UNDER THE HIGH PATRONAGE OF
MR EMMANUEL MACRON
PRESIDENT OF THE FRENCH REPUBLIC

HYBRID
Health Bioscience Research Industry Days

"NEXT GENERATIONS OF GENE AND CELL THERAPIES"

ACADEMIC BOOK 2024

Under the High Patronage of
Mr Emmanuel Macron
President of the French Republic

HYBRID
Health Bioscience Research Industry Days

HYBRID : Next generations of gene & cell therapies

8&9 February 2024

PROGRAM

Day 1: 8 February 2024

8:00am Welcome coffee

8:45am Official speech **Bruno Bonnell**, General Secretary for Investment – France 2030 (SGPI)

8:55am Opening ceremony **Thomas Lombes**, Deputy CEO for strategy, Inserm, **Thierry Hulot**, Chairman of Leem, **Marc Bonneville**, Chairman of Ariis, **Rosalie Maurisse**, Head of Health - Innovation Department at Bpifrance

Session 1: 9:15am - 12:45pm
The challenges associated with future viral vectors and genome editing approaches

From the main challenges, avenues and prospects for new vectors to optimized delivery and safety, and tomorrow's genome-editing strategies

9:15am **Overview:**
Oumeya Adjali, Inserm - Gene therapy using AAV viral vectors ; from vector engineering to preclinical evaluation

9:35am **Short talks:**
- **Ana Buj-Bello**, Inserm - Next generation AAV vectors for gene therapy of muscle disorders
- **Alexis Duvergé**, CNRS - Specific Targeting of Cancer Cells by Lentiviral Vectors
- **Maria Grazia Biferi**, Spark Therapeutics - Targeting CNS with AAV in a large animal

10:10 am Questions & Answers

10:30 am Coffee break

11:05 am **Short talks:**
- **Annarita Miccio**, Inserm - Genome editing approaches for beta-hemoglobinopathies
- **Aurélie Bedel**, Bordeaux University - How to secure CRISPR-Cas9 use?
- **Stéphane Boissel**, Sparing Vision - Pioneering Genomics to Save Sight

11:40am Questions & Answers

11:55am **Round table:**
- **Sitra Tauscher-Wisniewski**, Novartis
- **Anne Douar**, Vivet Therapeutics
- **Annarita Miccio**, Inserm

12:45pm Lunch break

B2B Meetings: 1:00pm – 3:00pm

PROGRAM

Session 2: 3:00pm – 5:00pm
New nucleic acid therapies : applications, vectorization

From prophylactic to therapeutic vaccines and the various potential uses of nucleic acids. Challenges and barriers associated with the uses of nucleic acids: shelf life, route of administration, targeting, etc.

3:00pm **Overview:**

- **Chantal Pichon**, Orléans University - The game changing potential of mRNA-based vaccines and therapeutics
- **Pierre Wils**, Sanofi - From Innovation to Industrialization: Challenges in Research and Development of mRNA Vaccines

3:35pm

- **Nathalie Mignet**, CNRS - Production of lipid nanoparticles formulations for gene and cell therapy
- **Philippe Barthélémy**, Bordeaux University - Therapeutic oligonucleotides: efficient and specific tools to combat diseases

4:00pm Questions & Answers

4:15pm **Round table:**

- **Paul Nioi**, Alnylam
- **Chantal Pichon**, Inserm
- **Anette Sommer**, Pfizer
- **Hélène Tran**, Servier
- **Pierre Wils**, Sanofi

B2B Meetings: 5:00pm – 6:30pm

Cocktail reception: 6:30pm – 8:00pm

PROGRAM

Day 2: 9 February 2024

8:00am Welcome coffee

8:30am Keynotes **Cécile Martinat** & **Christophe Junot** - National research program in biotherapies and bioproduction

Session 3: 8:45am - 12:00pm
Immuno cell based therapies

How can the uses of CAR be extended beyond current applications? How can we improve delivery to solid tumours, the role of the tumour environment, survival, etc.? Applications beyond cancer.

8:45am **Overview:**

- **Tamas Shisha**, Novartis - An Open-label, Multicenter, Phase 1/2 Study to Assess Safety, Efficacy and Cellular Kinetics of YTB323, a CAR-T Cell Therapy Targeting CD19 for Severe Refractory SLE (Preliminary Results)

9:05am **Short talks:**

- **Mitra Suman**, Inserm - Optimizing Adoptive Cellular Therapies in Physio-Chemical Tumor Environments for Superior Anti-Cancer Efficacy without systemic toxicity
- **Emmanuel Donnadieu**, CNRS - Predicting efficacy and toxicity of CAR T cells using an ex vivo human model
- **Marie-Caroline Dieu-Nosjean**, Inserm - Manipulation of tertiary lymphoid structures as the third generation of immunotherapy
- **Georges Lourenço**, Mnemo Therapeutics - A new class of tumor targets for next-generation cancer immunotherapies

9:50am Questions & Answers

10:15am Coffee break

10:50am **Round table:**

- **Alessandro Crotta**, BMS
- **Viggo VanTendeloo**, J&J
- **Sante Cundari**, Kite- a Gilead company
- **Karine Rossignol**, SmartImmune
- **Emmanuel Donnadieu**, CNRS

11:40am **Keynote:**

- **Lise Alter**, Head of AIS - The missions of the Health Innovation Agency

12:00pm Lunch break

B2B Meetings: 12:00pm - 2:00pm

PROGRAM

Session 4: 2:00pm - 4:30pm
Cell therapies and beyond

From the various therapeutic applications of cell therapies to exosomes: how to develop clinical uses of cell therapies, address characterisation issues, etc.? How to take full advantage of the coupling between therapeutic effect and vectorisation properties for exoxomes.

2:00pm **Short talks:**

- **Louis Casteilla**, Toulouse University - MSC-based ATMP: present and future
- **Christelle Monville**, Evry University - A stem cell therapy pipeline with focus on retinitis pigmentosa and other rare indications with high unmet medical needs
- **Frédéric Desdouits**, Treefrog Therapeutics - Unlocking cell therapy for all
- **Christian Jorgensen**, CHU of Montpellier - Next generation cell therapies for osteoarticular diseases

2:45pm Questions & Answers

3:00pm **Overview:**

- **Philippe Menasché**, Paris Cité University - Cell Therapy of Heart Failure: Cells, Exosomes, RNAs?

3:20pm **Shot talks:**

- **Grégory Lavieu**, Inserm - Extracellular Vesicle-based vector for targeted therapeutics delivery
- **Jérémie Laurent**, Astraveus - Lakhesys, the microfluidics powered benchtop factory for cell & gene therapies to develop better products and solve scalability
- **Jérome Bonnet**, Inserm - Programming bacteria as smart therapeutics

3:50pm Questions & Answers

4:10pm **Keynote:**

- **Alain Fischer**, Professor at the College of France, President of The French Academy of Sciences

Conclusive Keynote: 4:30pm
Marc Bonneville, Scientific and medical director of Merieux Institut, President of ARIIS

B2B Meetings: 4:30pm - 6:00pm

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Gene therapy using AAV viral vectors : from vector engineering to clinical evaluation

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Laboratory TaRGeT-Translational Research in Gene Therapy
City Nantes

Keywords

Abstract

Research area
Biotherapies
Gene Therapy
Viral vectors
AAV
Preclinical development
Bioproduction process development

Synopsis
TaRGeT laboratory (INSERM UMR1089, Nantes Université) is an internationally recognized expert in the field of gene therapy using viral vectors. Since 2004, the lab has progressively focused its research on the development of recombinant Adeno-Associated Virus (rAAV)-derived vectors covering the whole chain of their development from bench to bedside. Aside its research internal programs, the lab has put together a large number of skilled expertise within three (3) technological facilities that offer either services or collaborations to academic and industrial players in the field of gene therapy: (i) Translational vector core (CPV for "Centre de Production des Vecteurs" created in 1997 and dedicated to process and analytics developments in the field of AAV manufacturing. It is one, among the 8 integrators of the french national biotherapy acceleration strategy (FRANCE 2030); (ii) PAC for Preclinical Analytics Core, created in 2017 and expert in preclinical evaluation of in vivo gene therapy strategies in in vitro and in vivo models and (iii) GTI for Gene Therapy Immunology core, also created in 2017 and specialized in the monitoring of host immune responses against gene therapy products in preclinical and phase I/II/II/III gene therapy clinical trials.
Since 2004, the whole laboratory activities (both research teams and technological cores) are performed under a quality management system that is approved by Lloyd's Register Quality Assurance LRQA to meet requirements of international Management System Standards ISO 9001:2015.
For more info : https://umr1089.univ-nantes.fr/

Interests
Gene therapy;Gene editing;Viral vectors;Ophtalmology;Neuromuscular disorders;Rare diseases;Toxicology/Immune tolerance;In vitro models/ Organ-on-chip;In vivo models;Translational research;Bioproduction

No results presentation available

THERAPEUTIC OLIGONUCLEOTIDES

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Keywords
- Nucleic acids,
- RNAi,
- Oligonucleotides,
- Bioconjugates,
- Delivery,
- Antisenses

Abstract
Globally, ARNA pursue four main objectives: i) understand and decipher several key processes involved in gene expression at the molecular level; ii) interfere with these processes by the design, the synthesis and the delivery of oligonucleotides and its derivatives; iii) invent and develop cutting-edge methodologies in structural and molecular biology to study and characterize nucleic acid polymers; and iv) develop novel synthetic nucleic acid based bioconjugates for applications in medicinal chemistry.
Of note, the ARNA lab features a platform supported by INSERM TRANSFERT named “Optoligo”, which is dedicated to the design, the synthesis and characterization of modified oligonucleotides. It aims at developing innovative bioconjugates in order to increase their, stability, cellular internalization and therapeutic efficacy. For example, we have demonstrated that our modifications allow the delivery of the oligonucleotides without using any transfecting agents in different human cells lines. As part of the development of the “Optoligo hub”, our patented technologies have been applied to the modulation of protein expression in various pathologies and issues, including cancers, antibiotic resistance, allergies, rare diseases, chronic kidney diseases, neurodegenerative diseases, and several collaborative projects are currently under investigation. One of the main objectives of this platform is to bring these oligonucleotide technologies to clinical trials.

Research area
The ARNA unit is an interdisciplinary unit dedicated to the exploration of nucleic acids chemistry (DNA, RNA) in its widest sense. The unit combines world-class expertise in chemical synthesis, structural analysis of biological systems, and applications in both cell biology and medicinal therapy. ARNA is unique in its competence at a European level, and globally there are few units that could match ARNA’s excellence in expertise.

Synopsis
We are developing nucleic acid conjugates allowing the cellular internalization of therapeutic oligonucleotides modulating the expression of relevant genes. Our patented technologies

have been applied to the modulation of the protein expression in various pathologies and several collaborative projects are currently under investigation.

Interests

Non viral delivery systems;Oligonucleotides;Oncology;Rare diseases;Aging;Infectious diseases;In vitro models/ Organ-on-chip

THERAPEUTIC OLIGONUCLEOTIDES

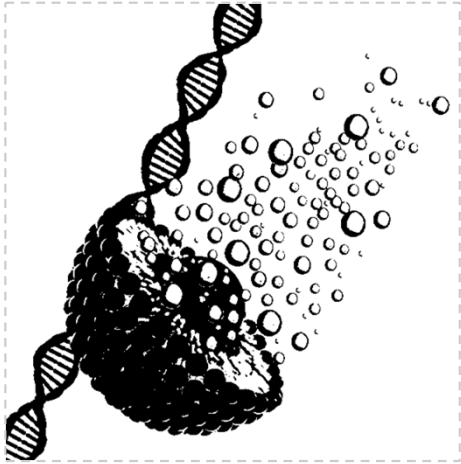
Philippe BARTHELEMY

ARNA, Bordeaux

- **Objectives:**
  - To design oligonucleotide conjugates
  - To demonstrate RNAi biological activities both in vitro and in vivo
  - To implement novel therapeutic oligonucleotides as new chemical entities (NCE) in oncology, rare diseases, neurodegenerative diseases…

- **Tools:**
  - Sequence selection
  - Innovative chemical modifications
  - Synthesis of novel oligonucleotide conjugates
  - Purification and characterization, Mass spectrometry, DSC, HPLC etc
  - Intra cellular delivery of oligonucleotides
  - Functional in vitro models (cancer cell lines)
  - Technology is compatible with a wide range of deseases:

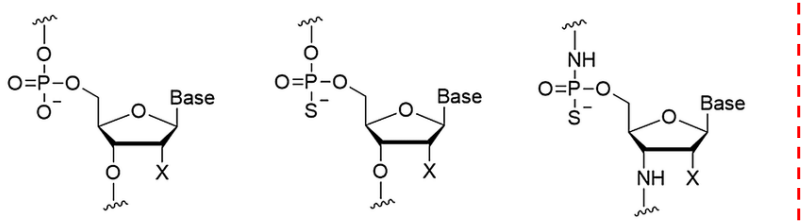
  Cancers, Rare diseases, Neurodegenerative diseases, Antibiotic resistance etc
  - Leading the Hub Optoligo (Inserm Transfert)

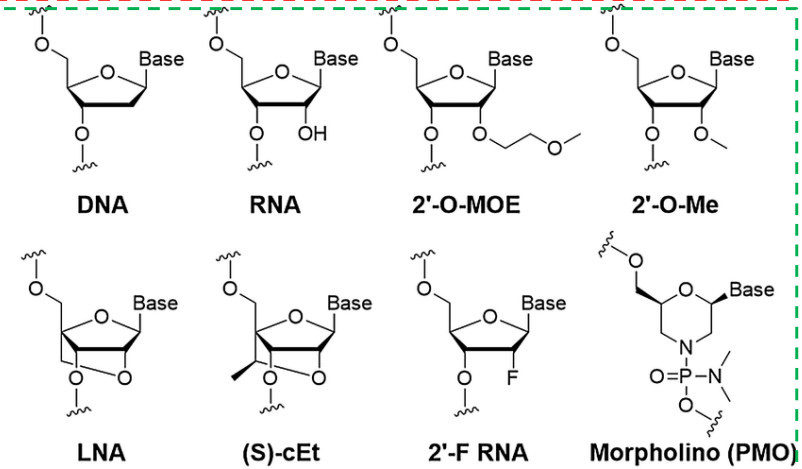


Modified Oligonucleotides

- **Results:**
  - Design of oligonucleotide sequences specific to a human gene.
  - Control of the mechanism of action *via* chemical modification (RNAse H, Steric blockers, RNAi…). Selection of the adapted oligonucleotides (SSO, ASO, Gapmers, Mixmers etc)
  - Integration of chemical modifications: Phosphorothiate (PTO), MOE, OMe, to stabilize the sequence
  - Chemical modifications to improve delivery, stability and efficay

Backbone modification
- *Nuclease stability*
- *Pharmacokinetics/biodistribution*



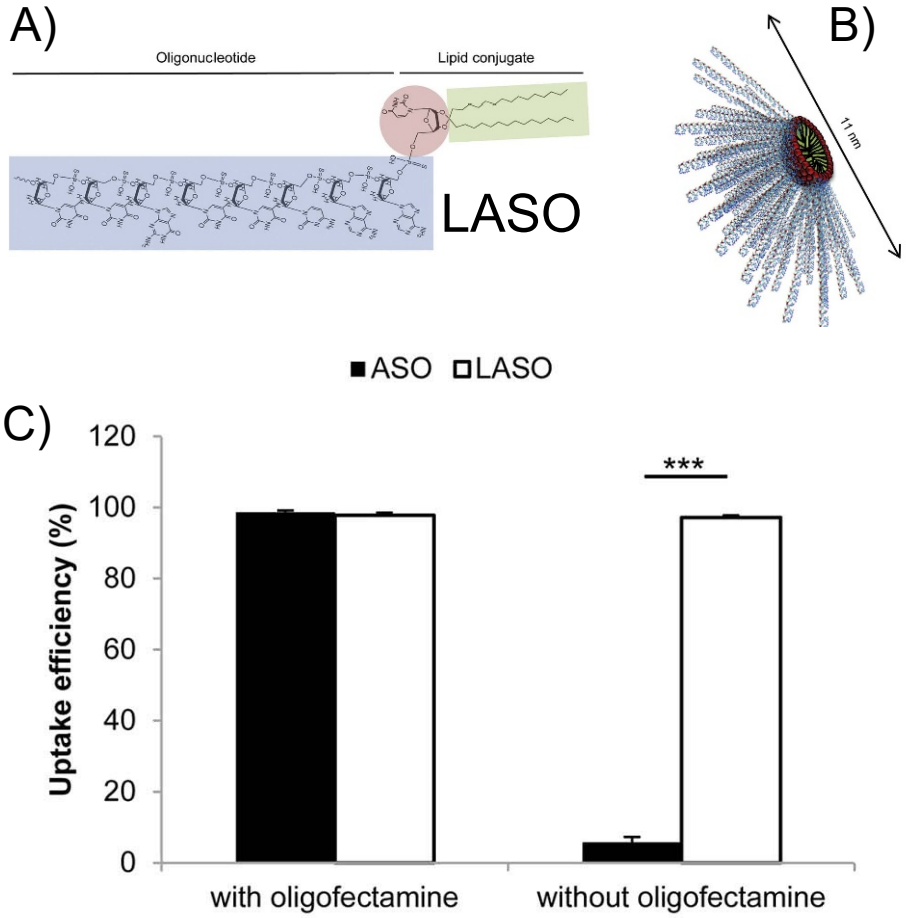


Sugar modification
- Nuclease stability
- Hybridization affinity

Adapted from Benizri, S.; Gissot, A.; Martin, A.; Vialet, B.; Grinstaff, M. W.; Barthélémy, P. (2019) Bioconjugated Oligonucleotides: Recent Developments and Therapeutic Applications. Bioconj. Chem., doi: 10.1021/acs.bioconjchem.8b00761.

Use of oligonucleotide conjugates for the delivery of antisense

- **Results:**
  - Conjugation of oligonucleotides with nucleolipids (A) induced the formation of micellar systems (B) allowing internalization into human cells (C).
  - This patented technology has been applied to the modulation of the protein expression in various pathologies and several collaborative projects are currently under investigation. Our innovative national platform is based on more than 15 years of research and development and is currently supported by INSERM TRANSFERT.
  - These projects take advantage of our expertise in the field of modified oligonucleotides, including the design and synthesis of all oligo types (DNA, ASO, SSO, siRNA, Aptamer, PMO, etc.) with or without conjugation.



The conjugates (Lipd antisense, LASO, A) are rapidly internalized into cells (C).
Flow cytometry was used to quantify the percentage of cells transfected with fluorescent oligonucleotides, 4 h after transfection with oligofectamine (positive control, left) and without (right).
In the absence of transfection agent (right) internalization into the cells is efficient for conjugates (100%) while unmodified oligonucleotides are little or not internalized (< 7%).

- **Perspectives:**

  - Determination of PKs and biodistribution profiles of oligonucelotide conjugates
  - Preclinical and clinical development of NCE based oligonucleotide
  - Applying oligonucleotides to personalized medicine

Unique selling points

Our solution enhances oligonucleotides performances:

- Efficacy
- Stability
- Lack of toxicity
- Biodegradability
- Targeting

Key benefits

- Cellular uptake
- Stability of sequences
- New Chemical Entities
- Bio-inspired and fully biodegradable
- Possibility to add targeting molecules
- Possibility to trigger controlled releases
- API for Nano-formulation (co-delivery)
- Strong intellectual property position

Our technology is compatible with a wide range of diseases:

- Cancers
- Rare diseases
- Neuro
- Antibiotic resistance

Selected bibliography

Publications (exempla):

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- Derre A, Soler N, Billoux V, Benizri S, Vialet B, Rivat C, Barthélémy P, Carroll P, Pattyn A, Venteo S. FXYD2 antisense oligonucleotide provides an efficient approach for long-lasting relief of chronic peripheral pain. **JCI Insight.** (2023).
- Kassahun GS, Farias ED, Benizri S, Mortier C, Gaubert A, Salinas G, Garrigue P, Kuhn A, Zigah D, Barthélémy P. Electro-polymerizable Thiophene-Oligonucleotides for Electrode Functionalization. **ACS Applied Materials & Interfaces** (2022).
- Nguyen PV, Aubry C, Boudaoud N, Gaubert A, Langlois M-H, Marchivie M, Gaudin K, Arpin C, Barthélémy P, Kauss T. Oligonucleotide Solid Nucleolipid Nanoparticles against Antibiotic Resistance of ESBL-Producing Bacteria. **Pharmaceutics** (2022).

Patents (exempla):

- Barthélémy P, Zigah D, Kuhn A, Kassahun GS, Gaubert A, Salinas G, Benizri S, Farias ED "Thiophene-modified oligonucleotides and their use in a process for preparing functionalized electrodes" **WO/2023/198914**.
- Kauss T, Arpin C, Barthélémy P. "Oligonucleotide solid nucleolipid nanoparticles for tackling antibiotic resistance." **WO2023135299A1**.
- Rocchi P, Camplo M, Barthélémy P, Ziouziou H, Siri O, Paris C. "Nanoparticles comprising a core with a phenazine derivative and a shell with a nucleolipid and uses thereof." **WO2022194926A1**.
- Kauss T, Arpin C, Nguyen PV, Barthélémy P. "Lipid oligonucleotide antisense against antibiotic resistance." **WO2020225371A1**.
- Venteo S, Carroll P, Benizri S, Barthélémy P, Pattyn A "Antisense oligonucleotides and their use for the treatment of pain." **WO2021069654A1**.

Expertise in large ON-target CRISPR-mediated genotoxicity and its prevention

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Keywords
- Gene therapy,
- Gene editing,
- CRISPR-Cas9,
- Genotoxicity,
- Safety,
- Palbociclib

Abstract
The CRISPR-Cas9 system has revolutionized our ability to precisely modify the genome. Gene editing is classically used to invalidate genes, to model or correct mutations. Clinical trials using this technology are already on going to treat monogenic diseases and cancers and are still using nuclease to invalidate genes in most of cases. However, this powerful tool can induce adverse genomic events. The off-target genotoxicity is well described, predictable, detectable, and can be resolved using new generations of Cas9 nucleases with high fidelity. In contrast, the ON-target genotoxicity due to a DNA double-strand break at the targeted locus is still underestimated. Comprehensive analysis of gene editing products at the targeted cut-site has revealed a complex spectrum of outcomes by incorrect or ineffective DNA repair and DNA damage response.

Large ON-target genotoxicity (chromosomal rearrangements) is underestimated with standard PCR-based methods and necessitates appropriate and more sensitive detection methods. Using cytogenetic approach after cell cloning (FISH, CGH-array, SNP-aray), we discovered the appearance of megabase-scale rearrangements in cell lines and in primary cells. We showed that LOH frequency depends on p53 status and cell division rate during editing. Importantly, G1-cell cycle arrest (by CDK 4/6 inhibitors, such as palbociclib) during editing suppresses the occurrence of LOH without compromising editing. These data were confirmed in human stem/progenitor cells, suggesting that clinical trials should consider p53 status and cell proliferation rate during editing to limit this risk by designing safer protocols.

When precise genome editing is required, the more prevalent ON-target unwanted events are InDels. To reduce Indels and improve homology-directed repair (HDR), we developed optimized CRISPR tools (nuclease and nickase), with template import at the targeted DNA cut site, “In the right place at the right time”. We obtained a 4-fold to a 30-fold increase of HDR with nuclease (DSB) or nickase (SSB), respectively. These data highlight the possibility to use SSB-mediated editing as an alternative to nuclease to prevent DSB genotoxicity.

Cell cycle blockade by CDK4/6 inhibitors and optimized nickase could offer opportunities to make CRISPR-based gene therapy protocols safer.

Research area
Biotherapies for genetic diseases and cancers

Synopsis
Expertise in large ON-target CRISPR-mediated genotoxicity and its prevention.

Interests
Gene therapy;Gene editing;Toxicology/Immune tolerance

Expertise in large ON-target CRISPR-mediated genotoxicity and its prevention.

Aurélie BEDEL
PharmD, PhD

INSERM U1312, Bordeaux University, CHU BORDEAUX
BioGO team (Pr Moreau-Gaudry/Pr Dabernat)

- **Objectives:**

CRISPR-Cas-mediated ON-target genotoxicity

- Decipher
- Understand the cell vulnerability
- Prevent
  - pharmacological approach to modulate cell susceptibility
  - molecular approaches to improve CRISPR tools

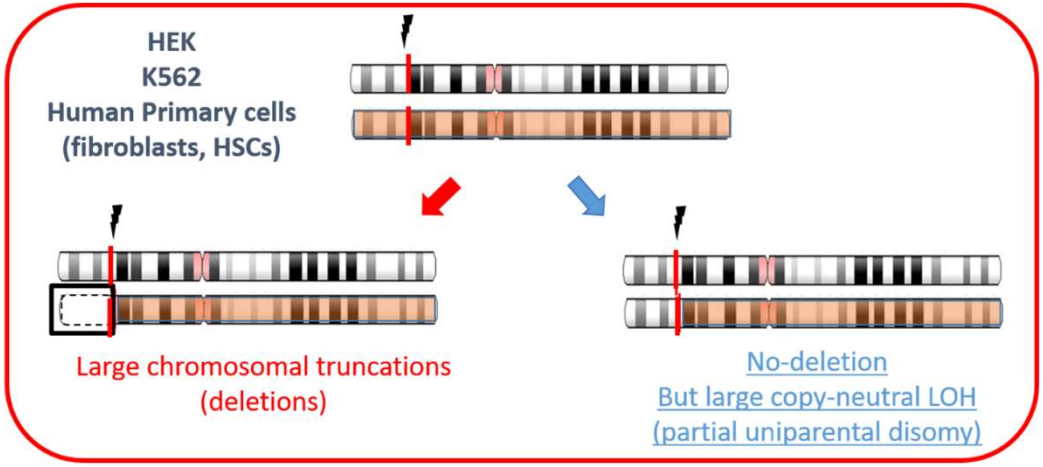
- **Tools:**
  - Molecular biology (PCR, sequencing, CGH-array, SNP-array, Bionano)
  - Sensitive LOH detection by cytometry (In-house FAMReD systems)
  - Molecular characterization of chromosomal rearrangements
  - Single cell DNA
  - Scientific expert for PEPR-Biotherapies et Bioproduction des thérapies innovantes
  - Member of SFTCG council

Subject 1:Large ON-target genotoxicty description

ON-target genotoxicity (unwanted events at the targeted locus) is still underestimated

Results:

- **Gene editing products at the targeted cut-site revealed a complex spectrum of outcomes by incorrect or ineffective DNA repair and DNA damage response.**
- **Discover of the appearance of megabase-scale rearrangements (LOH) in cell lines and in primary cells.**



Chromosomal rearrangements induced by CRISPR-DSB

Subject 2: Prevention of ON-target genotoxicity

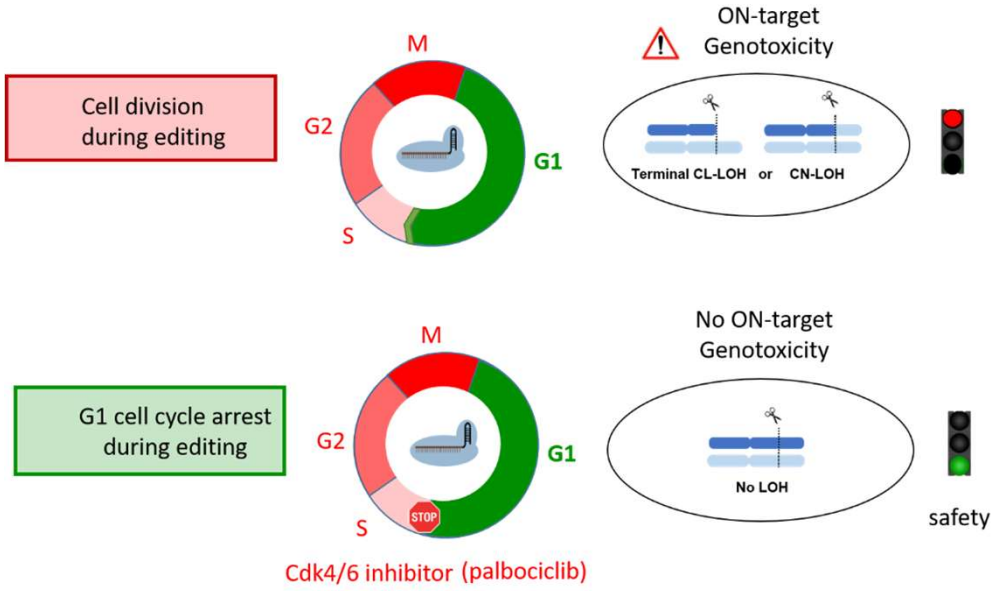
Knowledge and understanding of the mechanisms involved in ON-target genotoxicity allowed us to develop innovative solutions to prevent/mitigate this risk

- **Results:**
  - Improved CRISPR
    - Allele-specific gRNA
    - Import-CRISPR

    => To avoid INDELS
  - CDK4/6 inhibitors (Palbociclib)

⇒ to control cell cycle phase during editing

⇒ To avoid large chromosomal rearrangements



Cell cycle control drastically reduces ON-target genotoxicity for safer CRISPR-protocols

- **Perspectives:**
  - To validate *in vitro* and *in vivo* palbociclib use in edited HSCs for its use in clinical protocols
  - To validate Import CRISPR systems efficiency in different loci, in different primary cell types.
  - To propose Import CRISPR systems to private companies and academics
  - To develop quality control of edited cells
  - To develop safer clinical protocols

- **Unique selling points**
  - Biosafety management of CRISPR-Cas9 tools
  - International leader in genotoxicity understanding and preventing (Several publications in Top one-percent in life sciences journals)
  - Description of link between DSB-genotocivity and cell cycle
  - Palbociclib patent
  - Import-CRISPR patent in progress

Selected bibliography

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From therapeutic discovery to clinical translation

of advanced cancer cell therapies based on pluripotent stem cells

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Keywords
Induced Pluripotent Stem Cells, Genome editing, Bioproduction, Immunotherapy, Cancer Stem Cells, leukemia, oncology, 3D-Organoid, Biomarkers, Stem Cell Biotechnology

Abstract
Induced Pluripotent Stem cells are the game changer for next generation therapies. Our translational research focuses on malignant and therapeutic stem cell models with the discovery of biomarkers and novel targets enables the development of innovative immunotherapies against difficult-to-treat tumors. Using bioengineered Induced Pluripotent Stem Cells and precision medicine approach, we improve the immune response against resistant cancer stem cells. We are developing cancer vaccine and CAR therapies from engineered iNK, iMacrophages designed for the treatment of AML, glioblastoma and non-small-cell-lung cancer. Our Inserm Unit is paving the way for the clinical application of these disruptive therapies through the spinoff company IPSirius and close collaborations with oncologists, biotechnology companies or pharmaceutical companies.

Research area
From therapeutic discovery to clinical translation of disruptive cancer cell therapies based on pluripotent stem cells

Synopsis
Clinical translation of advanced Cell & Gene therapies and enable market access

Interests
Gene editing;Cell Therapy;Stem cells;Oncology;Immunology/Immunotherapies;In vitro models/ Organ-on-chip;Biomarkers;Bioproduction

oration of human primary B cells through sophisticated in vitro culture models replicating the intricate stages of B cell differentiation into plasma cells, our team is unraveling distinctive molecular pathways and factors potentially co-opted in the context of malignant lymphoproliferative diseases.

Interests
Non viral delivery systems;Oligonucleotides;mRNA;Oncology;Haematology;In vitro models/ Organ-on-chip;Translational research;Clinical research

From therapeutic discovery to clinical translation of advanced cancer cell therapies based on pluripotent stem cells

Annelise Bennaceur Griscelli

CITHERA 'Center for iPS Cell Therapy
UMS45/ UMRS 1310

- **Objectives:**

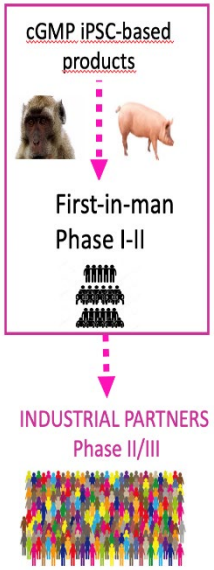
To develop breakthrough immune cell therapy in oncology and its translation into early stage clinical trials

To produce cGMP and universal IPS cells

To produce off-the-shelf scalable allogeneic immune cell therapy (CAR therapies and Cancer vaccine)

To modeling human cancer by 3D-organoids derived from patient-specific iPSCs

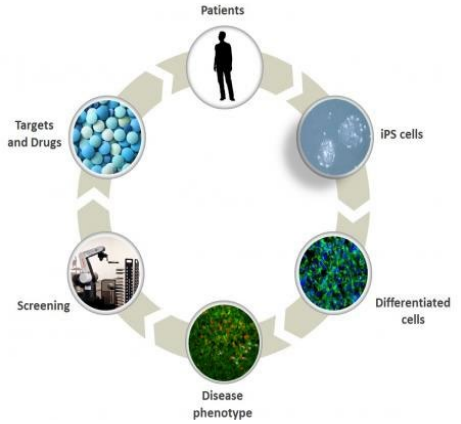
- **Tools:**
  - Reprogramming technology and genome editing
  - Patient-specific iPS cells and gene edited clones.
  - 3D-cancer organoid derived from iPSC (lung, breast, kidney, brain..)
  - NK cells, Macrophages, DC, T cells, MSC and Hematopoietic cells from iPSC
  - Advanced analytics & cutting-edge technologies for risk assesments
  - Suitable assays and test release: Functional potency assays and in vivo animal models



Topic 1 Pre-clinical iPSC-derived organoid models : target discovery for CAR Therapy

- **Results:**

Cancer-specific iPSC enable discovery of biomarkers and related cancer targets, based on bioinformatics and precision medicine approach.

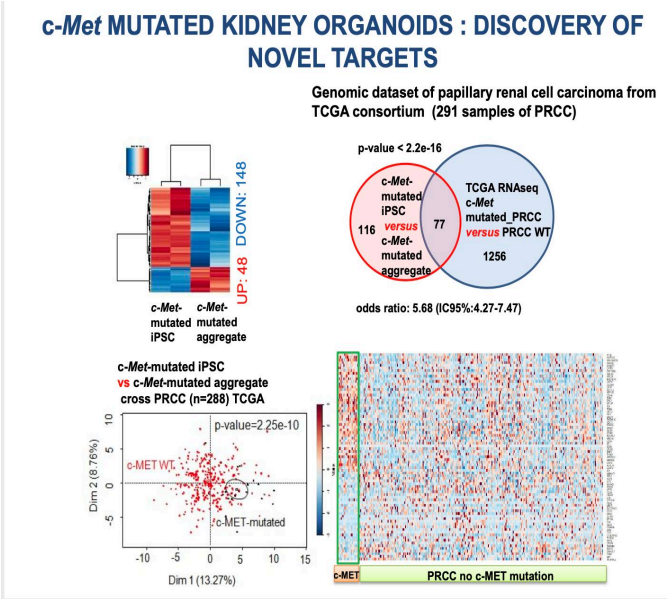


Proof of concept of the use of iPSC-derived cancer organoids:

- IPSC-derived organoids with oncogenic mutation as predisposition model of hereditary cancer and somatic cancer (Lung cancer, GBM, Breast cancer, Adenopancreatic cancer, papillary renal cell carcinoma type 1, AML .)

-Genomic data set, molecular landscape of cancer stem cells and novel biomarkers

-Identification of new targets for CAR Therapy



Topic 2: iPSCELL Platform for production of breakthrough iPS-Cell based therapies

- **Results:**

Manufacturing pharmaceutical grade iPSCs

-Collection of iPSC research grade and cGMP lines

- Genomic Data

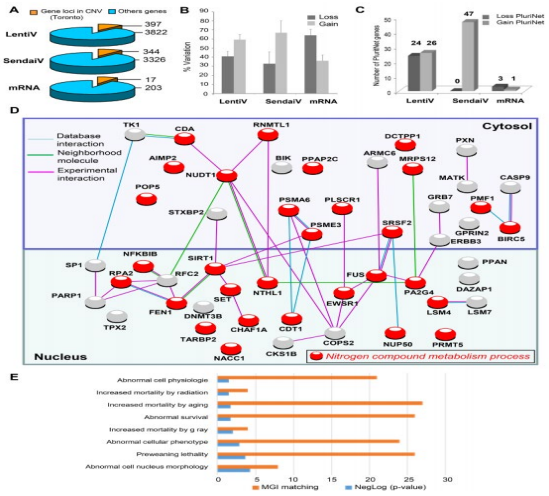
- Strategy of universal IPS cells (Hypo-immunogeneic)

Genetically engineered iPSC-based therapy

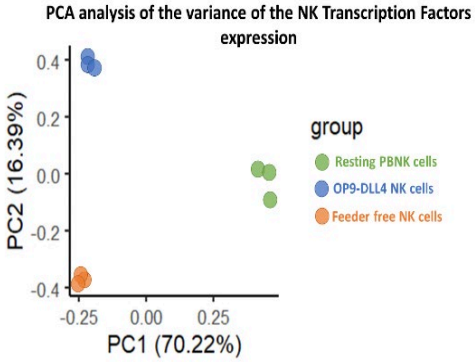
CAR-activated NK cells, macrophages and DC to serve multiple therapeutic approaches in oncology.

- Adaptation of RNA and gene editing to improve iNK iMAC and iDC efficiency

- Transcriptional analysis, metabolic, and functional properties of iNK, iMAC and iDC.



Genomic landscape of human iPSC



PCA analysis of the variance of the most significant transcription factors (n=164) of iPSC-derived NK cells compared to resting PBNK cells

- **Perspectives:**

To Translate innovative products to early-phase studies:

Improving differentiation and production of iPSC-CAR derived Immune cells

Providing genomic profiling, metabolic and functional assays of non exhausted and persistent CAR-iNK, iMAC and iDC.

Bioproduction scalable process

Potency assays and surrogate biomarkers to monitore FIH cell therapy

Industrial partnerships

- **Unique selling points**

GMP industrial transfer and technological innovation capacity to deliver sustainable growth and valuation :

CITHERA provide integrated cGMP platforms of iPSC production, expansion, differentiation and 3D engineering for next-generation immune cell therapy

-Leading the National Infrastructure INGESTEM (PIA4)

- Involved in PEPR France 2030 'Biotherapy and Bioproduction' program: IPSC-FRANCE

- Award of the EIC Pathfinder Cell &Gene Therapy Challenges

- Build partnership with SME/ Industry

Wordwide logistic International & European Networks:

- Board Director of Global Alliance for IPSC Therapy – facilitate the therapeutic use of clinical-grade iPSCs for the benefit of patients www.global.gait

- EU Cost Action Haplo-IPSC : cGMP IPSC generation from Haplo-selected UCB

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Targeting monocytes to harness by genetic engineering the power of myeloid cells to promote anti-tumor immunity

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Keywords
- Cell-based therapy
- CAR myeloid
- Solid tumors and metastasis
- myeloid payload
- innate immunity pathway
- anti-tumor immune response
- phagocytosis of tumor cells
- Cytokine secretion
- Macrophage polarization

Abstract
There is still an unmet need to treat solid tumors and metastasis. This is particularly the case for tumors poorly infiltrated by immune cells (immune desert). Our project, based on the engineering of autologous myeloid cells, takes advantage of their numerous functionalities. Solid tumors and metastasis continuously recruit monocytes which, after differentiation into macrophages, will contribute to the establishment of an immunosuppressive microenvironment. Our approach consists of purifying monocytes from the circulating blood of patients, modifying them by means of lentiviral vectors that code for receptors (CARs) that we have patented, so that once reinjected, these monocytes can be recruited by tumors and become activated upon contact with them. The activated cells then become capable of literally eating the tumor cells and initiating a tumor-specific inflammatory and immune response that will lead to tumor elimination. We have filed 3 patent applications based on our results obtained in vitro and in a preclinical model. Other more sophisticated strategies are under study. The Institut Curie's TTO is supporting this project.

Research area
Head of the Myeloid cells and Immunity team

Synopsis
Targeting monocytes to harness by genetic engineering the power of myeloid cells to promote immunity against solid tumors.

Interests
Cell Therapy;Chimeric Antigen Receptor (CAR)-T cells;Viral vectors;Genetic engineering;Oncology;Immunology/Immunotherapies;Translational research

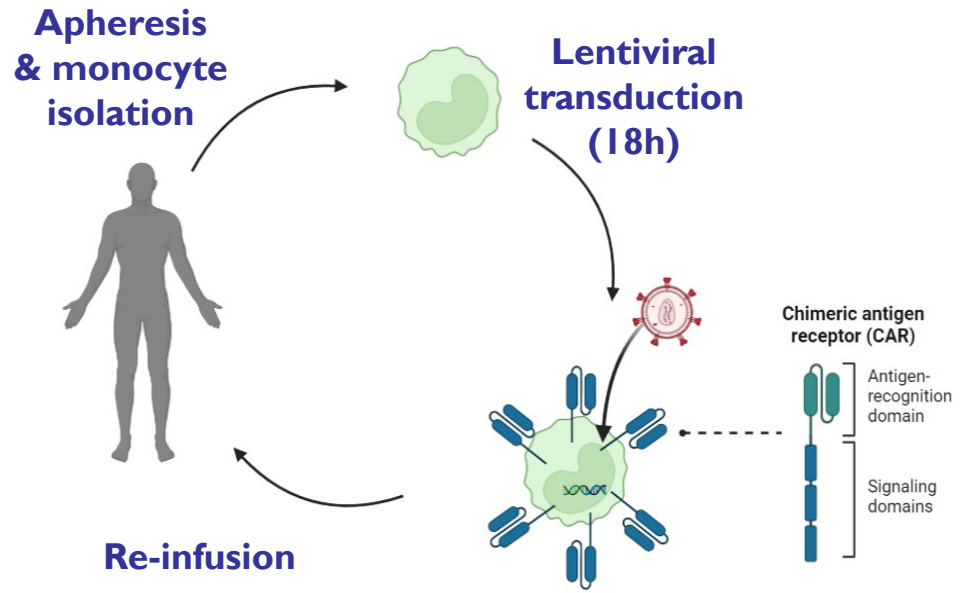
Targeting monocytes to harness by genetic engineering the power of myeloid cells to promote anti-tumor immunity

Philippe BENAROCH

Immunotherapy Cancer Center – Institut Curie – Paris
Inserm U932 "Immunity and Cancer"
Team "Myeloid cells and immunity team"

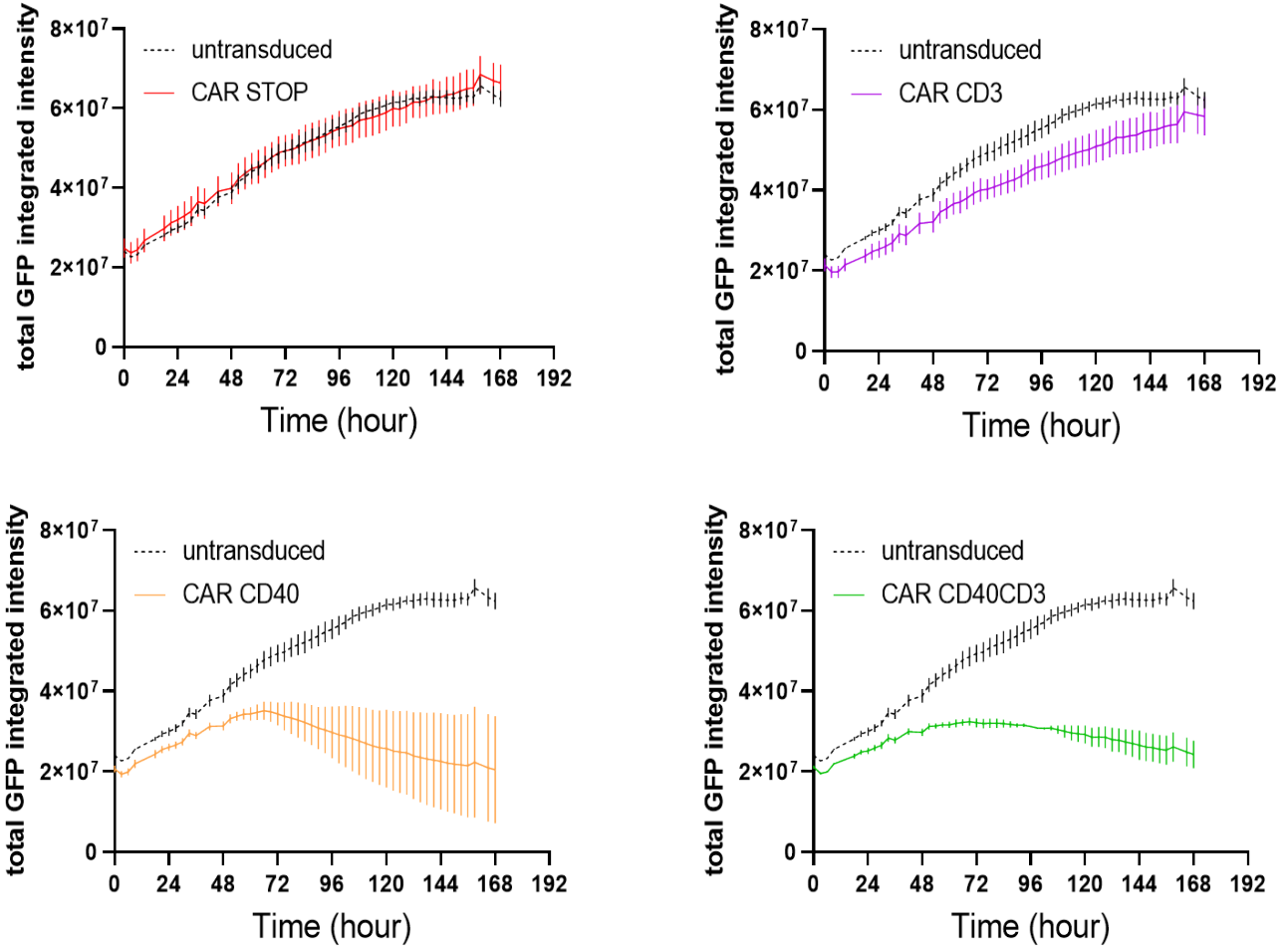
- **Objectives:**
  - To mobilize innate immunity pathways in monocytes at the tumor site to reprogram the TME
  - To develop monocyte-based therapy for a local action on the TME within solid tumors and metastasis and generate an efficient anti-tumor response.
  - To ensure spatio-temporal delivery of appropriate polarization signals to modify the TME

- **Tools:**
  - Fast and efficient process to genetically engineering autologous human monocytes to be reinfused
  - Original panel of CAR-M constructs able to mobilize innate immune pathways leading to phagocytosis, cytokine secretion, and Ag presentation
  - Original vectors able to generate monocytes carrying a CAR-dependent payload that can be used to integrate innovative therapies
  - Set of original functional assays such as a 3D platform to select the best CAR-expressing and functioning myeloid cells



Subject 1: CAR-M control cancer cell proliferation in spheroid models

- **Results:**
  - Our CAR monocytes differentiated into macrophages in vitro can control the growth of tumor spheroids
  - The capacity of the CAR to control tumor growth is linked to the presence of CD40 intracellular domain

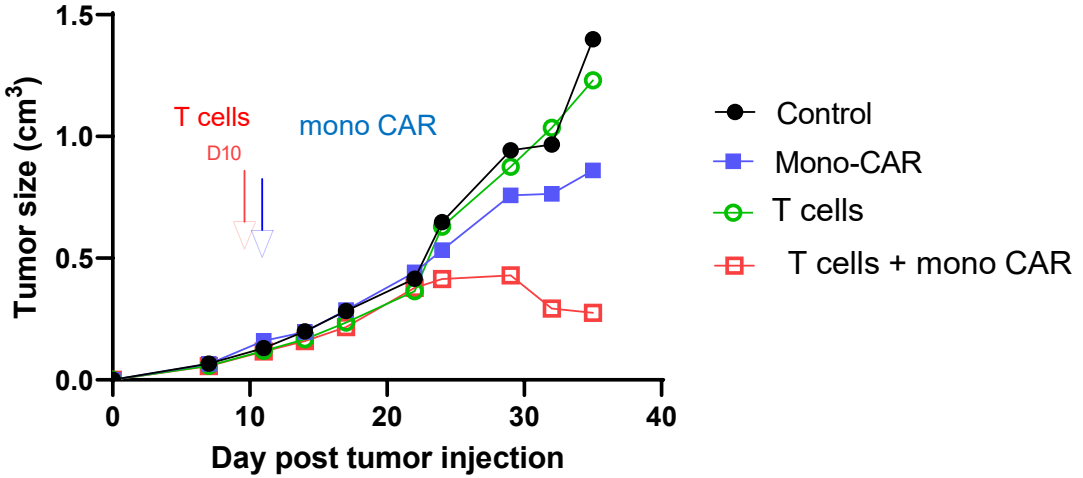


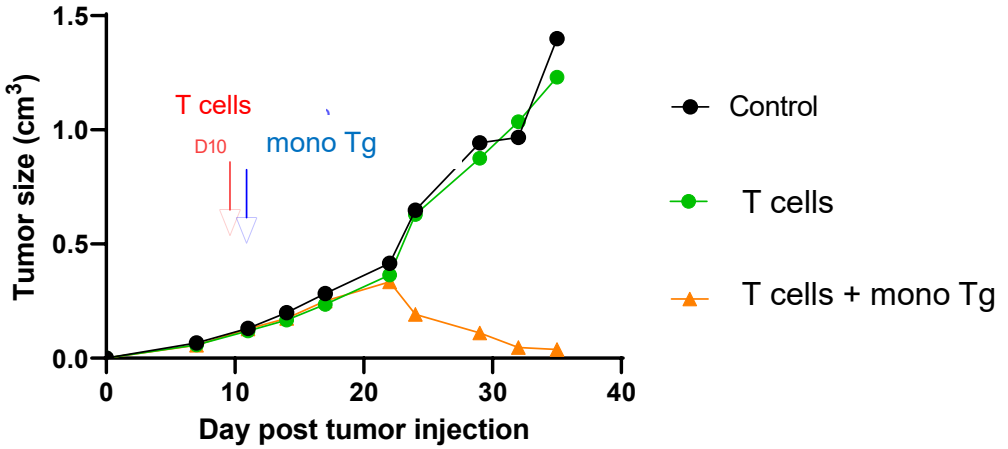
IncuCyte-based spheroid growth assay of breast cancer TNBC GFP+CD19+ cells co-cultured with the indicated CAR-macrophages. GFP intensity was followed by time-lapse microscopy. The black lines correspond to co-cultures with untransduced Mφ

Similar results have been obtained using a lung adenocarcinoma cell line

Subject 2: Preclinical validation in in vivo models of CAR-M strategies

- **Results:**
  - Car Mono can promote tumor growth control *in vivo* in cooperation with T lymphocytes (allogenic response)
  - Mono Tg were engineered to release a transgenic cytokine which can boost the allogenic response against the tumor
  - Mono can be used as a shuttle for cytokine or other proteins delivery into the TME





Model: Allogenic human breast tumor cells grafted s.c. in NSG mice

- **Perspectives:**
  - Deciphering the mechanisms of action of CAR-M
  - Optimization for selection of the best CAR constructs
  - Testing and selection of the best payload
  - Validation in preclinical models of the best indication
  - Streamlining the process of production

Unique selling points

- Harnessing autologous monocyte potential to be recruited by tumors by fast and efficient transduction
- CAR-Mono ensure spatio-temporal delivery of appropriate polarization signals to modify the TME
- Upon Ag stimulation, CAR-Mono mobilize several activation pathways to elicit efficient polarization and anti-tumor response
- Our proprietary 3D platform of functional assays allows for efficient in vitro screening of CARs
- Our next generation CAR technology offers the possibility to integrate innovative therapies
- 3 patent applications, expertise in immunity and myeloid cells.
- Unique infrastructure and know-how at Institut Curie.

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Pre-clinical development of cell culture processes and transfer to GMP production

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Keywords
- Bioreactors (2D and 3D cultures)
- Bioproduction optimization and modelling
- Human cells: Human Mesenchymal stromal cells, Human Immune cells
- CHO cells
- Monoclonal antibodies
- Online monitoring
- ATMPs
- GMP compliant
- Immunomagnetic selection
- Clinical trials

Abstract
Eric Olmos and Danièle Bensoussan are co-responsible for the Integrator Biotherapy Bioproduction MTInov in Nancy.
E. Olmos is a professor in bioproduction engineering at Lorraine University (Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires). His research activities include culture of animal and human cells in bioreactors with special focus on bioprocess modelling, simulation of hydrodynamics and cell kinetics, bioreactor scale-up and scale-down, bioprocess monitoring and performance intensification. Much effort has been put on the design of new MSC production processes in continuous perfused mode, on microcarriers. In the future, the main perspectives of MSC production engineering will concern the modulation of the secretome production and the impact of the expansion process on the phenotypical properties of MSCs.

Professor of Biotherapy and Immunology at Lorraine University, D. Bensoussan is a French biologist head of the department of Cell therapy, tissue and cord blood banking (UTCT) in Nancy University Hospital. In this unit, different ATMPs are generated in the context of clinical trials (MSCs, tri-virus VSTs) or clinical use (VSTs). She is co-head of a research team in a CNRS unit 7365: Cellular Engineering, Immunotherapy and Translational approaches. She is the co-founder and the scientific advisor of the start-up StemInov, promoting the patent filled on the research work she supervised on MSCs from Wharton's Jelly and Sepsis.

Research area
Bioprocess engineering, bioproduction
Animal and human cell culture in bioreactors
Fermentation and enzymes

Bioprocess modelling (CFD/kinetics)
Production of Advanced Therapy Medicinal Products (ATMPs) under Hospital exemption and Experimental ATMPs: Mesenchymal stromal cells (MSCs), Virus specific T cells (VSTs)

Synopsis
Today MTInov is the main academic team in France developing (animal and human) cell culture engineering in bioreactors, with special focus on process monitoring and control, intensification and optimization and also the only academic structure offering cell-based production from lab-scale to GMP production.

Interests
Cell Therapy;Viral vectors;Stem cells;Immunology/Immunotherapies;Infectious diseases;Process monitoring;Translational research;Clinical research;Bioproduction

Pre-clinical development of cell culture processes and transfer to GMP production.

Pr. Danièle Bensoussan (UTCT – CHRU – Nancy) &
Pr. Eric OLMOS (LRGP – Université de Lorraine – CNRS)

MTINOV
LARGE SCALE HUMAN CELL PRODUCTION

Objectives

- ***Human cell banking and 2D-3D expansion***
  - *Human Mesenchymal Stromal Cells (MCSs) extracted from umbilical cord (UC).*
  - *Expansion in culture flasks and bioreactors (from Ambr250 to 5 L)*
- ***Process scale-up and optimization***
  - *Bioprocess engineering (mode of cultures, intensification)*
  - *On-line monitoring and retro-control*
  - *Kinetic modelling, bioreactor scale-up*
  - *CFD simulation of bioreactor hydrodynamics*
  - *Multi-scale modelling of cell/support interactions*
- ***GMP production and clinical research***
  - *GMP production of Advanced Therapy Medicinal Products (ATMP).*
  - *GMP expansion of UC-MSCs in 2D and Bioreactors*
  - *Immunomagnetic isolation*
  - *French CAR-Net : Network for academic CAR T cell production*

Tools

- ***Bioprocess scale-up and intensification***
- ***Online and offline monitoring***
  - *On-line probes used during cell culture (RAMAN, Dielectric spectroscopy)*
  - *Off-line analysis*
  - *Cell characterization (Flow cytometry, Microscopy, MEB, confocal, AFM, Functional cell culture controls*
- ***Bioprocess modelling***
  - *Dedicated softwares (ANSYS Fluent, Matlab, Discovery studio, SuperPro Designer)*
- ***Customized Isolator (Comecer) for adherent cell cultures***
- ***CliniMACS Prodigy Platform for cells in suspension***

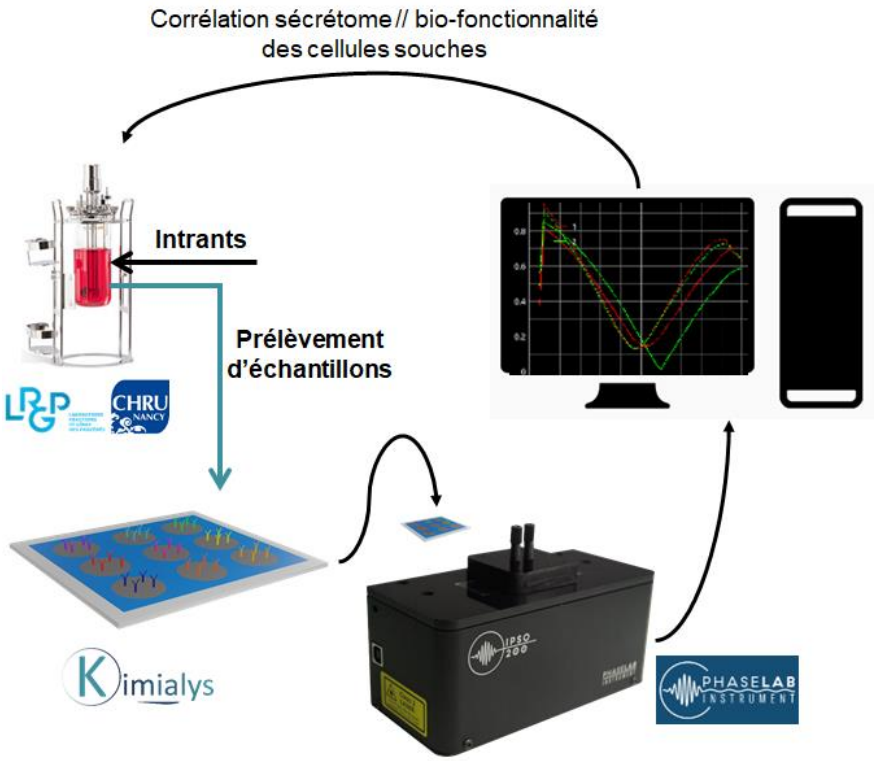



SEQRET Project

Development of an on-line quality control system based on the real-time analysis of the secretome of Mesenchymal Stromal Cells.

A semi-automatic and integrable quality control system to characterize the secretome

Granted in the call “Biotherapies and bioproduction of innovative therapies”, the SEQRET project aims to develop an on-line quality control system based on real-time analysis of the secretome of mesenchymal stromal cells (MSC) in production. It thus aims to meet two existing needs: to **establish correlations between the nature of the secretome and the bio-functionality of the cells** in order to optimize the culture conditions of MSCs by acting on them in real time, and **to achieve a semi-automated quality control module** that can be integrated into any type of production line for cell therapies or immune cells, which will make it possible to improve production yields.



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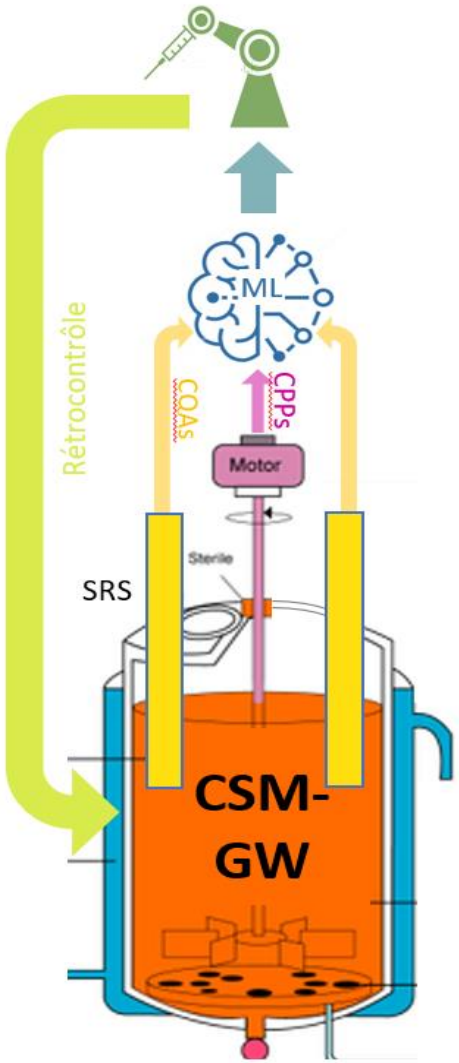
MTINOV
LARGE SCALE HUMAN CELL PRODUCTION

ACCESS project

Optimization of Mesenchymal Stromal cell production through the development of an on-line clinical grade production control system.



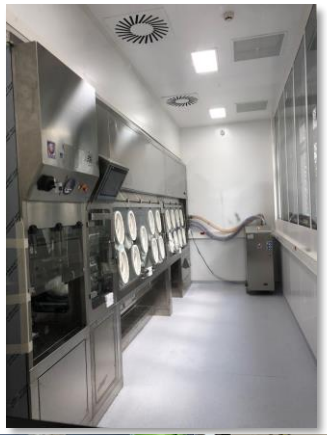
Granted in the Big Challenge "Biomedicines: improving yields and controlling production costs", **the ACCESS project will optimize the production of Mesenchymal Stromal Cells (MSC) through the development of an on-line control system for clinical grade production.** This system will be based on innovative spectroscopic sensors, Machine Learning algorithms and automated real-time feedback. The project will also develop the digital twin of this device as well as a Quality-by-Design (QbD) approach for process modelling. The ACCESS project will enable the automation and improve the robustness (repeatability) of the production of the UC-MSCs.

Perspectives

MTINOV
LARGE SCALE HUMAN CELL PRODUCTION

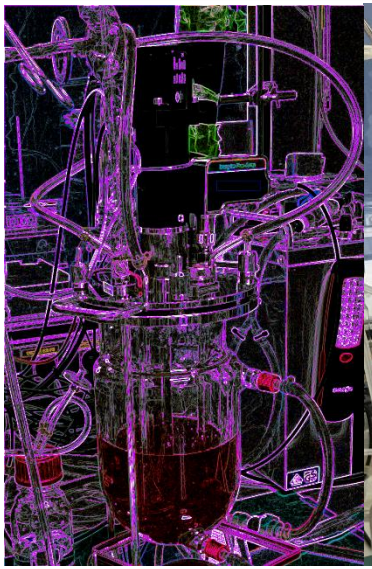
GMP Production of biotherapies

- Collaboration with start-up for development of Phase I/II clinical trials : quality controls and GMP facility and equipment provision.
- Production of academic ATMPs (French network under the aegis of SFGM-TC : *Virus specific T cells, UC-MSCs)*
- Contribution to the new French CAR-net network for academic CAR T cell production.



Bioprocess development

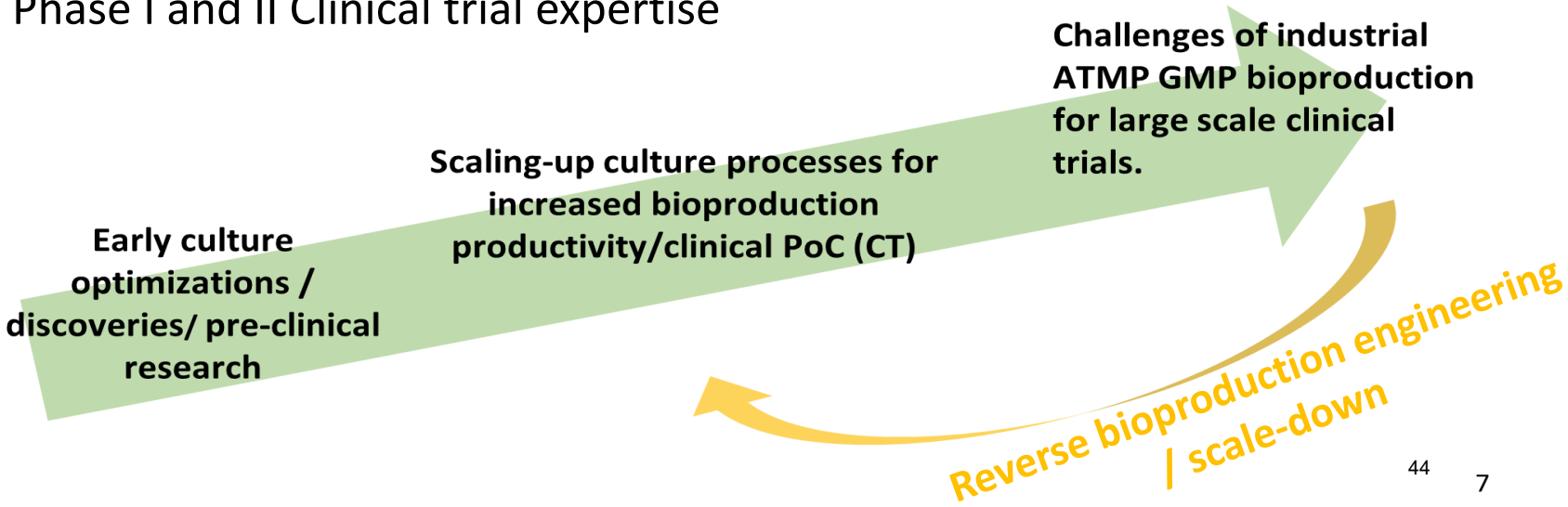
- Integration of AI in bioprocess modelling (CFD / digital twins).
- Modulation of the production of secretomes by human cells, in bioreactors (**Secretome Production Engineering**).
- Contribution to the transition of MSC products towards Industry 4.0
- Supporting process development for preclinical scale-up.



MTINOV
LARGE SCALE HUMAN CELL PRODUCTION

Unique selling points

- Providing an advanced "industrial-like" framework to carry out fundamental research studies on the therapeutic potential of biotherapies (especially products obtained from human cells) but also to integrate these developments in a reverse engineering approach
- Development of a multi-scale and multi-disciplinary approach to embrace the complexity of bioproduction engineering and intensification
- **Unique academic structure offering cell-based production from lab-scale to GMP production**
- Expertise in ATMP production in GMP conditions
- Phase I and II Clinical trial expertise



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Immunomodulation of tumor microenvironment and immunotherapy of thoracic cancers

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Keywords
- Thoracic cancers
- Targeted therapies
- Immunomodulation
- Oncolytic viruses
- 3D co-culture models
- Nanovectorization
- Tumor microenvironment
- Biocollections,
- Intercellular communication
- Endothelial compartment

Abstract
Our team has an expertise in developing novel therapies that exploit immunogenic cell death induction in order to improve the treatment of cancers that are known to be resistant to conventional therapies, like mesothelioma, a type of cancer due to exposure to asbestos, or lung cancer. Two main approaches are currently being developed in our laboratory: Targeted therapies and cancer virotherapy. The first approach deals with the development of new affinity agents and innovative nanovectors derived from biological world to specifically target cancer cells and increase immunogenicity and anti-tumor immune response. Our second approach consists in the use of oncolytic viruses that are able to target specifically tumor cells without harming the healthy ones. We demonstrated the antitumor and immunogenic properties of the attenuated strain of measle virus against mesothelioma, melanoma, lung and colorectal adenocarcinomas. To improve and develop our innovative therapies, our third goal is to better understand the tumor microenvironment (TME) of thoracic cancers. These works are based on validated biocollections of patient's samples (pleural fluids, blood, characterized derived cell lines). In the TME, stromal cells are corrupted by malignant cells, creating a permissive environment which drives cancer progression. In particular, we are studying the interaction/communication (soluble factors, extracellular vesicles) between malignant cells, tumor-associated macrophages (TAMs) and endothelial cells, with a focus on immune regulation. The objective is to identify new biomarkers/therapeutic targets. To compensate the difficult access to tumor tissue, we develop 3D co-culture models and microfluidic to integrate endothelial compartment.

Research area
The CRCI²NA teams focus on the death/survival decisions of malignant cells as well as their molecular evolution, the reactivity of immune cells and the cooperativity of stromal cells. They

combine a broad spectrum of expertise, including cancer genomics and computational biology, cell and molecular biology, cell models (3D and murine), immuno-oncology and imaging.
This multi-scale approach allows the identification of diagnostic and predictive tools, the deciphering of treatment resistance mechanisms and the design of innovative therapies and theranostic approaches, based on the molecular, phenotypic and radiomic specificities of each cancer.

Synopsis
Our projects, based on collections of patient's samples, aim at understanding the tumor microenvironment of thoracic cancers, especially malignant pleural mesothelioma and lung cancer, to develop innovative therapeutic strategies by combining their direct cytotoxicity with induction of specific antitumor immune responses.

Interests
Oncolytic viruses;Non viral delivery systems;Oncology;Immunology/Immunotherapies;In vitro models/ Organ-on-chip;Biocollections;Specific targeting;Biomarkers;Nanotechnology;Translational research

No results presentation available

Programmable bacteria for diagnostics and therapeutics

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Keywords
- Synthetic Biology
- Live Bacteria Therapeutics
- Cellular Programming
- Receptor Engineering
- Scalable Technology Platforms
- Custom Sensing
- Smart Therapeutics

Abstract
We work in the field of synthetic biology, the rational engineering of new biological systems and functions. Our expertise lies in cellular programming, the manipulation of cell function through genetic engineering, enabling fine control of cellular behavior.

We focus on engineering bacteria to function as intelligent therapeutics. Our research aims to create a suite of reconfigurable bacteria-based therapeutic platforms, each tailored to address a distinct disease or condition. We have developed scalable technology platforms for custom sensing and logic programming, equipping bacteria with the ability to perceive and respond to their surroundings. This approach empowers bacteria to act as therapeutic agents, monitoring body locations, detecting pathological biomarkers, and responding to exogenous signals to produce therapeutic effectors at the right place and at the right time. We have successfully engineered various receptors detecting biomarkers along with cellular logic and memory devices operating in living cells. Coupled together, these systems enable the integration of multiple signals to finely control therapeutic activity.
The development of reconfigurable platforms for custom sensing and logic programming holds immense promise for tackling various diseases. By integrating these platforms into bacteria, we envision a future where these smart therapeutics can autonomously monitor and treat specific disease states. This approach holds immense potential for personalized medicine and minimally invasive therapeutics

Research area
Synthetic Biology, Structural Biology, Biophysics

Synopsis
Our research in synthetic biology focuses on engineering programmable bacteria as smart therapeutics, uniquely addressing challenges in targeted drug delivery with precision, adaptability and safety.

Interests

Non viral delivery systems;Genetic engineering;Sensors and biosensor;Single cell manipulation;Translational research

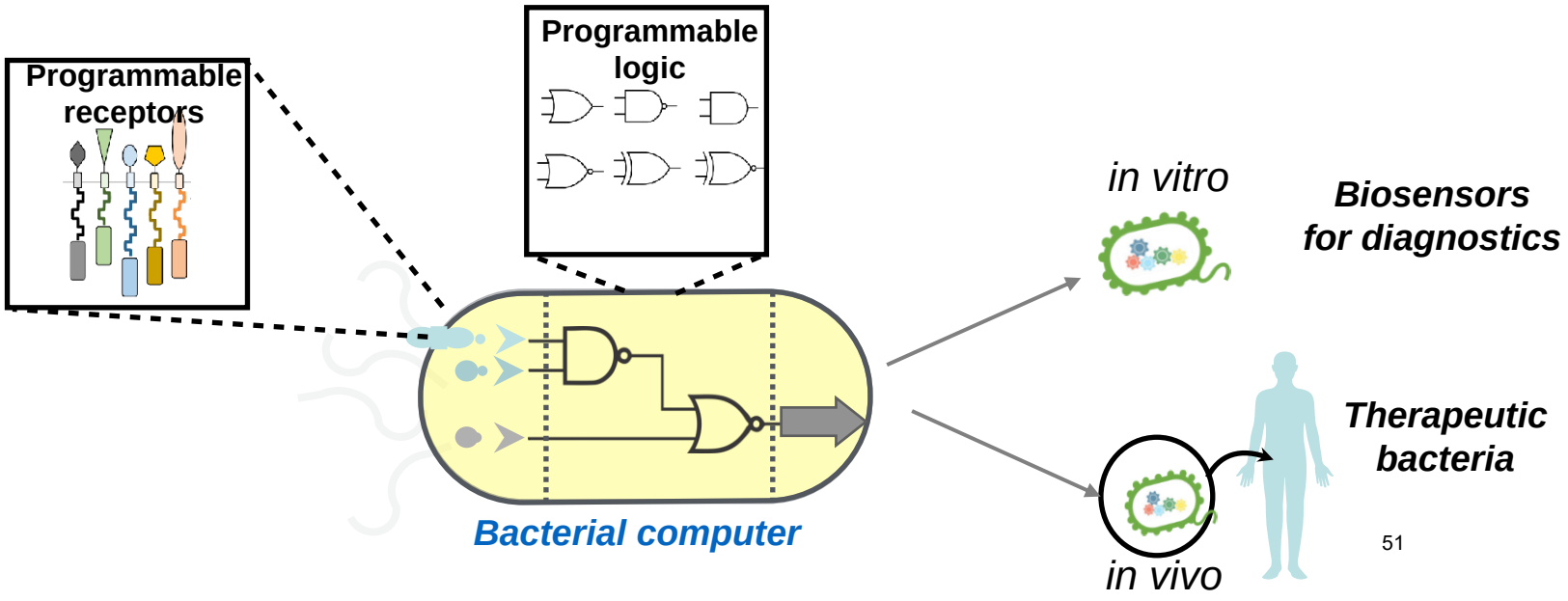
Programmable bacteria for diagnostics and therapeutics

Jerome BONNET

Centre de Biologie Structurale (CBS),
Inserm U1054, CNRS UMR5048,
University of Montpellier

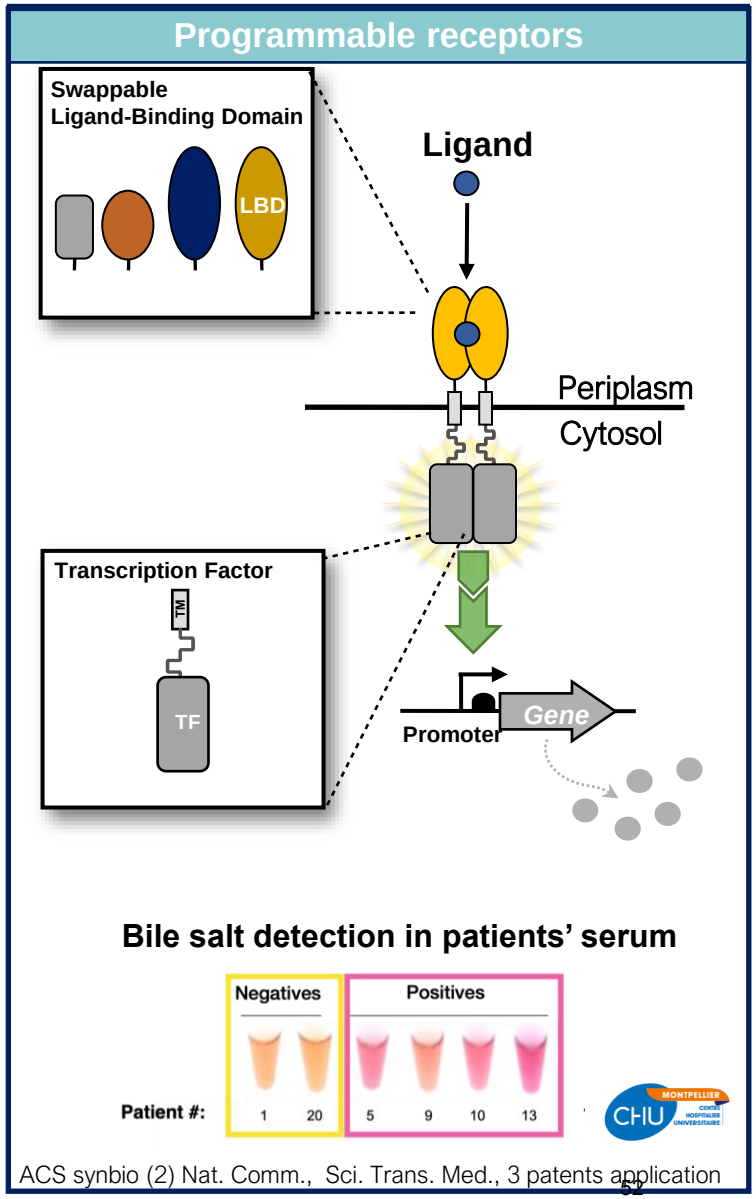
- **Objectives:**
  - Engineer bacteria as living computers to solve pressing challenges in healthcare
  - Use bacteria as smart biosensors for detecting pathological biomarkers
  - Program bacteria as smart therapeutics to detect and treat diseases *in situ*

- **Tools:**
  - Scalable synthetic receptor platform for custom detection of signals of interest
  - Genetic memory systems to store transient signals
  - Genetic logic gates to compute sophisticated responses to multiple signals



Programming bacteria for *in vitro* diagnostics

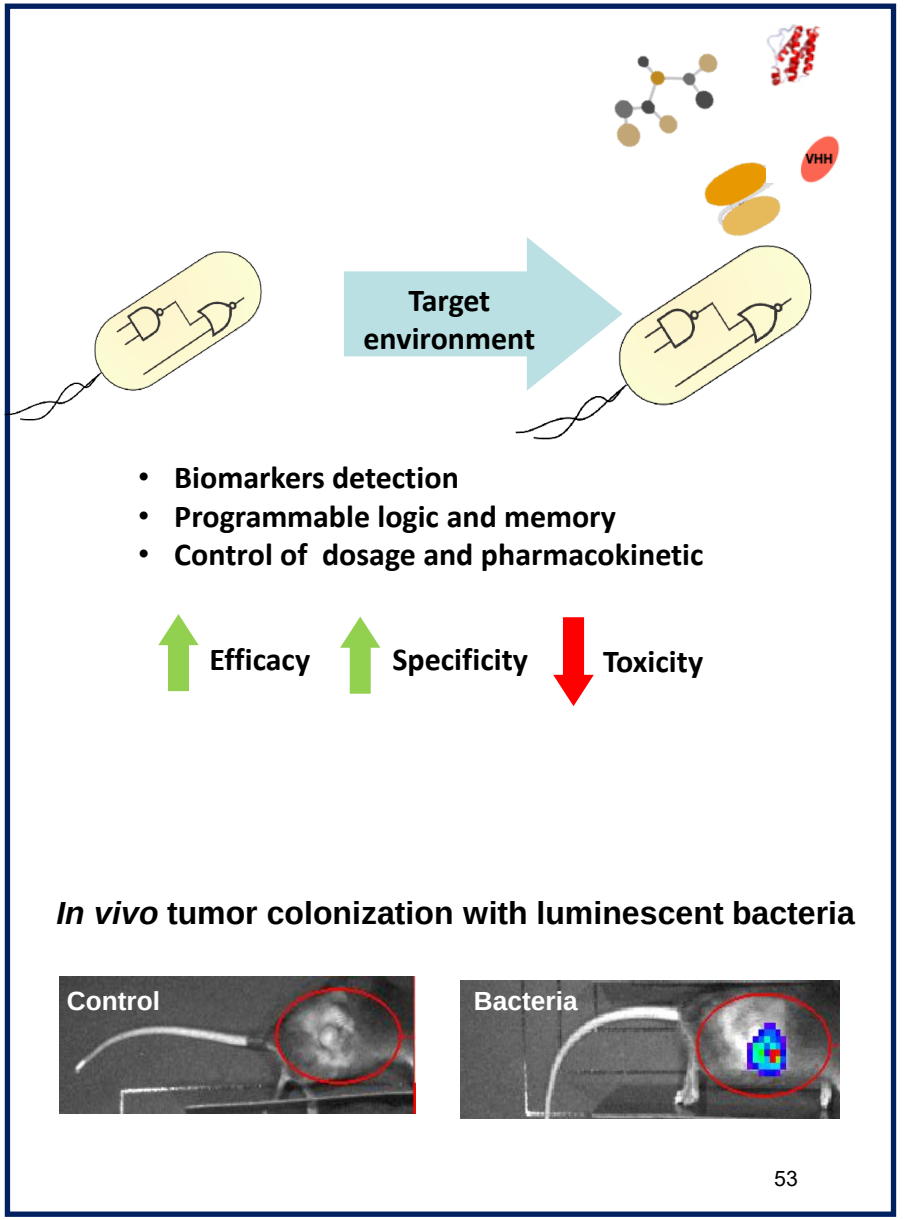
- **Results:**
  - Bacteria equipped with engineered genetic circuits can detect biomarkers of disease in patient samples. First example with detection of glycosuria in diabetic patients.
  - Programmable receptors enable detection of biomarkers of liver dysfunction in patient serum.



ACS synbio (2) Nat. Comm., Sci. Trans. Med., 3 patents application

Programming bacteria for cancer therapy

- **Results:**
  - Bacteria can colonize tumors and secrete effectors *in situ.*
  - Synthetic receptors enable conditional control of bacterial therapeutic activity *via* detection of tumor specific biomarkers and the administration of exogenous molecules.



- **Unique selling points**
  - Unique modular receptor platform for reprogramming specificity to detect new ligands of interest.
  - Pioneering work in genetic logic and memory enabling cellular computation in response to the environment.
  - Coupling sensing with logic supports thigh control on therapeutic activity location, activation threshold, levels, and duration.
  - Wide array of possible therapeutic effectors including antibodies, enzymes, chemokines…
  - Living bacterial therapeutics are micro-factories synthetizing drugs *in situ,* improving efficacy and specificity, and reducing side effects.
  - Biology is a self-manufacturing technology, reliably and rapidly producing billions of diagnostics or therapeutic agents autonomously.

- **Perspectives:**
  - Synthetic receptors and genetic logic enable custom sensing and control of cellular response.
  - Next generation diagnostic devices using autonomous living cells that can detect different signals and perform multiplexed computation in a test tube.
  - New paradigm in treatments with living cellular therapeutics that can sense and respond to their environment and be controlled externally for targeted and enhanced therapeutic activity.

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Next generation AAV vectors for gene therapy of muscle disorders

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Keywords
- Gene therapy
- AAV vectors
- neuromuscular disorders
- myopathies
- motoneuron diseases
- translational research.

Abstract
Our research activities focus on developing AAV-based gene therapies for neuromuscular disorders with a major interest in clinical translation. We performed pioneering preclinical work on gene replacement therapy for myotubular myopathy that led to the first clinical trial in patients. Our goal is to develop novel therapies for genetic diseases that affect muscles and/or the nervous system with a clear unmet medical need using appropriate preclinical models and optimized technologies for gene transfer, in particular bioengineered AAV vectors.

Research area
Development of gene-based therapies for muscle and motoneuron diseases and bioengineered adeno-associated viral (AAV) vectors for gene transfer.

Synopsis
Innovative gene therapies for neuromuscular diseases: from POC studies to clinical translation

Interests
Gene therapy;Viral vectors;Neuromuscular disorders;In vitro models/ Organ-on-chip;In vivo models;Translational research;Clinical research

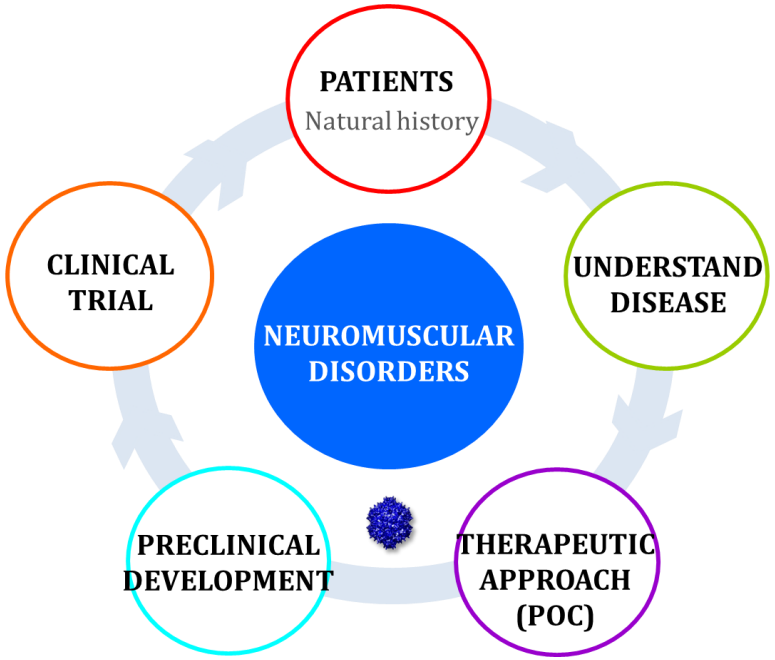
Next generation AAV vectors for gene therapy of muscle disorders

Ana BUJ BELLO

Inserm U951, University of Evry – Paris Saclay, Genethon, Evry-Courcouronnes

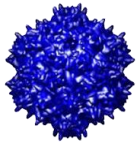
- **Objectives:**
  - Development of innovative AAV-based gene therapies for neurological diseases, with a focus on neuromuscular disorders
  - Proof-of-concept studies in animal models based on the understanding of the genetics and pathophysiology of the disease
  - Translational research leading to clinical trials
  - Optimization of AAV vectors for gene transfer in target cells

- **Tools:**
  - Adeno-associated viral (AAV) vectors
  - Animal models
  - Molecular, histological and functional assays
  - Patient-derived samples



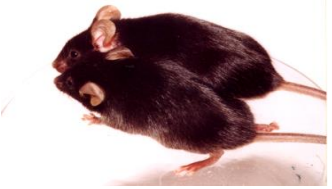
Subject 1: Gene therapy of myotubular myopathy

- **Results:**
  - Proof-of-concept studies on the efficacy of an AAV-based therapy in animal models
  - Preclinical development of an AAV8-MTM1 vector
  - Prospective natural history study in patients
  - First clinical trial in patients with myotubular myopathy showing efficacy and safety profile

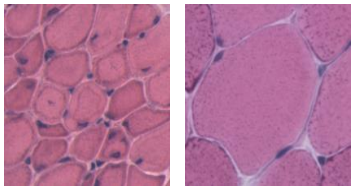


Vector
AAV8-MTM1

Animal models



Untreated Treated



Childers et al. *Sci Transl Med* 2014

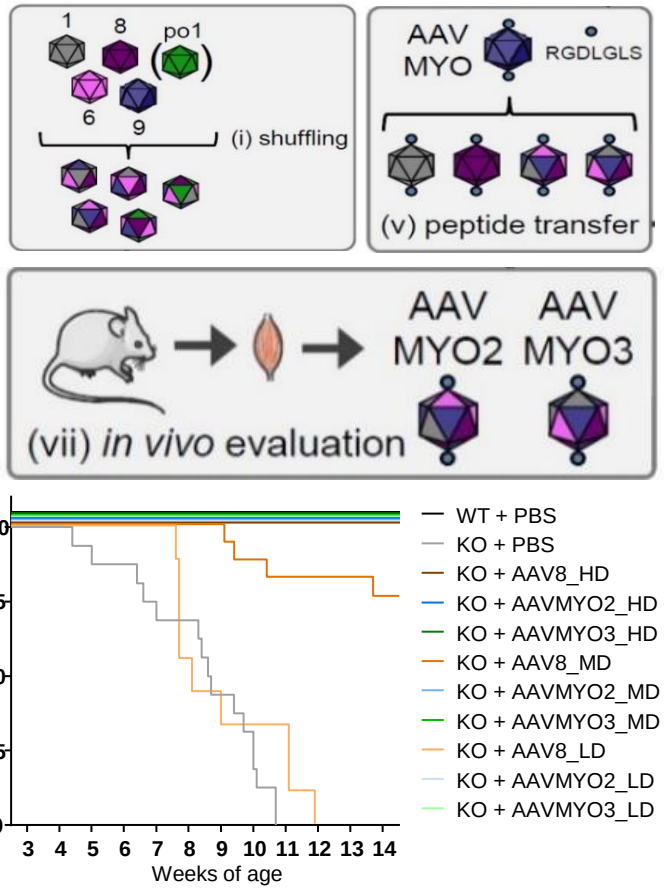
Clinical trial

Safety and efficacy of gene replacement therapy for X-linked myotubular myopathy (ASPIRO): a multinational, open-label, dose-escalation trial

Shieh P. et al. *Lancet Neurol*. 2023

Subject 2: Bioengineering of AAV vectors with increased potency and specificity for skeletal muscles

- **Results:**
  - Generation of two novel AAV capsids (AAVMYO2 and AAVMYO3) by semirational engineering that show increased tropism and specificity for skeletal muscles in mice (collaboration)
  - Efficacy studies in a mouse model of myotubular myopathy with reduced vector doses as compared to a natural serotype



Mtm1 KO mice; HD: high dose; MD: mid dose; LD: low dose

Al-Andari J. et al. Sci Adv. 2022

- **Perspectives:**

  - To develop gene therapy approaches for neurological (neuromuscular) disorders with unmet medical need
  - To perform multi-level analyses on the therapeutic response in disease models
  - To generate and characterize novel AAV vectors with increased tropism and specificity for muscle and CNS
  - To assess the efficacy and safety of novel gene therapies in patients

- **Unique selling points**

  - Long-standing expertise in the fields of gene therapy and neuromuscular disorders
  - Capacity to perform translational research leading to first in human clinical trials
  - Internal platforms with expertise in preclinical development and vector production at small to large scale
  - Network with scientists, clinicians and patient associations

Selected bibliography

- **Safety and efficacy of gene replacement therapy for X-linked myotubular myopathy (ASPIRO): a multinational, open-label, dose-escalation trial.** Shieh PB, et al. *Lancet Neurol.* 2023 Dec;22(12):1125-1139.
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- **Gene therapy prolongs survival and restores function in murine and canine models of myotubular myopathy.** Childers MK, et al. *Sci Transl Med*. 2014 Jan 22;6(220):220ra10.

Universal CAR-MAIT cell platform : an opportunity to treat solid tumors

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Keywords
Chimeric Antigen Receptor (CAR)-T cells; immune cell therapy; anti-tumor response; Graft-versus-host-disease; Alloreactivity; Mucosal-Associated Invariant T (MAIT) cells

Abstract
Our projects address the pathophysiological mechanisms of immune responses in immunocompromised subjects, mainly in the context of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Besides, we consider new immune cell therapy approaches for manipulating immune responses.

More specifically, we exploit the properties of human MAIT cells, a population of semi-invariant T cells abundant in barrier tissues and endowed with potent effector functions, to provide a new platform for the production of universal, off-the-shelf, chimeric antigen receptor (CAR)-expressing cells. Having demonstrated the lack of alloreactive potential of MAIT cells, we have established the conditions for the production of CD19-directed CAR-MAIT as a proof of concept, demonstrated their efficacy in controlling CD19+ tumor cell growth in vitro and in a preclinical leukemia model, and showed their accumulation in tissues without causing graft-versus-host-disease (GVHD). Using solid cancer models, we now show the killing efficacy of CAR-MAITs in 3D tumor spheroids and in a relevant preclinical model (lung metastasis of breast cancer). We propose to use MAIT cells from healthy donors as a source of immune cells with strong tropism for tissues to produce a novel platform for CAR engineering for the treatment of solid malignancies, in particular the liver and lung.

Research area
Immune responses in the immunocompromised host and new therapeutic approaches for manipulating them.

Synopsis
Exploiting the functions of human Mucosal-associated invariant T (MAIT) cells in the allogeneic setting.

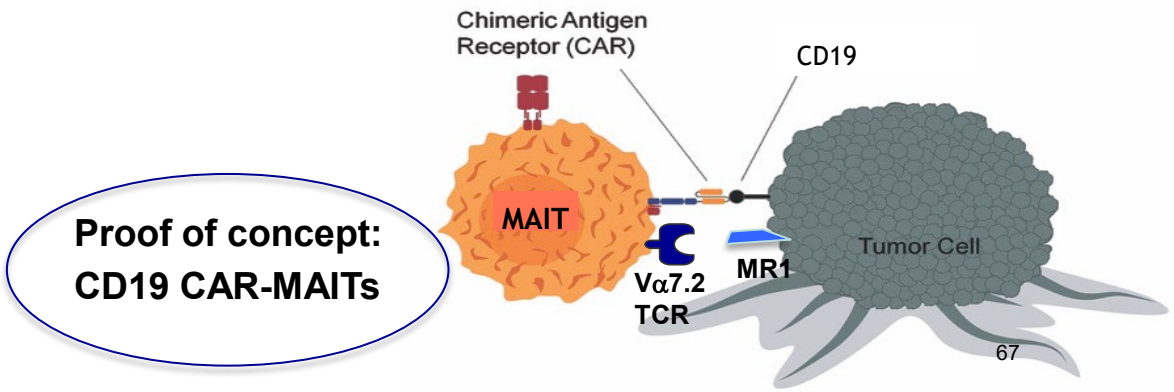
Interests
Cell Therapy;Chimeric Antigen Receptor (CAR)-T cells;Stem cells;Oncology;Immunology/Immunotherapies;In vitro models/ Organ-on-chip;In vivo models

Universal CAR-MAIT cell platform :
an opportunity to treat solid tumors

Sophie CAILLAT-ZUCMAN

INSERM UMR976, St-Louis Research Institute, Paris

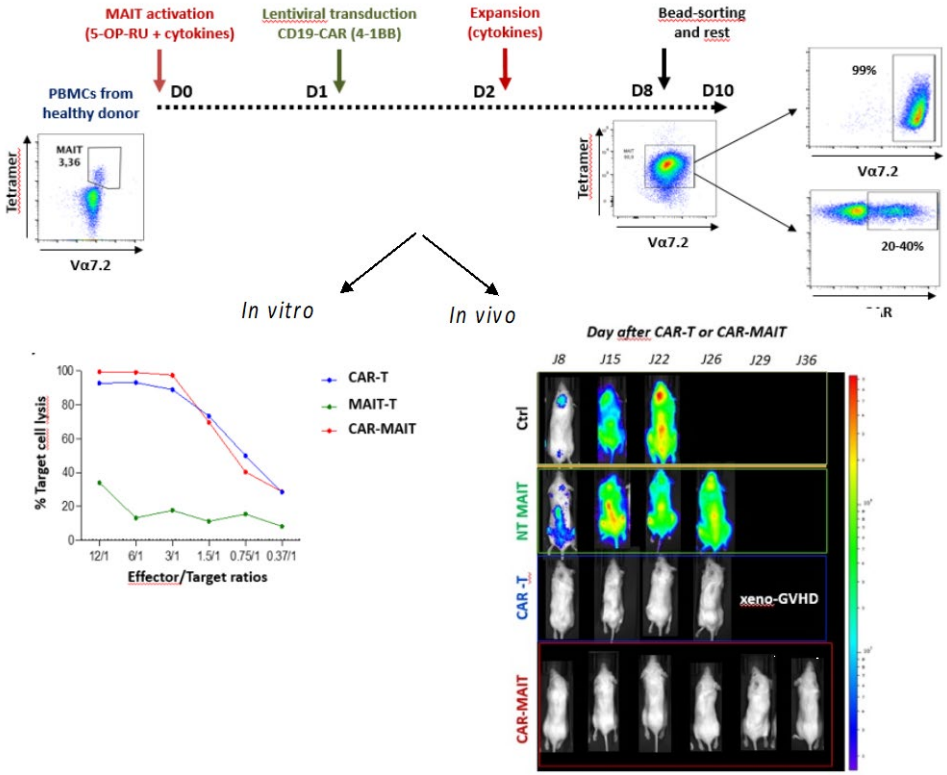
- **Objectives:**
  - To demonstrate that CAR-MAIT cells control tumor growth in relevant preclinical models without causing xeno-GVHD, by evaluating their efficacy in :
    - leukemia models as Proof of Concept and
    - in solid tumor models
  - To establish the optimal conditions for their production (with diverse CAR constructs) using CAR-MAIT cells directed to CD19 as a model antigen
  - To show that CAR-MAIT cells kill $CD19^+$ tumor targets as efficiently as CAR-T cells
- **Tools:**
  - Synthetic MR1 ligand (5-OP-RU) to activate MAIT cells
  - Lentiviral CD19-CAR constructs
  - $CD19^+$ tumor cell lines (constitutively or artificially expressing CD19)
  - NOD/Scid/Il2rγ−/− (NSG) mice engrafted with $CD19^+$-luc tumor cells



Subject 1

PoC of CD19 CAR-MAIT efficacy in the Nalm6 leukemia model

- **Results:**
  - The CAR-MAIT production process has been established
  - CAR-MAIT kill Nalm6 targets *in vitro* as efficiently as CAR-T cells
  - CAR-MAIT control Nalm6 leukemia growth in NSG mice and do not cause xeno-GVHD



MAIT cells from healthy donor are activated, transduced with a lentiviral CD19-CAR, expanded and purified before being evaluated.

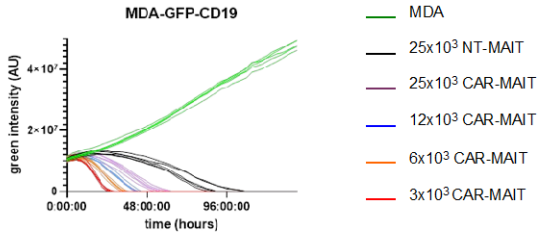
CAR-MAIT or CAR-T cells are cultured with Nalm6-luc targets for 12 hrs. The percentage of target cell lysis is measured by bioluminescence.

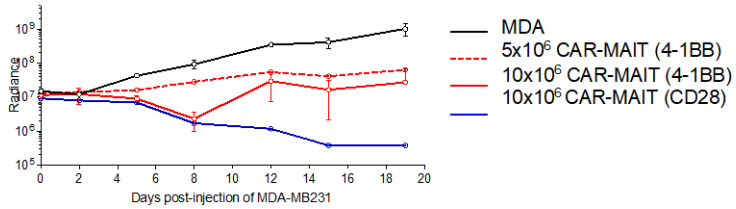
NSG mice are injected i.v. with 10^5 Nalm6-luc cells and 12hrs later with $5x10^6$ CAR-MAIT or CAR-T cells. Tumor growth is monitored twice weekly.
CAR-T cell treated mice were euthanized at D28 due to severe xeno-GVHD.

Subject 2

Evaluation of CD19 CAR-MAIT in a solid tumor model

- **Results:**
  - CAR-MAIT efficiently kill CD19⁺ MDA-MB231 cells (triple-negative breast tumor cell line) in a 3D-spheroid tumor model
  - CAR-MAIT migrate to the lungs and control lung metastases of MDA-MB231 tumor in NSG mice
  - CAR-MAIT equipped with a CD28 costimulatory domain appear more efficient than those with a 4-1BB domain





MDA-MB231 -CD19-GFP cells were cultured in low-attachment 96-well plates. After 3 days, non-transduced MAIT or CAR-MAIT cells were added and tumor cell growth was quantified using Incucyte Live-Cell Analysis system

Irradiated NSG mice were injected i.v. with 1x10⁶ CD19+ MDA-MB231-luc cells and 5-10x10⁶ CAR-MAIT 12 hrs later. Tumor growth was monitored by bioluminescence twice weekly

- **Perspectives:**

  - Determining the best CAR construct (with the best costimulatory domain) for CAR-MAIT cell efficacy and persistence in the MDA-MB231 lung mestastasis model
  - Profiling CAR-MAIT cells and related efficacy and persistance
  - Extend the results to other solid tumor models:
    - Orthotopic breast cancer model (MDA-MB231 injected in the breast fat pad, followed by resection of the tumor before occurrence of lung metastases)
    - Orthotopic hepatocellular carcinoma model (HepG2 cells injected in the liver)

- **Unique selling points**
  - Advantage of universal, off-the-shelf CAR-MAIT compared to conventional CAR-T (no requirement of TCR-gene editing)
  - Tissue homing properties of CAR-MAIT exploited for the treatment of solid tumors
  - Patent PCT/EP2021/085062 *"MUCOSAL-ASSOCIATED INVARIANT T (MAIT) CELLS EXPRESSING CHIMERIC ANTIGEN RECEPTORS"* covering treatment methods using MAIT cells as CAR therapy, in the context of cancer, infectious diseases or autoimmune diseases
  - Capacity to go from basic research laboratory work to first in human clinical trials on the site of St-Louis hospital (Meary MTI platform)

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- Boulch et al. Tumor-intrinsic sensitivity to the pro-apoptotic effects of IFN-g is a major determinant of $CD4^+$ CAR T cell anti-tumor activity. **Nat Cancer**. 2023 Jul;4(7):968-983. PMID: 37248395
- Boulch et al. A major role for CD4+ T cells in driving cytokine release syndrome during CAR T cell therapy. **Cell Rep Med** 2023, Sep 19;4(9):101161. PMID: 37595589

Multidisciplinary approaches for stroma based regenerative and rejuvenative medicine

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Keywords
- Regenerative medicine
- MSC
- ATMP (Advanced Therapy Medicinal Product)
- Organoid
- Stroma
- Adipose tissue

Abstract
Our research interest focuses on mesenchymal stem cell (MSC)-based regeneration in adults. The therapeutic prospects are wide-ranging, as MSC support the parenchyma in all tissues and are essential for the proper functioning of any organ. Since our preclinical demonstration of the therapeutic interest of adipose-derived mesenchymal cells (ASC), we have been managing the facility for control qualities of ATMP, the only one of its kind in France, and have participated in various clinical trials using ASC as ATMP. More recently, we have turned to implementing artificial Intelligence, in silico modelling, tissue engineering and up to date imaging methodologies in our research. We set-up an AI pipeline to define personalised physiological age to stratify patients for regenerative and rejuvenative medicine. Using an in silico modelling, we discover that an early and transient treatment right after injury unlocks repair processes in adult mammals for regeneration. We are presently investigating its translation according to the age. We have also implemented an engineering process to generate mesenchymal organoids at different scales that are suitable not only for drug screening but also for transplantation as future ATMPs. The proof of concept has been demonstrated for human beige adipose tissue providing rejuvenation of energy homeostasis in adults.

Research area
Stroma based Regenerative and rejuvenative therapies, tissue engineering

Synopsis
Multidisciplinary approaches for stroma based regenerative and rejuvenative medicine

Interests
Cell Therapy;Stem cells;Patient cohorts;Modelling/Digital Twin;Artificial Intelligence (AI);Clinical research

Multidisciplinary approaches for stroma based regenerative and rejuvenative medicine

Louis Casteilla

RESTORE Institute, Toulouse

Guided self-Organization of Tissue for Innovative Therapeutics - GOT-IT Team

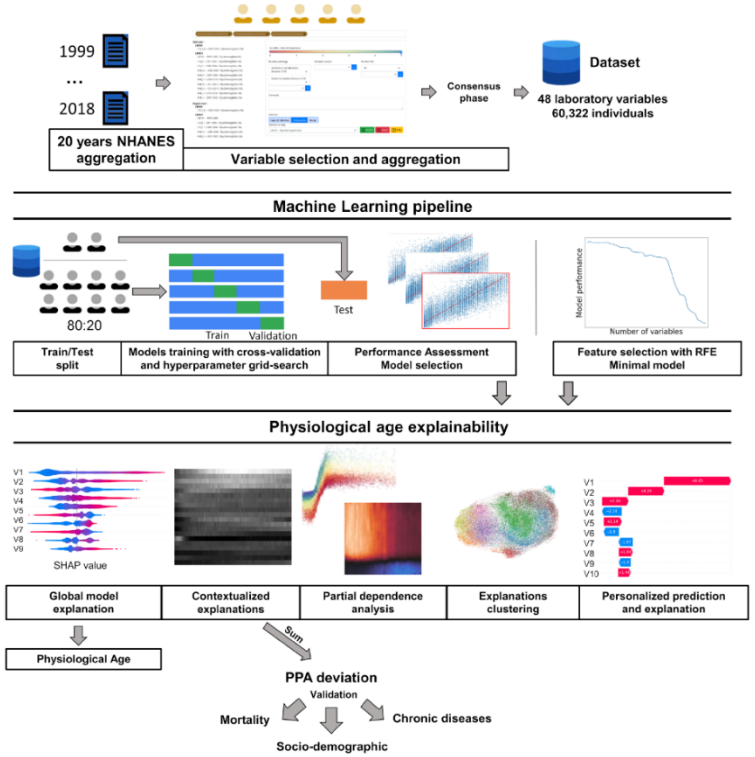
- **Objectives:**
  - Unlock inhibitory cues for regeneration in adult and aging mammals using multi-disciplinary approaches.

- **Tools:**
  - Explainable Machine learning
  - Up to date 3D imaging (light sheet, …)
  - MSC/ASC based tissue engineering (OBBI: industrial demonstrator)
  - ATMP quality control facility (Ecellfrance national infrastructure)
  - Innovative in vivo repair models

Subject 1: Explainable machine learning framework to predict personalized physiological aging (PPA), *David et al, Aging cell 2023, patent*

- **Results:**
  - We set-up a generic explainable machine learning pipeline to define a personalized physiological age from any initial chronological dataset
  - We apply this pipeline to NAHNES dataset (60 000 individuals with 48 biological variables) to specify the personalized physiological age that predicts chronic diseases and mortality
  - Furthermore, this prediction and its explainability provide a precise metric to monitor the physiological age and stratify individuals in different ageotypes

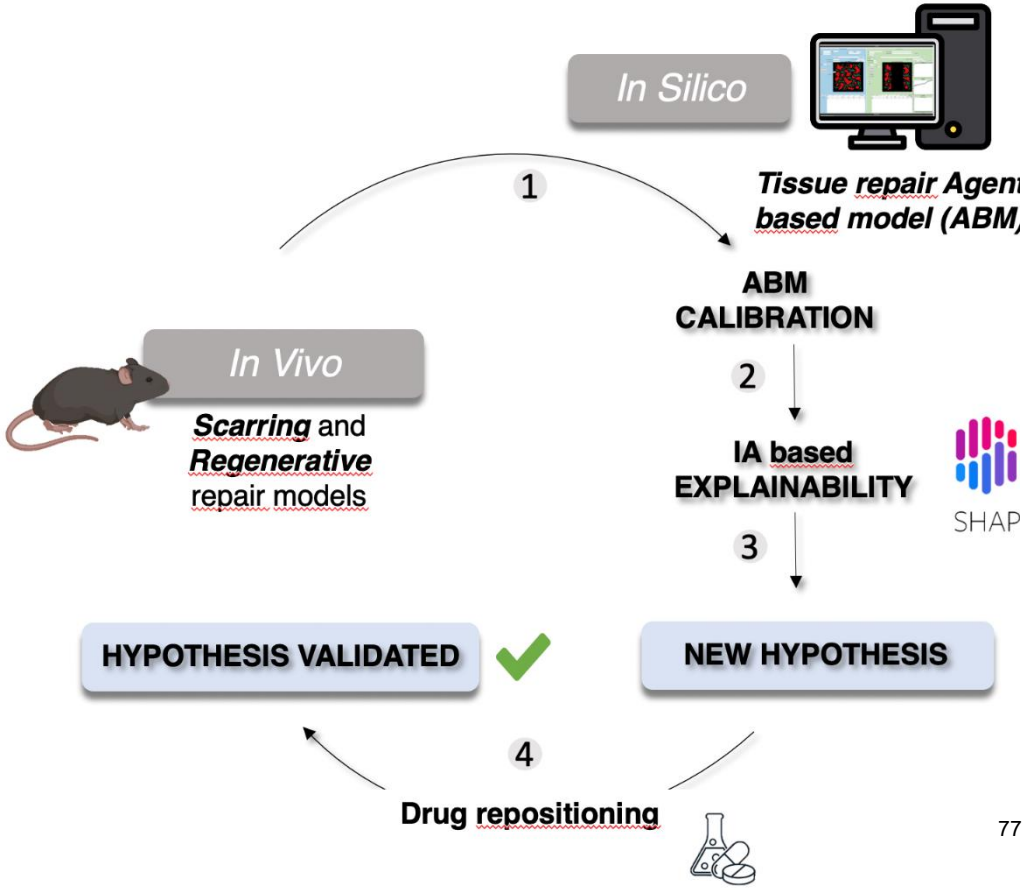
Graphical abstract:
Framework of PPA: after a careful pre-processing of the initial datatset, the best algorithm is defined to predict PPA. SHAPS (SHapley Additive exPlanations) analysis reveals the most important variables with their respective metrics and different ageotypes.



Subject 2: A digital twin identifies an inhibitory cue to unlock the repair process in adult mammals for regeneration. *Pacary et al, submitted, patents*

- **Results:**
  - In silico investigations on a calibrated agent based model (ABM) of tissue repair identify that an early transient treatment with an inhibitory compound (on going patent) unlocks healing repair in adult mammals for regeneration
  - The repositioning of molecule validates in vivo the hypothesis elaborated from in silico investigations.

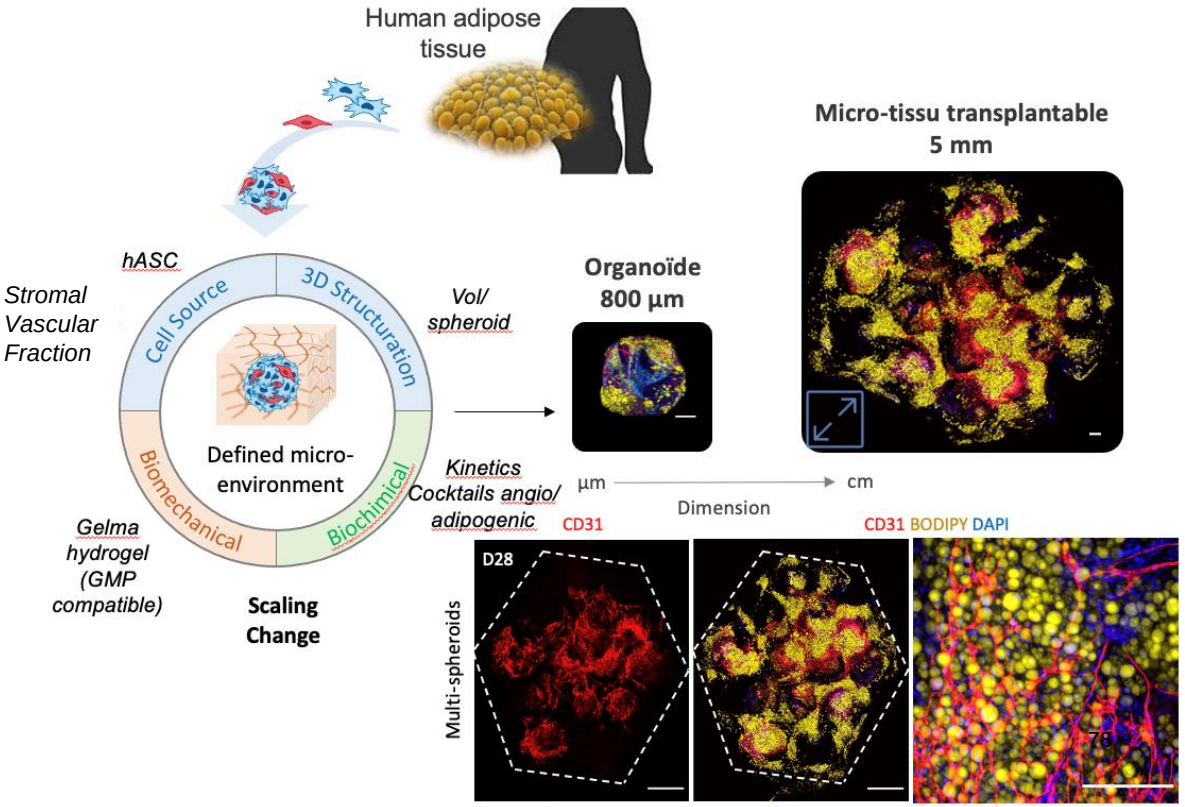
Graphical abstract:
A digital twin to investigate repair processes.
Back and forth between in vivo and in silico models has enabled us to validate an innovative target for regeneration in adult mammals.



Subject 3: Scalable Generation of Pre-Vascularized and Functional Human Beige Adipose Organoid. *Escudéro et al, Advanced science, 2023, 2 patents*

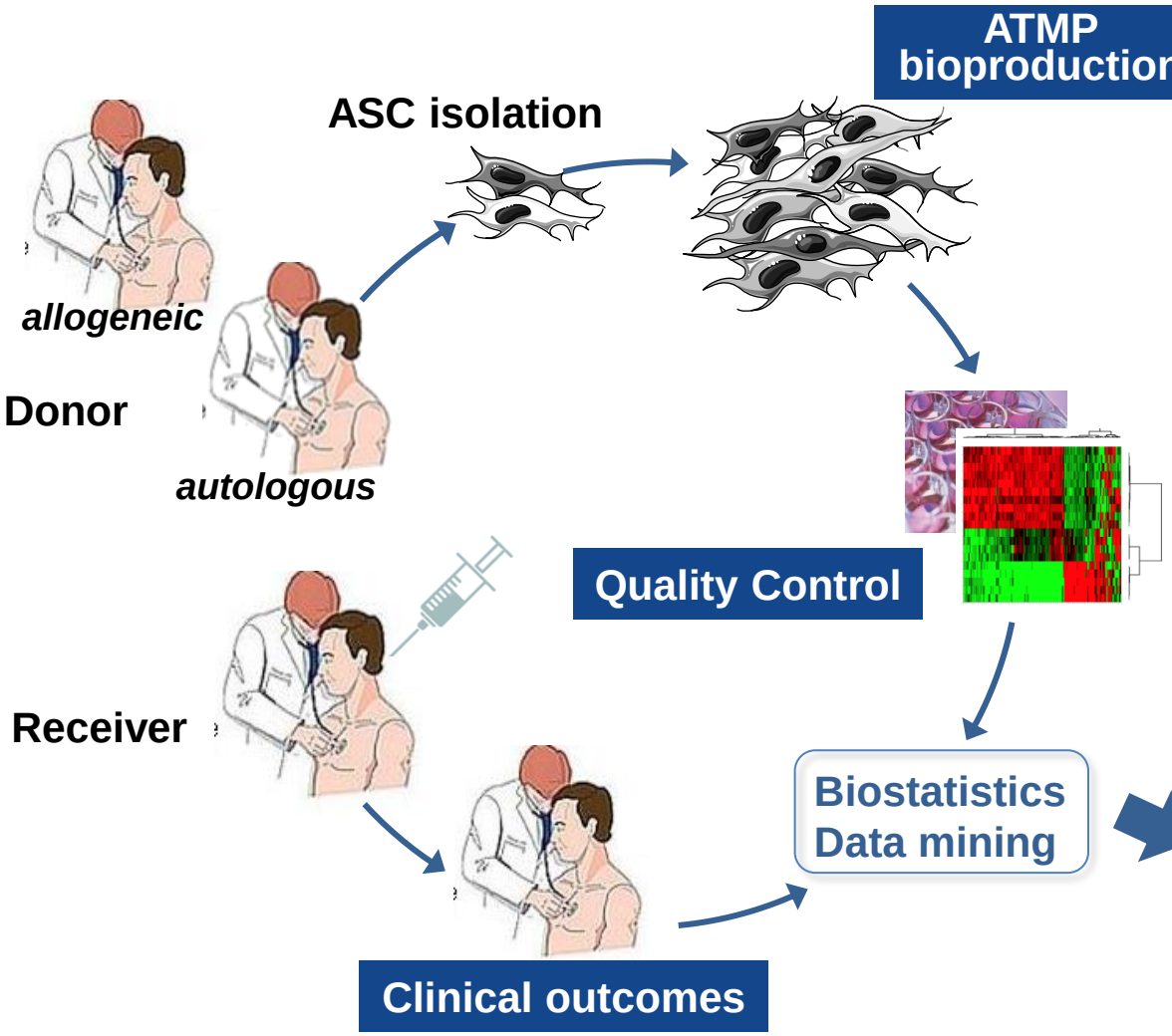
- **Results:**
  - By defining the adequate combination between a tunable hydrogel, adipose derived stroma cells and a differentiation cocktail, we propose a scalable bioengineering process to generate human mesenchymal organoids at different scales that are suitable not only for drug screening but also for transplantation as future Advanced Therapeutical Medicinal Products.
  - We perform a first proof of concept by generating human beige adipose tissue avatars at different scales

Graphical abstract: Biomechanical and biochemical initial conditions lead to scalable generation of vascularized human beige adipose organoids. CD31 is an endothelial marker, bodipy and DAPI stain neutral lipids and nuclei respectively.



Subject 4: A unique Quality Control facility to evaluate MSC and ATMP. *ECellFrance infrastructure*

- **Results**



Extensive phenotyping
- *Omics & signature*

Conventional MSC properties
- *Paracrine activity*
- *Multipotency*
- *CFU-f*

Safety assays
- *hTERT*
- *Replicative senescence*

Dynamic challenges
- *Angiogenic support*
- *Myofibroblastic activity*
- *Senescence hallmarks*
- *Oxidative stress resist.*
- *Metabolic flexibility*
- *Mitochondria integrity*
- *Immuno-inflam. Challenges*
- *Migration*

New QC
New targets
Biomarkers
Donor/Receiver selection

Subject 5: A double-blind randomized controlled trial in a dog model of a chronic aged-related disease with no reliable treatment shows the safety and efficacy of a hydrogel embedded adipose-derived stromal cells ATMP: Proof of concept in periodontitis *Monsarrat et al, submitted*

- **Results**
  - Aged canine model is invaluable periodontitis model that closely mimics human periodontitis - a pervasive, chronic inflammatory age-related disease of tooth-supporting tissue with no therapeutic option
  - Rigorous regulatory demands are met, utilizing control, randomization through a robust randomized double-blind methodology mirroring human trials.
  - ASC in fibrin hydrogel ATMP treatment compared to biomaterial support alone and conventional surgical treatments demonstrate safety and significant efficacy in clinical attachment, pocket depth, gingival index, bleeding on probing, and histological tissue reparation that suggests periodontal regeneration.

Trial design to test ASC based ATMP in periodontitis

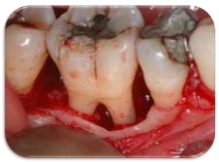
adipose tissue

ASC Qualification of cultured ASC for predictive biomarker of efficacy

+ Platelet Lysate hydrogel

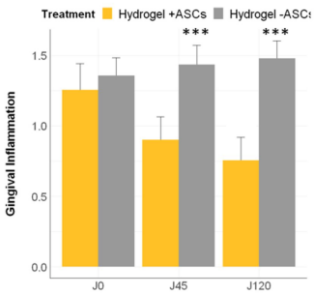
15 aged dogs
In double double-blind randomized controlled

Hydrogel embedded cultured ASC

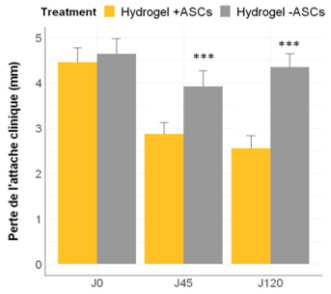


Periodontitis: a dysbiotic chronic inflammatory aged-related disease in therapeutic impasse. 35 à 50% of adults with quality-of-life alterations and extra-oral consequences

Multimodal assessment of ATMP efficacy

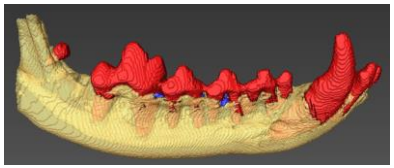


Clinical gingival inflammation decrease



***: 0,001 vs -ASC
**: 0,01 vs -ASC
*: 0,05 vs -ASC

Clinical periodontal tissue gain



Alveolar bone tissue gain (in blue TDM acquisition, day 120)

- **Perspectives:**
  - Human trials (periodontitis, allogenic ASC in ischemia)
  - POC of the efficacy of transplantable organoids
  - The improvement of the digital twin to further investigate regenerative and rejuvenative processes including fibrosis reversal.
  - Personalized regenerative therapies
  - Bioengineering building blocks for organoid bioproduction

- **Unique selling points:**
  - Multidisciplinary approach for stroma based regenerative medicine
  - Innovative targets for regenerative medicine
  - AI pipeline for Personalized Physiological Age (PPA Index)
  - A unique facility for ATMP QC (Ecellfrance infractructure)
  - Preclinical file for the filing of an IMPD for an innovative ATMP targeting periodontitis
  - Scalable pre-vascularized human beige adipose tissue organoids
  - Industrial integrator (OBBI labeled France 2030) for EVs and organoid bioproduction

Selected bibliography

- **Scalable Generation of Pre-Vascularized and Functional Human Beige Adipose Organoids.** Escudero M et al, **Adv Sci (Weinh). 2023;10(31):e2301499. doi: 10.1002/advs.202301499**

- **Cell Aggregate Assembly through Microengineering for Functional Tissue Emergence. Eke G et al, Cells. 2022 Apr 20;11(9):1394. doi: 10.3390/cells11091394**

- **Explainable machine learning framework to predict personalized physiological aging.** Bernard D et al, **Aging Cell. 2023;22(8):e13872. doi: 10.1111/acel.13872**

- **Characterization and Safety Profile of a New Combined Advanced Therapeutic Medical Product Platelet Lysate-Based Fibrin Hydrogel for Mesenchymal Stromal Cell Local Delivery in Regenerative Medicine. Canceill T et al, Int J Mol Sci. 2023;24(3):2206. doi: 10.3390/ijms24032206.**

Developing Bespoken Antibody Releasing B-cells (BAR-B cells) for therapy

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Keywords
- B-cell
- immunoglobulin genes
- genomic edition
- immunotherapy

Abstract
The team's contribution to B cell immunology and immunopathology has resulted in more than 200 scientific publications and 8 patents (3 under active licence form mice producing chimeric antibodies). Some of these studies have been devoted to the elucidation of Ig gene alterations associated with lymphoproliferative disorders, in particular heavy chain disorders and Ig deposition disorders, which are severe complications of monoclonal gammopathies. We are also involved in studies of class switching, in particular analysing elements that regulate Ig gene transcription and recombination in B cells, with a particular interest in regulatory elements located 3' of Ig gene loci. We showed that the IgH 3' regulatory region (3'RR) is a major enhancer of all AID-mediated remodelling events affecting the locus, regulating class-switch recombination (CSR) and somatic hypermutation (SHM) of VDJ regions, but also responsible for deregulation of those oncogenes translocated to the IgH locus, thus participating in lymphomagenesis. We have shown that the 3'RR, by constituting itself an AID target, promotes locus suicide recombination in human and mouse B cells. We have shown that the unique palindromic structure of the 3'RR is essential for the optimal activity of this superenhancer. We are now translating our knowledge of B cell genetics into remodelling their genome for therapeutic purposes.

Research area
My team works on B cell immunology, immunopathology with applications to immunotherapy. After developing expertise in the regulation of transcription, hypermutation and recombination in Ig gene loci, often based on transgenic and knock-out mouse models, I moved to Rennes 3 years ago with strong support from the EFS. This helped to develop new topics dedicated to the genomic editing of human primary B cells for immunotherapy targets.

Synopsis
As B cell experts, we have recently validated innovative strategies for hijacking immunoglobulin expression in human B cells, notably based on an original and patented single-chain full antibody format. We are now working on a number of applications where B-cell reprogramming therapy could address unmet needs.

Interests

Gene editing;Cell Therapy;Immunology/Immunotherapies;In vivo models

Developing *Bespoken Antibody Releasing B-cells (BAR-B cells)* for therapy

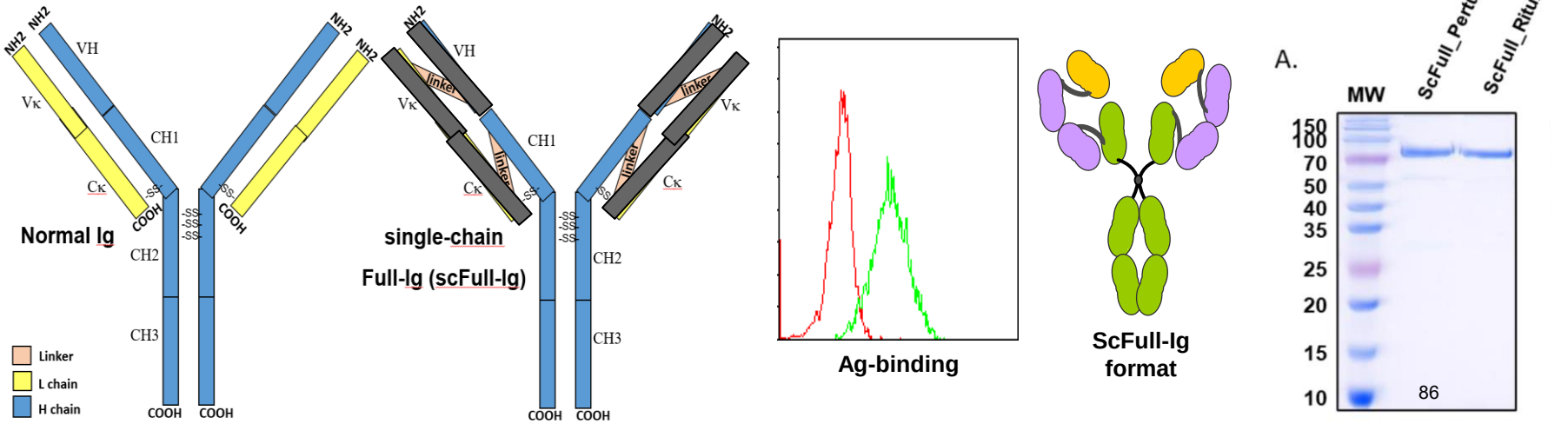
Michel COGNÉ

Inserm U1236, EFS, Rennes University, RENNES, France

BAR-B cells for therapy

- **Objectives:**
  - *Ex vivo* edition of primary B cells for therapy
  - *In vivo* production of therapeutic antibodies for which permanent infusion is needed and/or production *in vitro as for* classical biotherapy is not mastered
  - Therapeutic manipulation & class switching of B-cell receptor expression in edited B-cells
- **Tools:**
  - Mastering of immunoglobulin gene edition in mouse and human cells
  - Immunochemistry, cellular immunology, immunomonitoring , immune repertoires
  - Hu-SCID mouse models grafted with human hematopoietic stem cells or primary B-cells

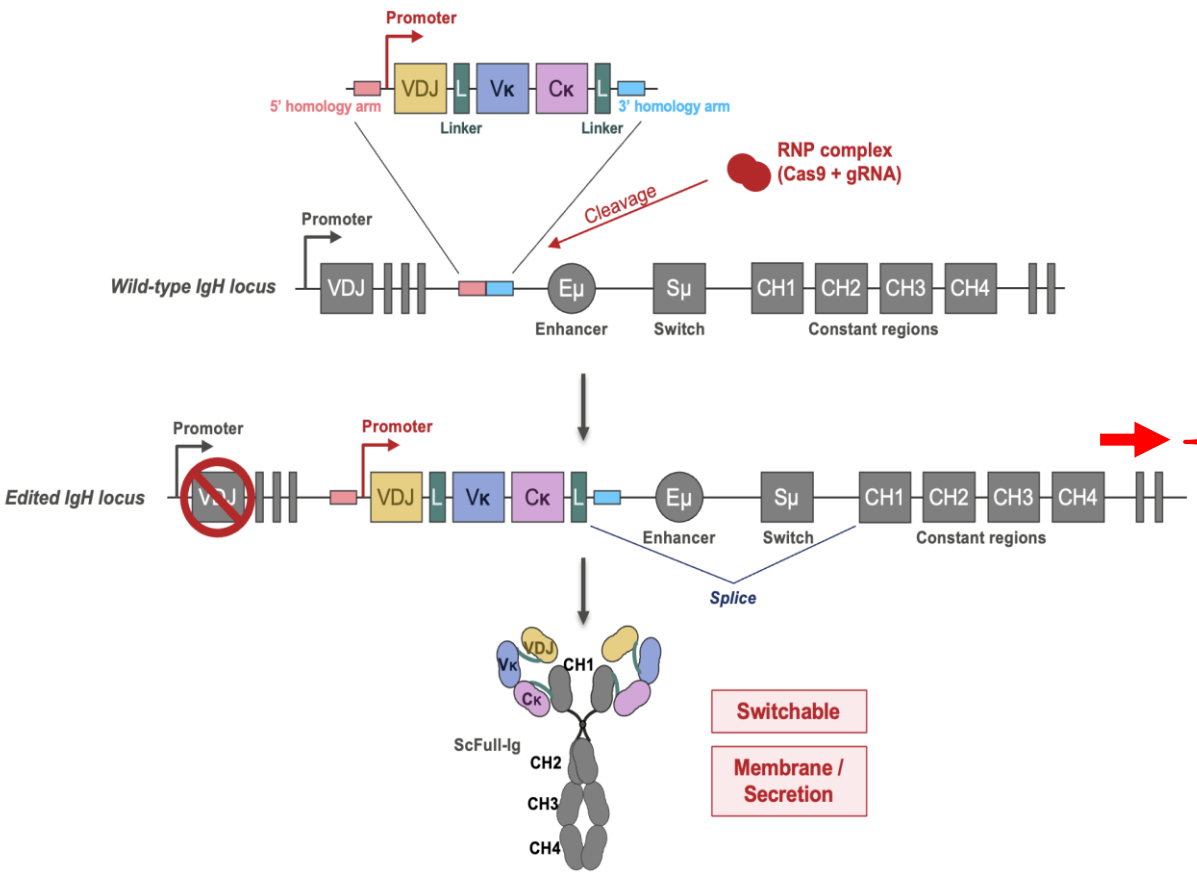
Work with a complete Ig molecule under a single-chain format: the « scFull Ig:

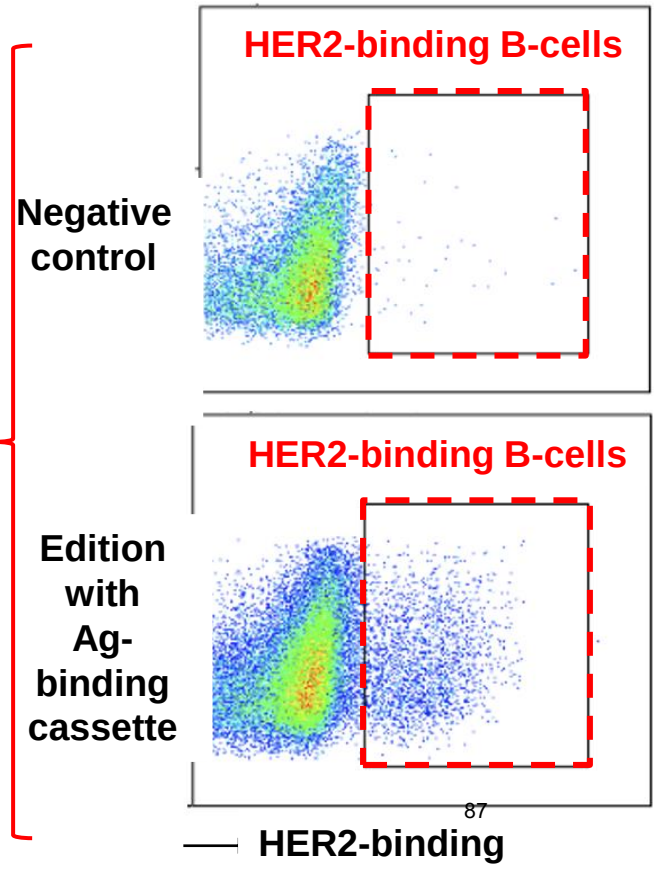


Topic 1: Edition of human primary B-cells for on-purpose binding of a tumor-specific antigen

Results:

- Classical model of the HER2 antigen
- Design of a pertuzumab-based single cell cassette for hijacking BCR expression in primary B cells
- Bespoken expression of an anti-HER2 single-cell B-cell receptor



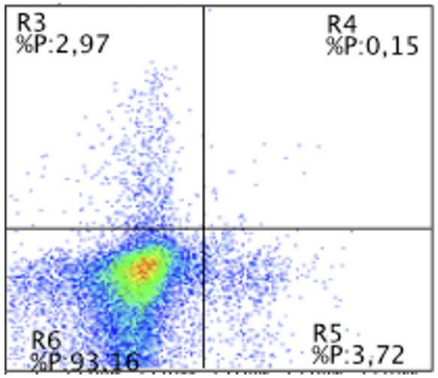


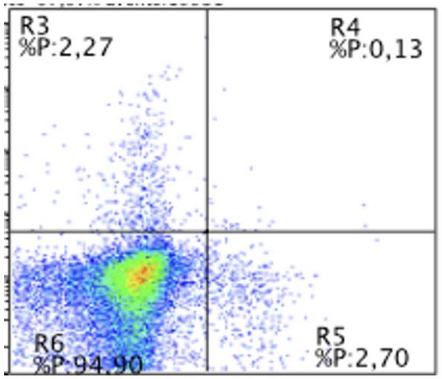
Topic 2: On-purpose class-switching in primary B-cells (example of the IgA2 isotype)

Results:

- Human primary B cells can be converted ex vivo in variant cells expressing a new Ig class (IgA2) with immunomodulatory functions that differ from IgM or IgG

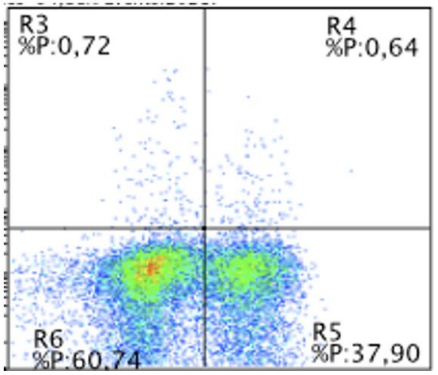
Control untreated primary B-cells



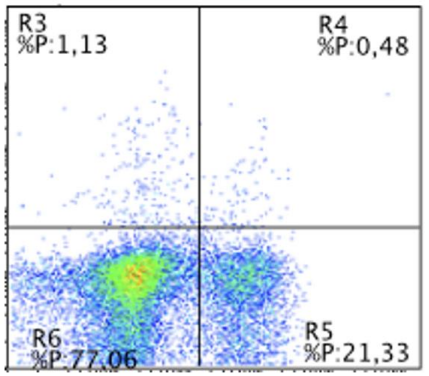


Experimental genetic switch

Buffy 1 (J7 : 1/08/2023)



Buffy 4 (J7 : 1/08/2023)



→ IgA2 BCR expression →

BAR-B cells for therapy

Perspectives:

- Optimization of the single-cell mAb format
- Application to new targets, for which unmet needs are defined in terms of Ab format
- Pre-clinical trial in Hu-SCID mice and later in primates
- Specific applications of the on-purpose class-switching strategy
- Optimization of gene edition and vectorization

Unique selling points

- The B-cell editing strategy can bring innovative solutions to a number of unmet needs with biotherapies (long-term therapy; therapy with short-lived or unstable mAb format, therapy with non-IgG classes…)
- On-purpose class switching can serve unique goals for modulating B-cell function and/or antibody function
- The team & unit UMR1036 are supported by Inserm, EFS and University Hospital providing unique access to human samples from donors and patients
- The team & unit master a broad range of methods from basic genetics, to cellular immunology, immunochemistry and immunomonitoring of biotherapies
- The team include clinicians involved into clinical trials with mAbs and/or CAR-T cells
- The team belongs to an academic consortium starting in 2023, with other teams involved into vectorization, immunomonitoring, trials in primates, gene edition (ANR PEPR 2023)
- The team belongs to Institut Carnot CALYM, encouraging industrial partnerships about lymphoma therapy

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Therapeutic innovation in pancreatic cancer

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Keywords
Pancreatic cancer
Patients cohorts
Biomarkers
Patients stratification
Animal models of cancer
Therapeutic resistance
First-in-man clinical trial
Intracellular single-domain antibodies
Oncolytic virus

Abstract
Our team, specializing in "Therapeutic Innovation in Pancreatic Cancer," has a strong focus and excels in identifying and targeting the mechanisms that underlie therapeutic resistance in pancreatic cancer. To achieve this, we leverage the most extensive collection of samples procured from patients at various stages of the disease, which includes comprehensive clinical data (the Bacap cohort, P.I. Prof. B. Bournet, with over 1500 patients enrolled to date). This unique resource encompasses primary cultures and PDX and is crucial in identifying patients' molecular profiles that predict the response to the therapeutic innovations we are currently developing. Our expertise centers on developing single-domain antibodies, engineered to selectively and efficiently disrupt protein-protein interactions or initiate the targeted degradation of proteins associated with tumor resistance to chemotherapy, particularly when combined with E3 ligase domains (P.I. Dr N. Béry). Additionally, we are pioneers in translating discovery research results into gene therapy first-in-man clinical trials involving over 100 patients to combat resistance to chemotherapy. This approach involves intratumoral injection using ultrasound endoscopy, in a close collaboration with the CHU of Toulouse (led by P.I. Prof. L. Buscail). Presently, we've transitioned our focus to proprietary oncolytic viral strains. These strains specifically replicate within and eliminate pancreatic cancer cells, offering the potential to induce a robust anti-tumoral immune response. For the latter, we have developed unique syngenic, experimental animal models of the disease (P.I. Dr P. Cordelier).

Research area
Oncology

Synopsis
We are expert in devising biotherapies to defeat therapeutic resistance to treatment, especially in pancreatic cancer, from discovery research to first-in-man clinical trials