucleotide
- Radiotherapy
- Chemotherapy
- Synergistic treatments
- Precision medicine

Abstract
Prostate Cancer (PC) ranks as one of the most prevalent cancers worldwide and the third leading cause of cancer-related deaths in men. Although early-stage disease is curable, 40% of patients are diagnosed late at advanced stages. While androgen deprivation therapy works well as a primary treatment for advanced PC, patients become resistant to treatment (TRPC) and inexorably progress towards a severe and fatal form.
Our lab previously has provided significant scientific contribution in this field and demonstrated that the heat-shock protein 27 (Hsp27) is a key driver of therapy resistance in PC. We developed an antisense oligonucleotides (ASO) targeting HSP27 mRNA (Apatorsen, OGX-427) that restore chemotherapy sensitivity (Rocchi, P., cancer research 2004 and 2005; Patent PCT no 10/605, 498 2005). This approach has been evaluated in the setting of clinical trials in collaboration with Ionis Pharmaceutical Company. Our lab demonstrated that Hsp27 fosters TRPC by safeguarding partner proteins like DDX5 from ubiquitin-proteasome degradation via its chaperone activity and define DDX5 as a novel therapeutic target for TRPC. DDX5 overexpression is strongly correlated with aggressive tumor features, and notably with TRPC. ASO-based inhibitor developed in our lab targeting DDX5 mRNAs inhibits cell proliferation in pre-clinical models, and particularly restores treatment sensitivity of TRPC (Le, T.K., molecular therapy, 2022; Patent PCT19031, 2020). However, the high variability in gene expression among tumors highlights the need for tailored therapies targeting individual oncogenic drivers.
In parallel, we have initiated a work on the identification of more reliable PC models. Prostate tumor derived organoids (PTDOs) have recently emerged as a reliable model to understand the development and treatment response of TRPC and design precision medicine in PC.
Our project relies on the combination of our experience in several aspects of our research: 1/ Validate our technology targeting mRNA for personalized therapies combining ASO therapy and radiation or chemotherapy in preclinical models and 2/ Develop PTDO for stratifying PC patients into different sub-groups according to their oncogenic drivers. This approach is

expected to increase the therapeutic efficacity of all components through synergistic effects and demonstrated its relevance for personalized therapies of CRPC.

Research area

Inserm Unit ERL1326 “RNAnoTher” focuses on translational research to fight treatment resistance in prostate cancer. Our principal research interest is focused on antisense oligonucleotide (ASO)-based medicine targeting stress-induced proteins mRNA for personalized therapy and imaging

Synopsis

We focus on developing new medicines for mRNA stress-induced proteins involved in treatment resistance to restore therapy-sensitivity of prostate cancer

Interests

Non viral delivery systems;Oligonucleotides;Oncology;In vitro models/ Organ-on-chip;Patient cohorts;Translational research;Clinical research

Pluripotent Stem Cells in Mammals

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Keywords
- Pluripotent stem cells
- embryonic stem cells
- reprogramming
- cell signalling
- systemic chimeras
- human, monkeys, rabbits

Abstract
We possess strong expertise in the field of derivation and genetic engineering of pluripotent stem cells, whether they are embryonic pluripotent cells, known as ES cells, or induced pluripotent stem cells, known as iPSCs. Our know-how extends from mice to humans, including rabbits and non-human primates such as the marmoset, macaque monkey, and hominid monkeys, allowing us to benefit from the advantages of different biological models to answer various questions. Our work develops along three complementary axes:
1. Deepen our understanding of the mechanisms regulating the state of cellular pluripotency in order to improve the stability and adaptability of human iPSCs for clinical applications. Our efforts focus on the characterization of new ligands and receptors that capture iPSCs in an immature pluripotent state called the 'naive' state.
2. Produce somatic and germinal chimeras from ES and iPSCs in rabbits and macaque monkeys with the aim of creating new models of human diseases. Our work focuses on the characterization of signaling pathways involved in the 'chimerization' process.
3. Develop innovative models of inter-species chimeras using primate iPSCs, with the goal of modeling human development. In the long term, these new models may pave the way for the production of human tissues and organs in animals. Our current work focuses on iPSCs from hominoid monkeys and the creation of hybrid chimpanzee:macaque embryos

Research area
Stem cell biology

Synopsis
We manipulate primate-induced pluripotent stem cells to empower them to produce chimeric and cloned animals, both for human disease modeling and for generating human organs within large animals.

Interests
Genetic engineering;Stem cells;In vitro models/ Organ-on-chip;In vivo models;Biocollections;Biomarkers;Epigenetics;Single cell manipulation;Imaging

SBRI Stem-cell & Brain Research Institute Lyon

Pluripotent Stem Cells in Mammals

Pierre SAVATIER

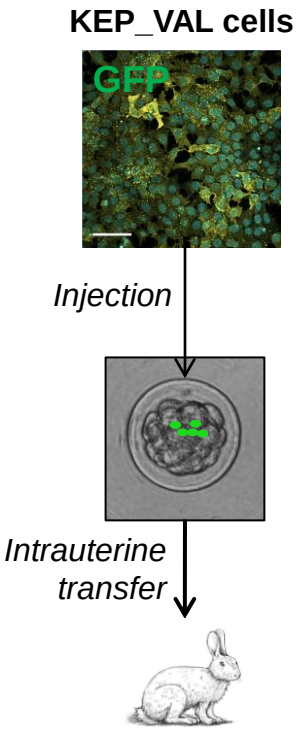
INSERM U1208
Stem Cell and Brain Research Institute
Lyon

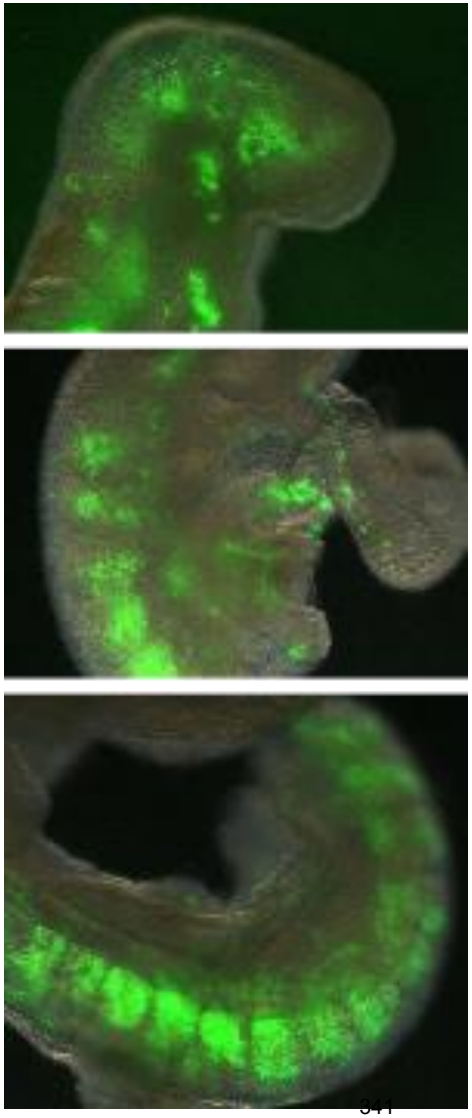
- **Objectives:**
  - Developing the next generation of human induced pluripotent stem (iPS) cells to improve their fitness and genetic stability.
  - Developing a proof-of-concept for systemic chimerism using monkey iPS cells in the prospect human disease modeling.
  - Developing a proof-of-concept for monkey cloning using using iPS cells as source of donor nuclei in the prospect human disease modeling.
  - Advancing the proof-of-concept for inter-species chimerism in non-human primates utilizing pluripotent stem cells derived from apes, with the ultimate goal of growing human organs in large animals.
- **Tools:**
  - A unique collection of embryo-derived and iPS cells in rabbits, monkeys (marmoset, macaque), and apes (chimpanzee, bonobo, gorilla, orangutan)
  - A monkey breeding facility for embryo production, employing assisted reproduction technology (intracytoplasmic sperm injection), fetus collection, and phenotypic analysis. This includes a state-of-the-art cell culture facility (L2 and L3).
  - World-renowned expertise in pluripotent stem cells (PSCs) and PSC-based systemic chimerism in rabbits as a surrogate model for non-human primates.

Generation of somatic and germline chimeras in rabbits

- **Results:**

Rabbit iPS cells engineered using novel cocktails of genes, growth factors, and small molecules, resulting in a significant capacity to participate in fetal development following injection into rabbit morulae (*unpublished*)



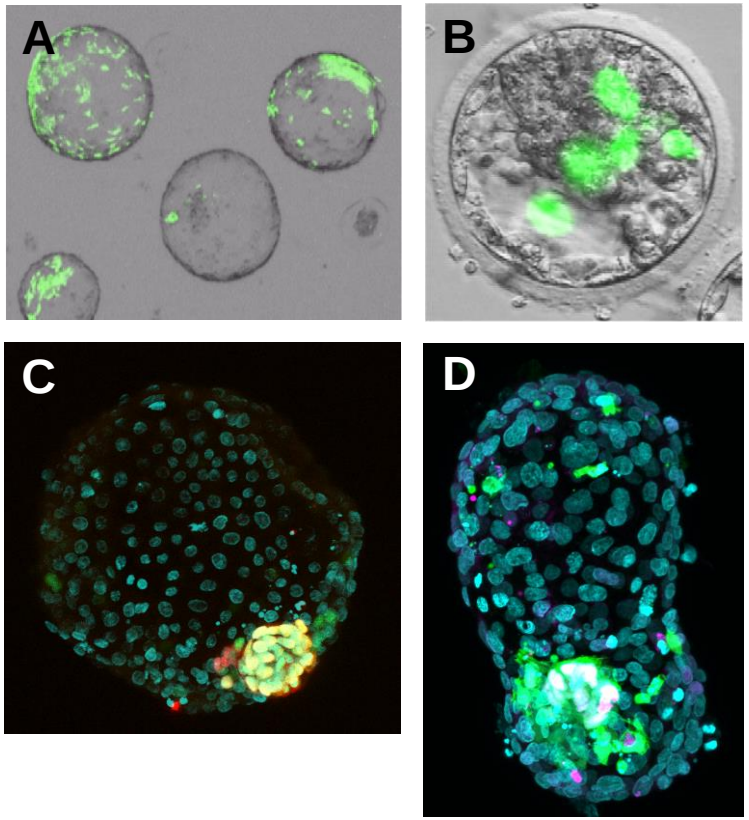


Left panel: Schematic of the experiment.
Right panel: A rabbit chimeric fetus at day 10 of development, obtained after the injection of optimized rabbit iPS cells (expressing GFP) into rabbit morulae and subsequent transfer to surrogate females. The injected cells participated in organ development with high efficiency) (Perold et al., submitted)

Interspecies embryo chimeras in mammals

- **Results:**
  - We identified key signalling pathways (MEK-ERK and PI3K-AKT signalling pathways) involved in the growth and survival of primate iPS cells following their introduction into host embryos and subsequent colonization.
  - We generated iPS cell lines from humans and chimpanzees, as well as embryo-derived pluripotent stem cell lines from macaque and marmoset monkeys, capable of efficiently colonizing rabbit and macaque monkey embryos.
  - We generated interspecies embryos, including human:rabbit, human macaque, chimpanzee:rabbit and chimpanzee:macaque combinations.
  - We developed a cell competition assay to measure the fitness of iPS cell lines.

  (*Unpublished*)



(**A**) Chimpanzee:rabbit chimeric embryos obtained using chimpanzee iPS-GFP cells. (**B**) A chimpanzee:macaque chimeric embryo obtained with chimpanzee iPS-GFP cells. (**C**) A chimpanzee:rabbit chimeric embryo obtained with chimpanzee iPS-GFP cells. Immunostaining with a SOX2 antibody (in red) demonstrates that most pluripotent cells (in yellow) are of chimpanzee origin. (**D**) A human:rabbit chimeric embryo obtained using human iPS-GFP cells.

- **Perspectives:**
  - Perform high-throughput screening of small molecule libraries on rabbit, chimpanzee, and human iPS cells. Then, identify new compounds that enhance fitness and subsequent embryo colonization.
  - Generate chimpanzee:macaque and chimpanzee:pig chimeric fetuses and newborns using our optimized iPS cells and protocols. Study organogenesis from the single-cell to the physiological level. Determine the impact of the phylogenetic distance between the host and iPS cells on organogenesis.
  - Generate cloned monkey fetuses using a two-step nuclear transfer protocol, and utilizing our optimized iPS cells with bovine and macaque monkey eggs (in collaboration with Chulalongkorn University and Suranaree University of technology, Thailand).

- **Unique selling points**

  - A unique set of human, ape, monkey, and rabbit iPS cell lines and culture protocols, enpowered to generate systemic chimeras
  - A world-unique expertise in rabbit systemic chimeras
  - A unique European expertise in assisted reproduction technologies for macaque monkeys

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Your partner in pharmaceutical bioprocess innovation

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Keywords
- Innovation
- Bioproduction
- Biotherapy
- Formulation
- Technological platform
- Virus
- Gene therapy
- Cell therapy

Abstract
The development of biotherapies is a growing market. Today, one out of two drugs in development in the world is a biomedicine, and pharmaceutical industries aim to produce more. InnoBioVir, winner of the call for projects IRICE (company-centered research and innovation facilities), is an academic technological platform resulting from a synergistic alliance between research laboratories (LAGEPP, VirPath), technological platforms (VirNext) and educational platforms (BIOTOP, IUT Biological Engineering) aimed at supporting innovation in the fields of production and formulation of innovative therapies and evaluation of vaccine antigens. Thanks to a high-quality service, ensured by our solid expertise covering the entire production chain of a biomedicine, access to state-of-the-art equipment and partnerships of excellence, InnoBioVir offers pharmaceutical industries, start-ups and academics custom services, R&D collaborations and industry-oriented trainings. Thus, with proven know-how in bioproduction and formulation, InnoBioVir is committed, through its service offering, to accelerating the marketing of these advanced therapy medicinal products through the development of innovative solutions meeting the needs of a booming sector.

Research area
Innovation in process development to accelerate production and marketing of biotherapies

Synopsis
InnoBioVir: a cutting-edge technological platform at the service of innovation in the fields of production, formulation and evaluation of viral antigens and innovative therapies

Interests
Gene therapy;Cell Therapy;Infectious diseases;Translational research;Bioproduction

Your partner in pharmaceutical bioprocess innovation

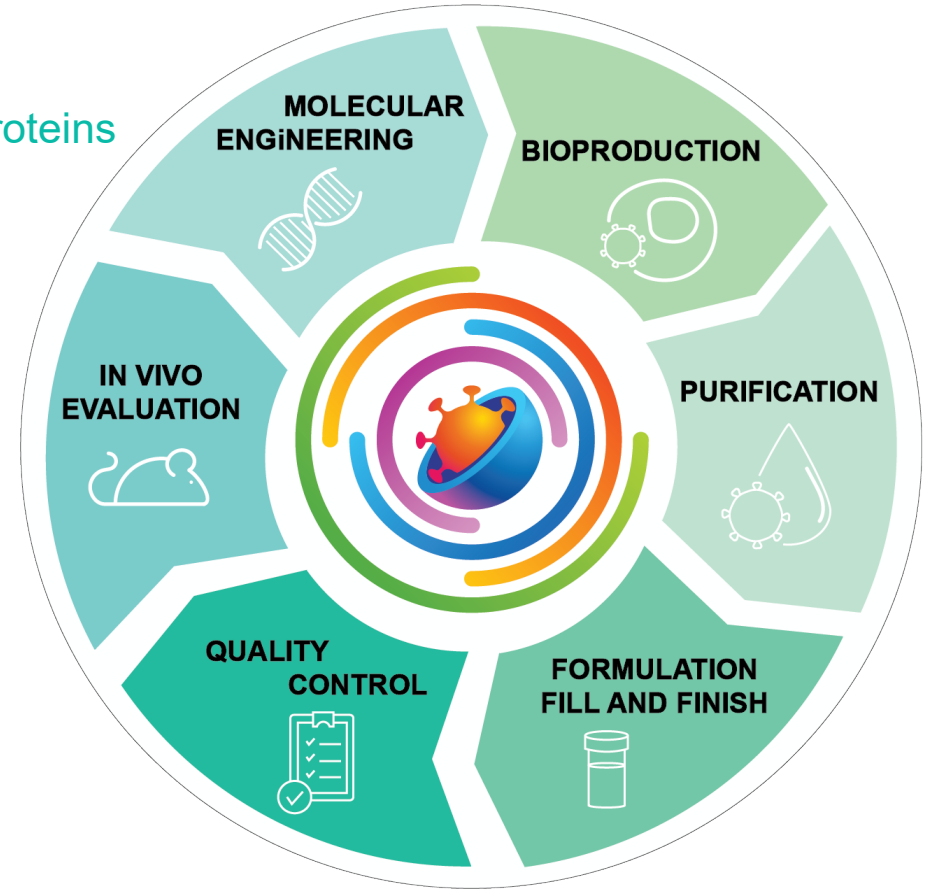
Vernier Mathieu, PhD

Université Claude Bernard Lyon 1 (UCBL), Lyon

innobiovir
RESEARCH, SERVICE & TRAINING IN BIO-PRODUCTION

Objectives:

- Tailor-made offer for process modelisation and process development
- R&D collaboration
- Training and Tech-transfert
- Production of viruses and recombinant proteins



Cross-competences alliance for full value chain integration:

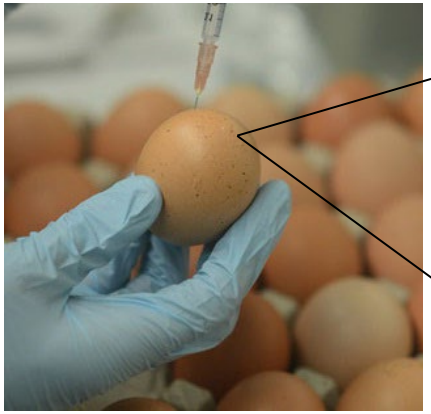
- 2 research laboratories
- 3 technical plateforms
- Expertise in all aspects of the development chain
- State-of-the-art R&D facilities

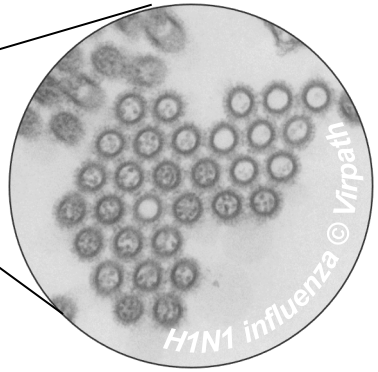
LAGEPP
Laboratoire d'automatique, de génie des procédés, et de génie pharmaceutique.
cnrs
VirPath
Inserm
ipil INSTITUT DE PHARMACIE INDUSTRIELLE DE LYON
ISPB Faculté de Pharmacie de Lyon
IUT Lyon 1
l'excellence technologique
Lyon 1

Expertises and capabilities

Mol. Eng. | **Bioprod** | Purif. | Formul. | Quality | Eval.

Upstream process





Embryonated Eggs

Mammalian cell culture Bioreactor
1L to 50L

BSL-2 & BSL-3 facilities

Expertises and capabilities

Mol. Eng. | Bioprod | **Purif.** | Formul. | Quality | Eval.

Downstream process
Clarification / Purification / Polishing

Ultra centrifugation

Tangential Flow Filtration / UF / Diafiltration

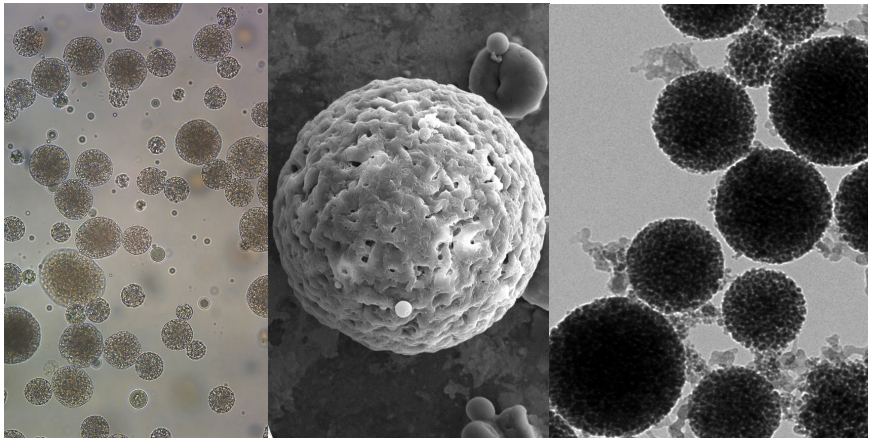
Chromatography

Expertises and capabilities

Mol. Eng. → Bioprod → Purif. → **Formul.** → Quality → Eval.

Formulation - Fill and finish

Solids/liquids

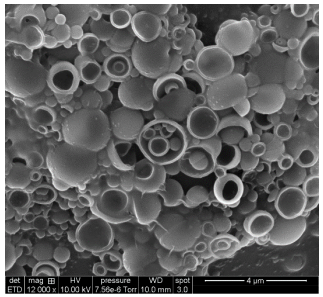


Emulsification
Encapsulation
Vectorization

Semi-industrial filling
Lyophilization

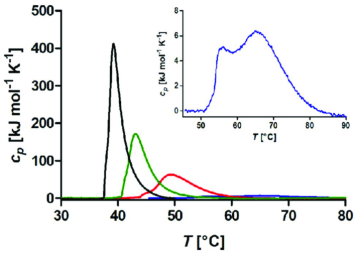
Expertises and capabilities

Mol. Eng. → Bioprod → Purif. → Formul. → **Quality** → **Eval.**



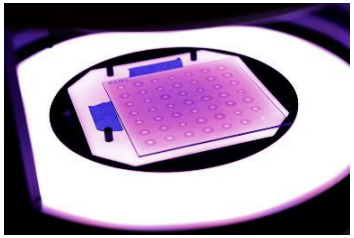
Physical characterisation of biomolecules

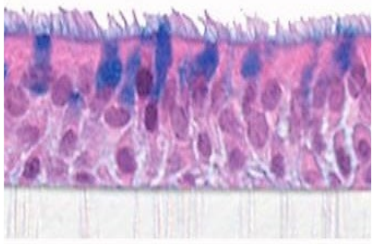
MET/MEB, Granulometrie, Calorimetrie (DSC)



Viral quantification

HA/TCID/RT PCR/ SRID





Models for evaluation

Antigens / Antiviral / Proviral

in vitro & in vivo test (Souris & Furet)

Unique selling points:

- One unique interlocutor for a global project
- Tailored-made services, R&D collaboration and tech-transfert
- Broad cutting-edge expertise in biotechnology and bioproduction
- Training and process development in state-of-the-art facilities industry oriented

Tech-transfert and training

- Industry oriented training program
- Pedagogic plateforms « Clean room »
- Upstream process
  - Cell culture (adhesion/suspension)
  - Bioproduction
- Downstream process
  - Purification
  - Formulation
  - Fill & Finish
- Viral characterization and quantification (model Influenza)

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Nanodelivery systems: From their design to their use as vaccine candidates against infectious diseases.

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Keywords
- Infectious diseases
- Nanomedicine
- Nanodelivery
- Adjuvants
- Protein vaccine
- mRNA vaccine
- Vaccine response
- Mucosal immunity
- Whole body imaging
- Administration route

Abstract
In recent years, nanodelivery systems have raised huge interest as platforms for drug delivery or vaccine development, as they offer multiple options for improving the safety and efficacy of therapeutic agents. For example, side effects might be decreased upon encapsulation of drugs or adjuvants. Although the use of nanodelivery systems has open a wide area of research in nanomedicine, it should be implemented with caution as their safety is a main issue.
We are focusing our work in this field on three aspects:
- Elaborate innovative particles using a biodegradable core made of Poly-Lactic Acid, PLA): alone they could encapsulate immune molecules (TLRs ligands) and could be coated with vaccine antigens (peptide/proteins). When covered by lipid layers, they could vehiculate DNA or mRNA for therapeutic purpose (immunotherapy with TLR3 ligand, etc) or vaccine design.
- By deciphering in vivo biodistribution and the fate of these innovative particles according to the routes of administration, we could adapt their size or charge to target specific area such as draining lymph nodes, mucosa using mice or zebrafish models and whole-body imaging tools.
- As therapeutic challenges, we focus our work on infectious diseases targeting mucosa (HIV-1 and Influenza), with a strong emphasis on designing therapeutic formulations able to cross biological barriers and induction of long-lasting immunity.

Research area
Versatility of nanodelivery systems: from their design to their use as vaccine candidates able to carry either protein moieties or mRNA or both to induce long lasting immunity

Synopsis

Biodegradable nanodelivery systems as a critical tool for designing sub units or mRNA vaccines for One Health purpose.

Interests

Non viral delivery systems;Immunology/Immunotherapies;Vaccine;Infectious diseases;In vivo models;Translational research

Nanodelivery systems:

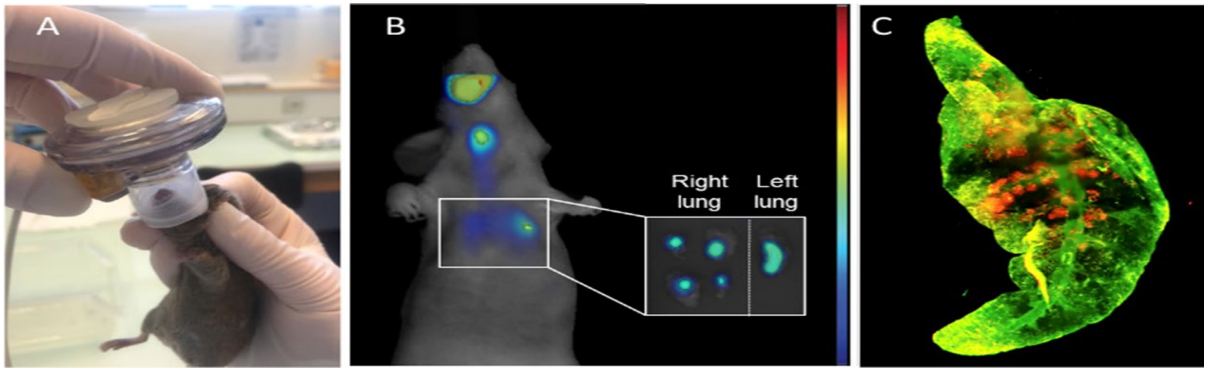
From their design to their use as vaccine candidates against infectious diseases.

Bernard VERRIER

UMR5305 Laboratory of Tissue Biology & Therapeutic Engineering
Lyon (France)

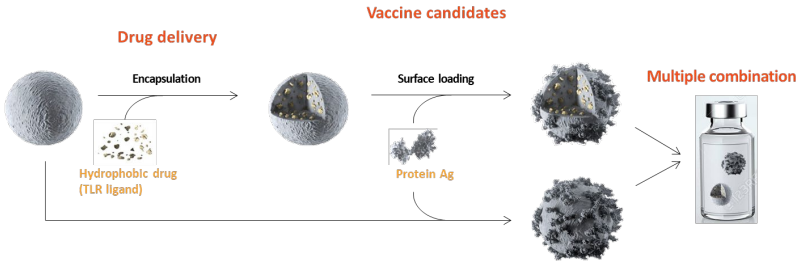
- **Objectives:**
  - Design & elaborate nanoparticles using a biodegradable core made of Poly-Lactic Acid (PLA)
  - Nanoparticles dedicated to protein or mRNA delivery
  - Therapeutic challenge: focus on infectious diseases targeting mucosa (HIV-1 and Respiratory Diseases)

- **Tools:**
  - Green chemistry and colloidal characterization of nanoparticles
  - *In silico* design of protein vaccine antigens and epitope exposure after formulation onto particles
  - Design of mRNA vaccine sequences (AI) and characterization upon formulation onto particles
  - Zebrafish and mice models to monitor the biodistribution & immune responses



Design of two versatile nanodelivery systems for protein or mRNA delivery

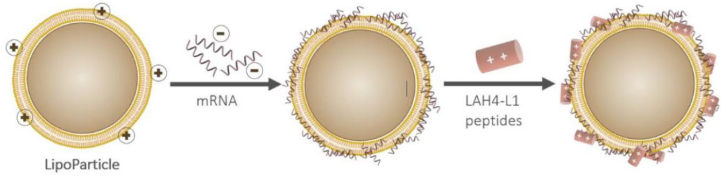
- **Protein moieties delivery:**
  - Viral mimicry by adsorbing several antigens at the surface (BcR entrapment)
  - Encapsulation of TLR ligands inside the solid core acting as adjuvants



Outline of Protein formulation process
(Lamrayah M. *et al.* 2023)

- **mRNA vaccine delivery:**
  - Identification of specific motifs to increase mRNA expression
  - Design of LipoParticles as alternative to LNPs (LipoNanoParticles)

• The LipoNanoparticle: polymeric core and lipid coating



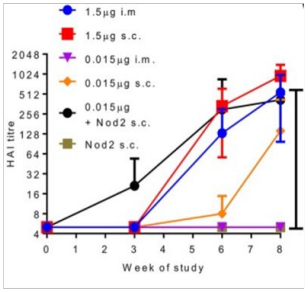
✓ Mucopenetrant
✓ Resist to the shear stress (aerosolization, nebulization…)

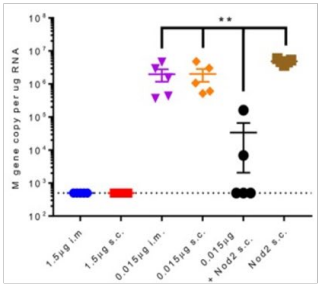
Outline of mRNA formulation process
(Ayad C. *et al*. 2021, 2023)

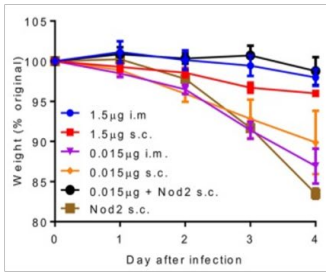
Immune responses an biodistribution studies
ex: Influenza & Sars-CoV2

- **Protein delivery**
  - Long lasting immune response
  - Use of dedicated ligands to guide quality of immune responses

PLA NanoParticles
Encapsulated NOD2 agonist: mifamurtide
Adsorbed antigen: ciliate derived rHA A/Cal (0.015 µg)

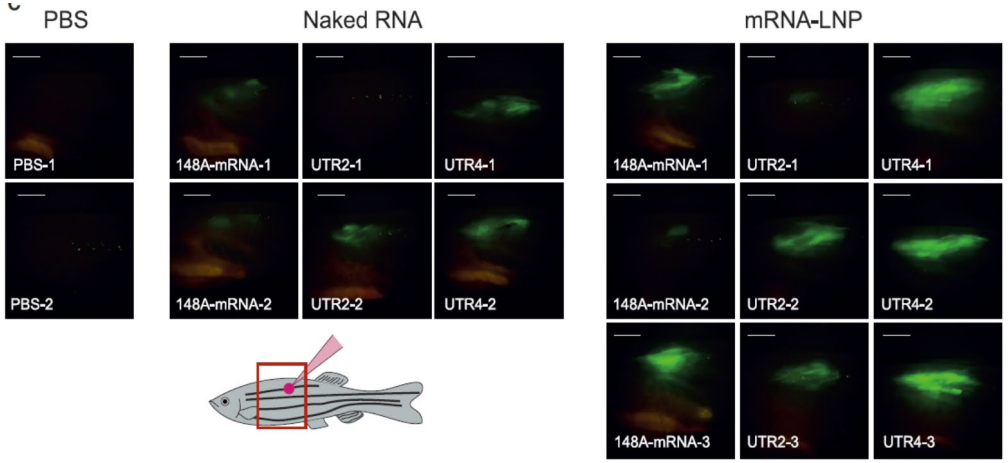






➢ **Combining rHA antigen with i-Particles(NOD2) led to similar levels of protectives antibody with much lower levels of antigen → enabled dose sparing (Jawinski et al., Front Immunol. 2019**

- **mRNA delivery**
  - Design of predictive *in vivo* mRNA expression using zebrafish as tools
  - Ongoing experiments on HIV-1, Influenza, Sars-CoV2 & RSV



Linares et al, Molecular Therapy, 2021

Perspectives:

- **Designing particles able to cross the mucus for nasal route**
  - Particles must swim in the mucus to reach targeted immune cells

- **Co-delivery of adjuvants for inducing long lasting mucosal immunity**
  - Repurposing existing immune molecules to target different pathways while diminishing their side effects

- **mRNA cancer vaccines with targeted delivery:**
  - Identifying new surface proteins appearing at the precancerous state as vaccine candidates

Safe by design nanoparticles for protein or mRNA delivery against infectious diseases

- **Proteins vaccine delivery**
  - Clustering of vaccine antigens at the surface controlled by the particle size
  - Viral mimicry
  - Protective effect in Influenza challenge model

- **mRNA vaccine delivery**
  - Use of a solid core with a lipid corona instead of pure Lipid NanoParticles
  - Encaspsulation in the solid core of any molecules with hydrophobic properties

- **Drug delivery**
  - Design of bi-or multiple immuno-therapeutic tools, by encapsulating one molecule into the core, the other ones being loaded at the surface or entrapped in the lipid bilayer

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