

Bioproduction de vésicules extracellulaires : alternatives aux thérapies cellulaires et nano-vecteurs thérapeutiques

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MSC Med -> NABI

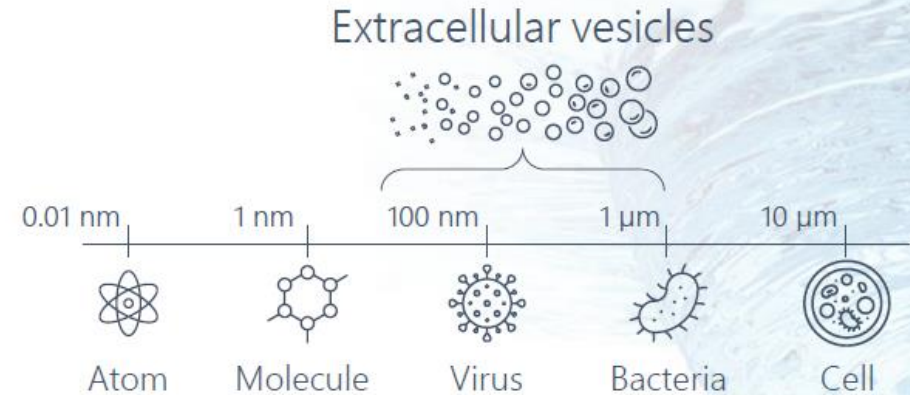
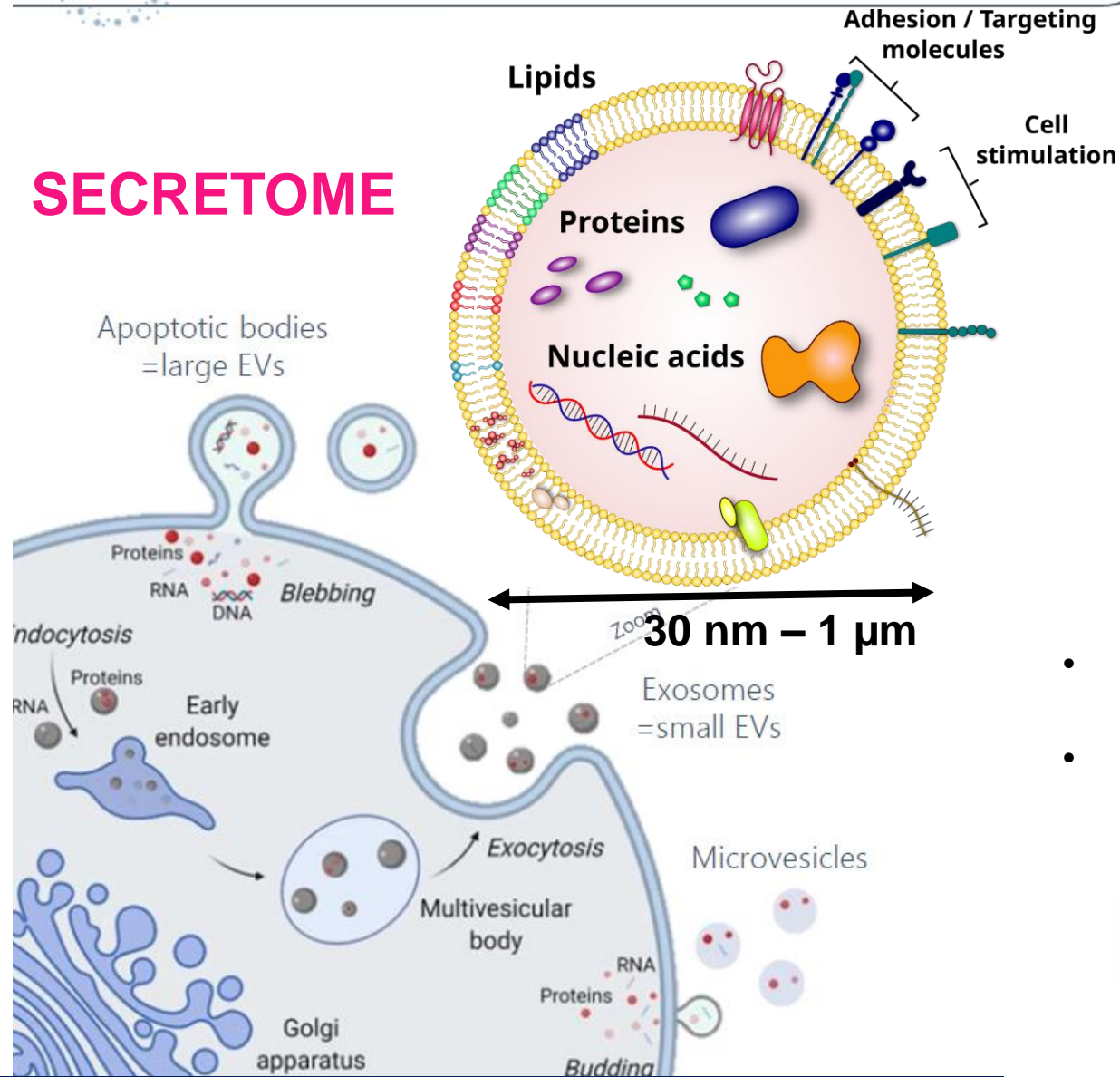
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4èmes Rencontres académie-industrie du CNC
Les usines du vivant : Génération et Transformation
de matériaux et de principes actifs
5 décembre 2024



What are extracellular vesicles (EVs)?

SECRETOME



Sub-cellular particles released by cells in healthy context or in response to stress

Contain proteins, nucleic acids and lipids on membrane or/and in cytoplasm

- Are actors of **intercellular** communications
- Keep the biomolecular **signature** and the **properties** of the secreting cells.

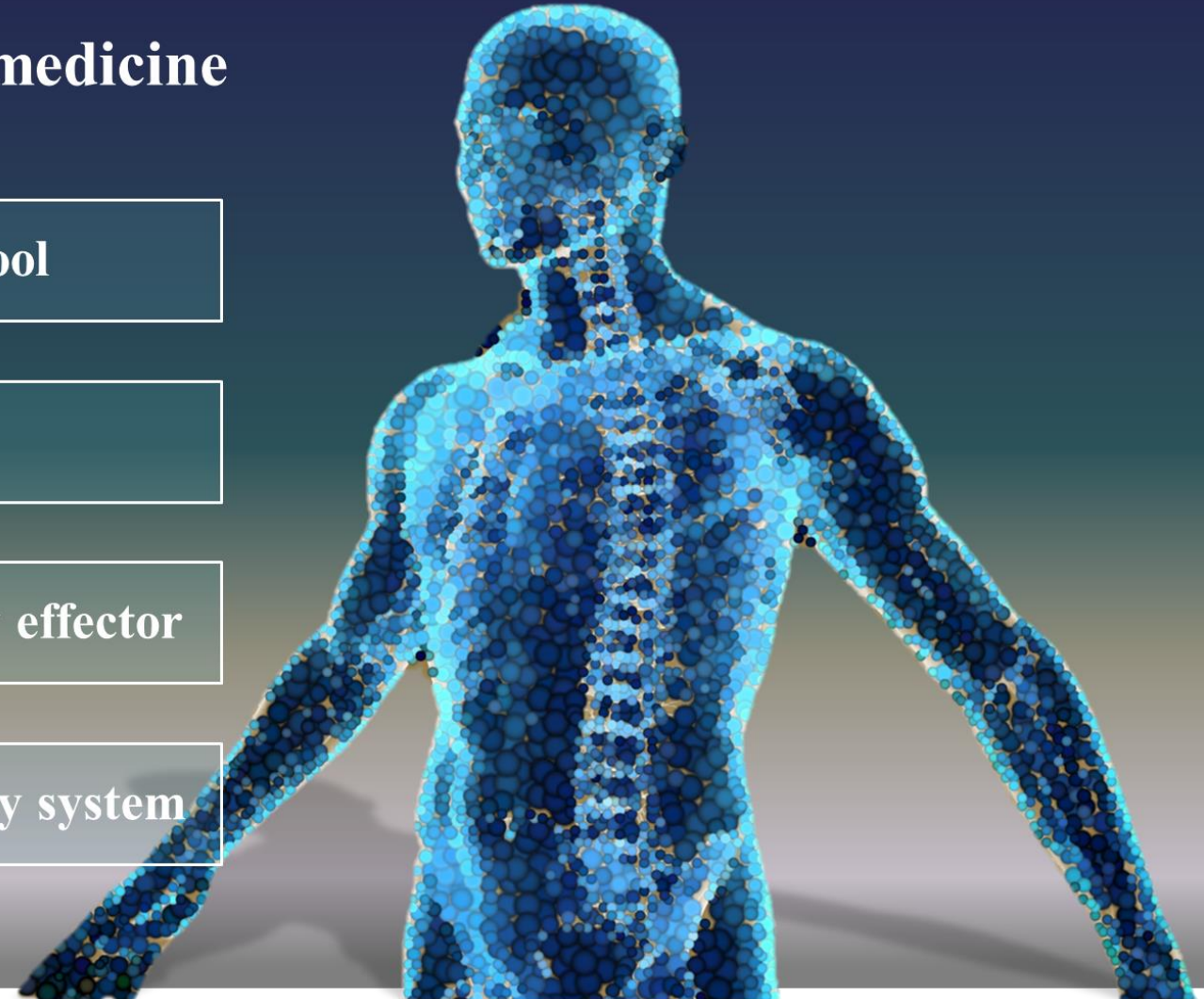
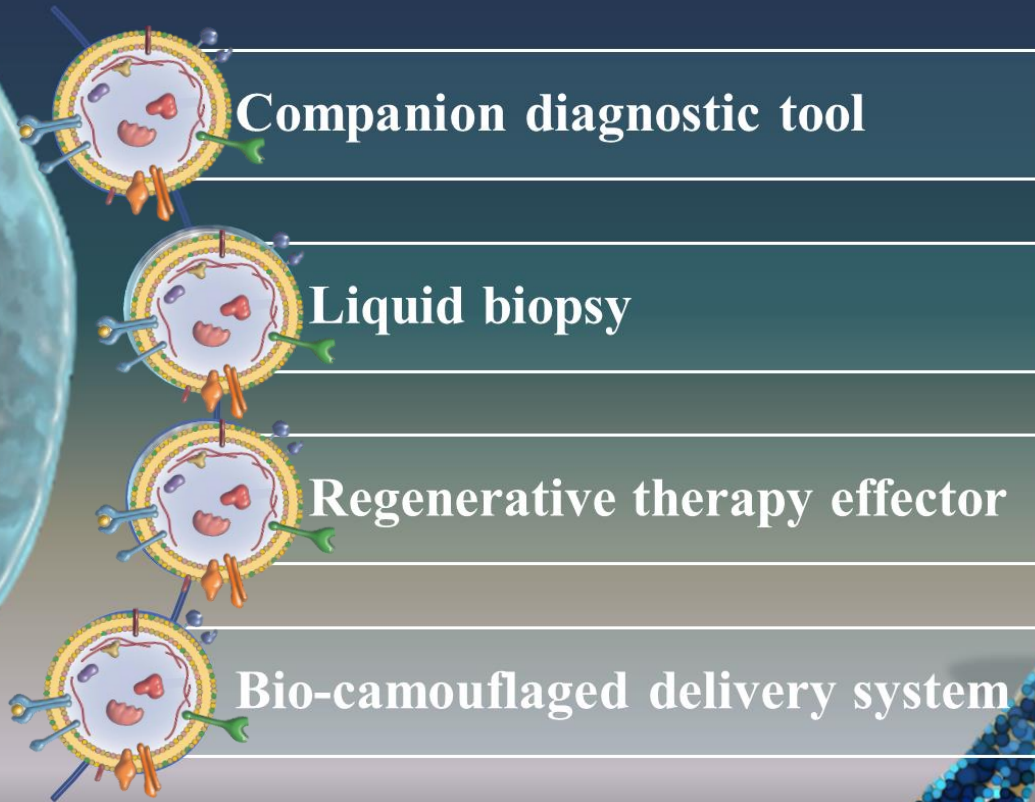
Ten times more EVs than cells in the body

EVs for diagnostics

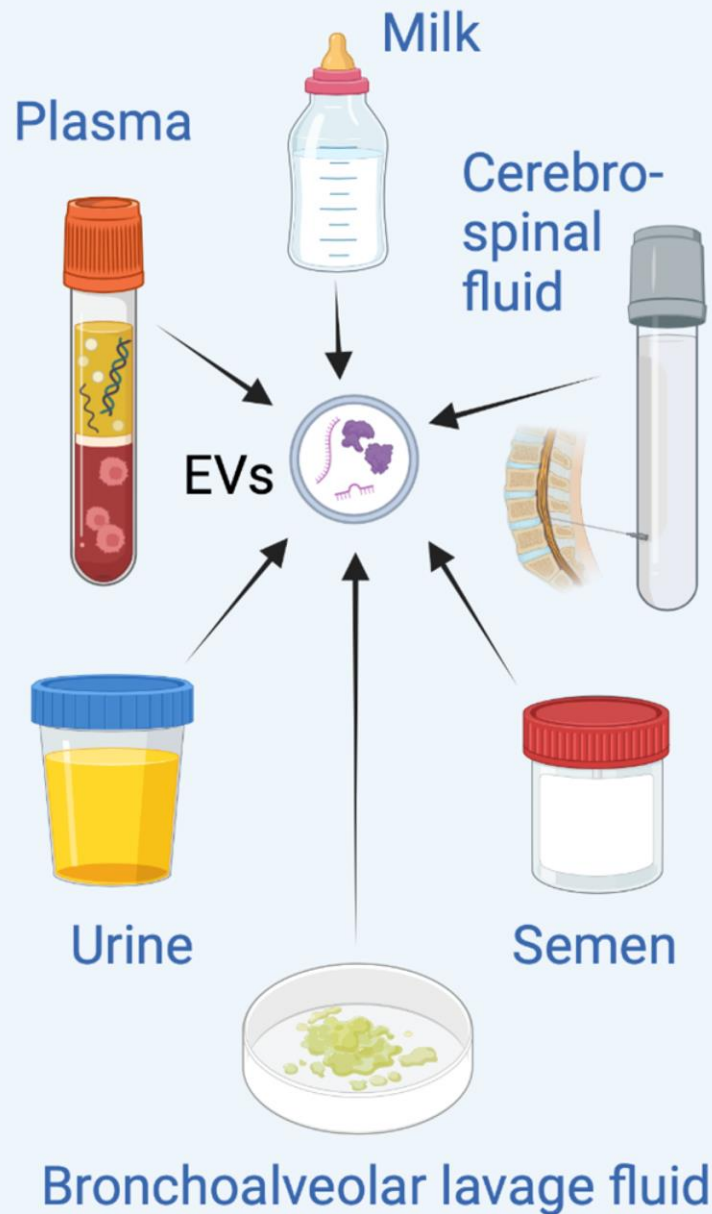
EVs for therapy

Introduction: Extracellular vesicles (EVs)

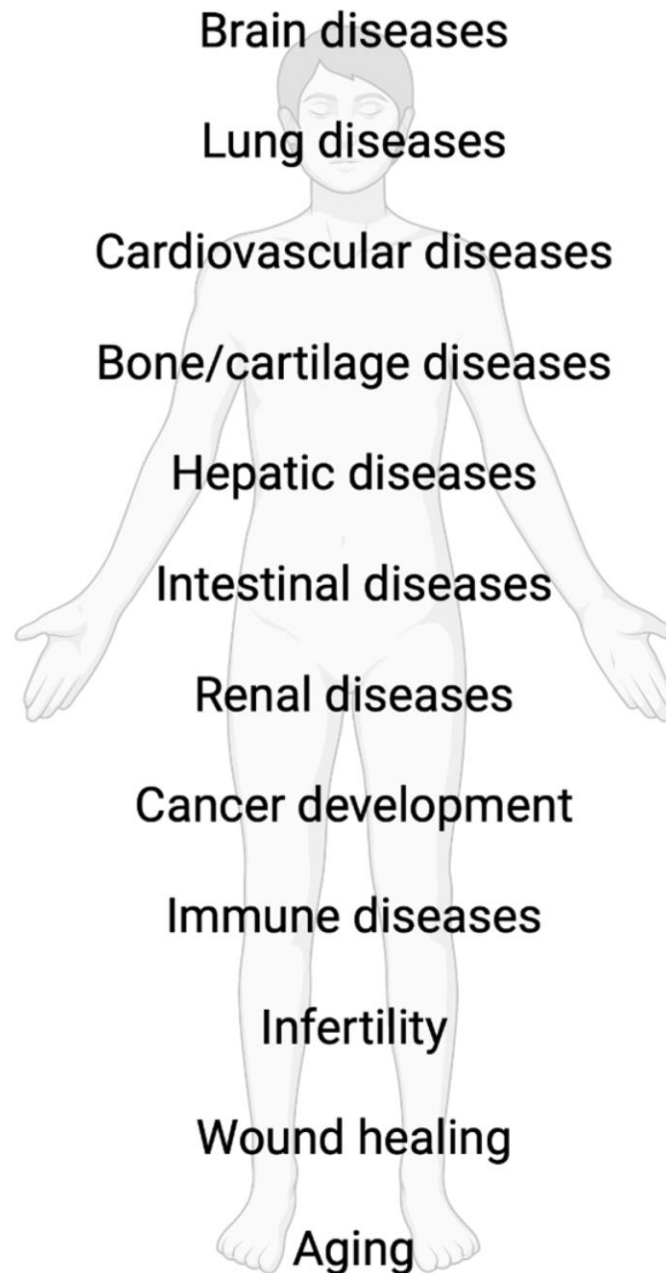
Extracellular vesicles in medicine



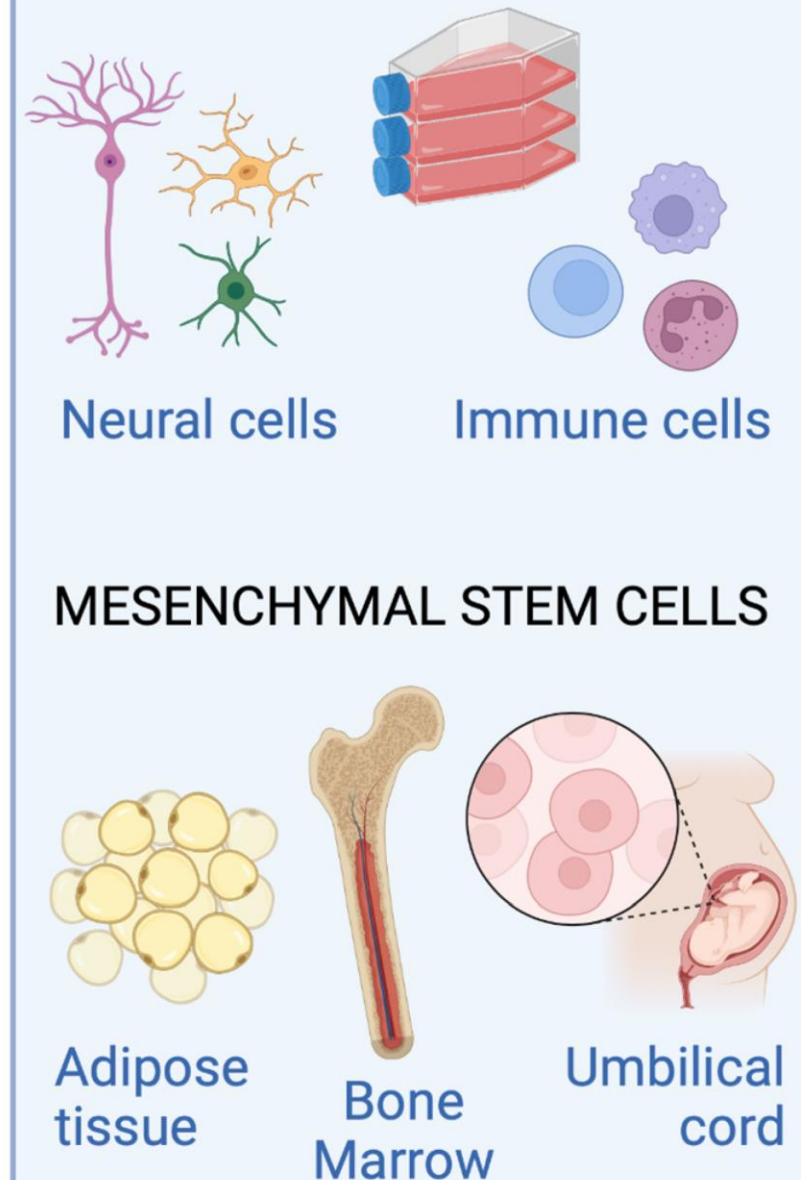
EVs from body fluids



EVs therapeutical uses



EVs from cell culture



Extracellular vesicles: a booming therapeutic modality



VS.



Alternative to cellular therapies

EVs are a condensate of their parental cells and keep their properties

No replication and no differentiation

Nanoscale size: no risk of occlusion of the blood capillaries

Low immunogenicity

Allogenic: several patients could be treated with one batch of EVs

Off the shelf product

Lyophilization and -20°C storage



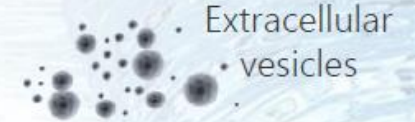
Viral vector



Synthetic Nanoparticle



VS.



Drug delivery vector

Biocompatible

Non-toxic

Possible modifications of the cargo (Proteins, RNAs, DNAs and lipids) or the surface proteins

Cross biological barriers

→ even the blood-brain barrier?

Targeting of a cellular type possible

Delivery efficiency



EVs for Therapy - A wide range of indications with key advantages

Main effects

- ✓ Regenerative potential
- ✓ Immunotherapy
- ✓ Can be engineered for drug delivery

Key advantages

SAFETY

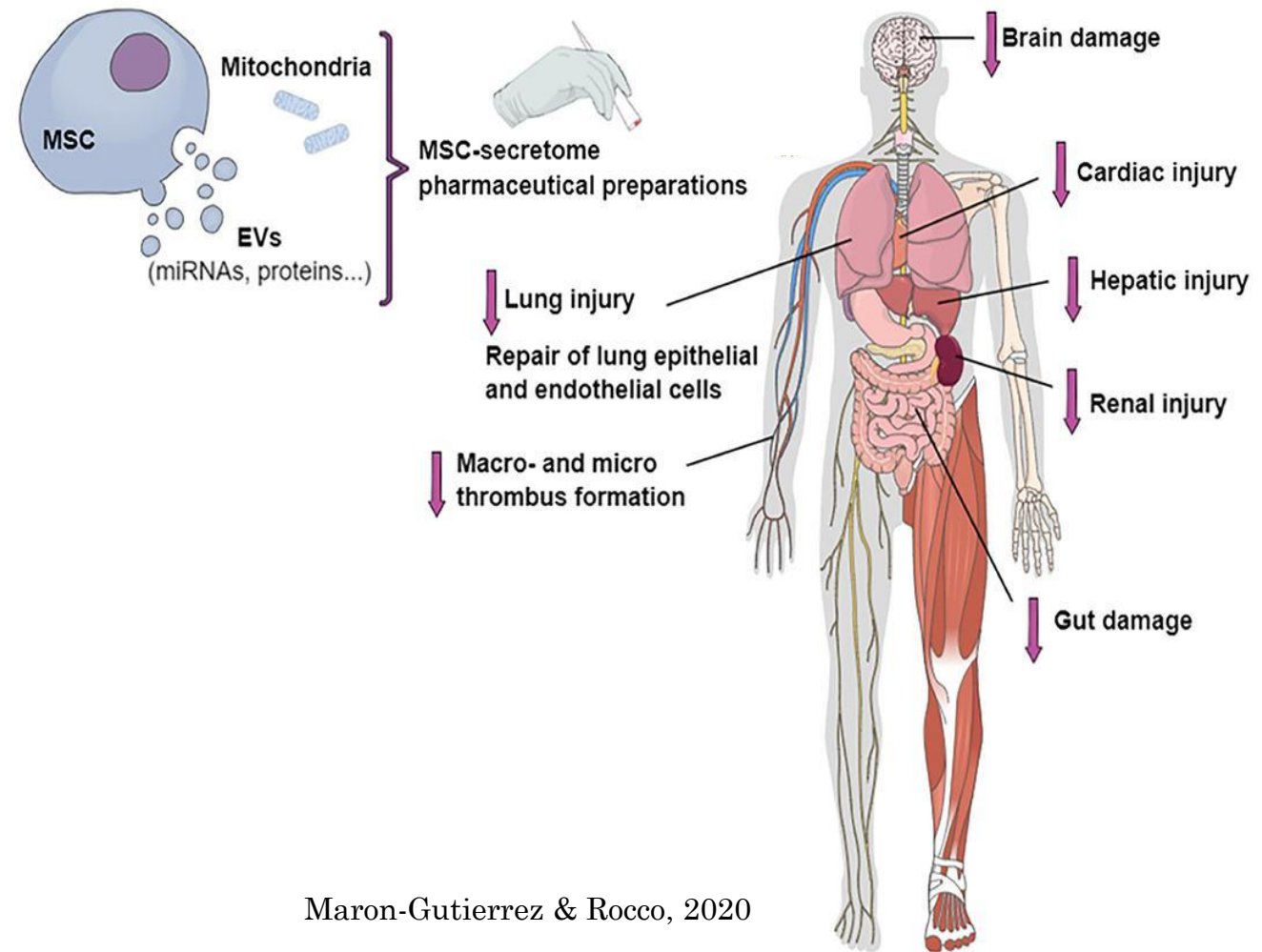
Better **benefit-risk balance** for patient than cell therapy

EFFICIENCY

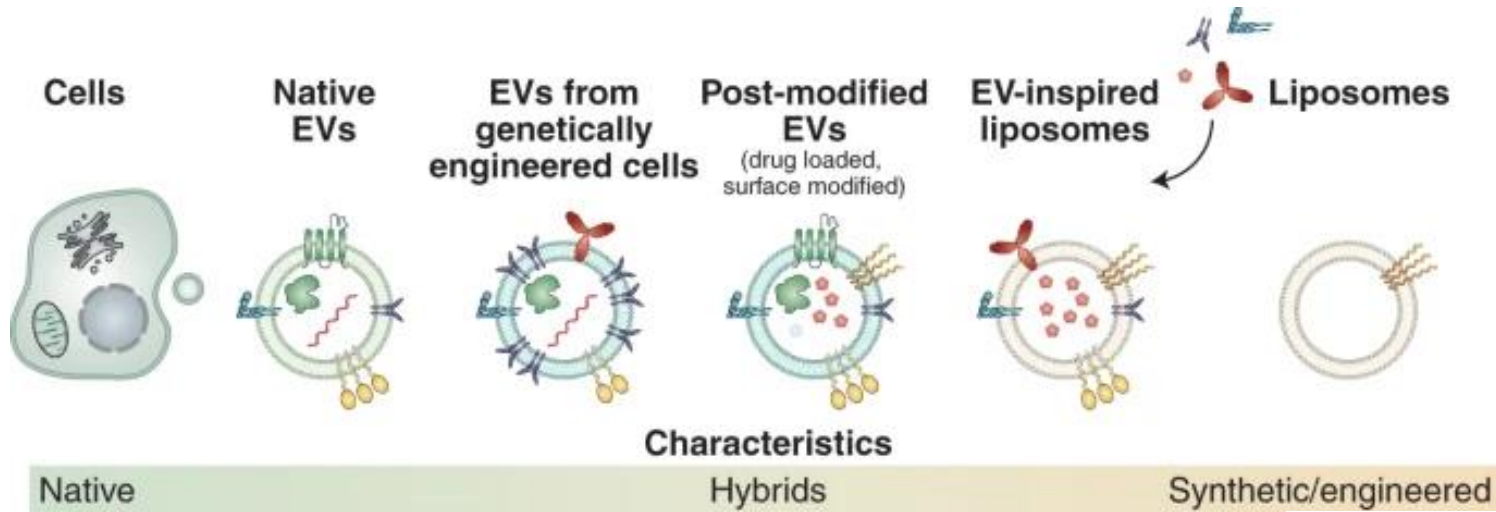
Cross biological barriers

ECONOMICAL POTENTIAL

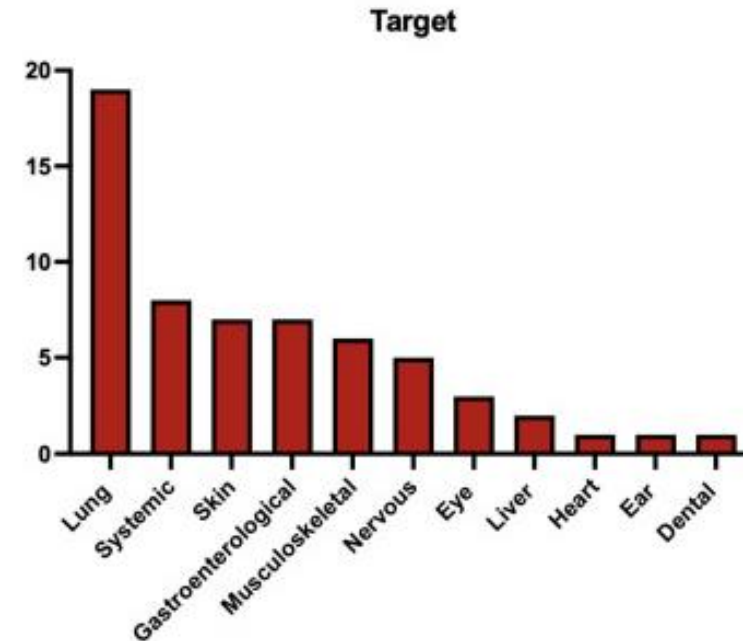
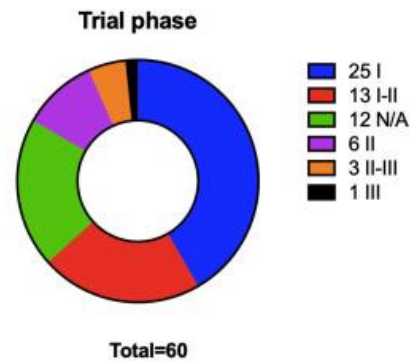
“Off-the-shelf” product



Extracellular vesicles : clinical applications



- Native EV, loaded or genetically modified
- >60 clinical trials
- Phase 1 - 2



How to expedite EV-based diagnostic, monitoring and biotherapies to the clinic ?



National Integrator Biotherapy-Bioproduction

IVETH innovation hub

Extracellular Vesicle Production, Engineering, and Characterization
for therapy and diagnostics



A. Silva



F. Gazeau



Université
Paris Cité

Why an integrator dedicated to extracellular vesicles?



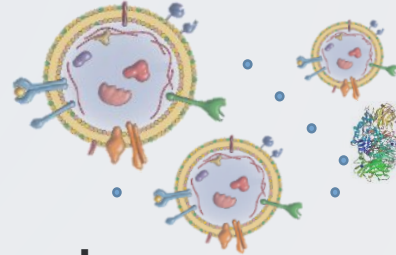
Specific features compared to more classical biotherapies/ biomarkers

Cell therapy



Secretome

EVs (microvesicles, exosomes, apoptotic bodies), soluble factors



Distinct nanosize range, complexity and heterogeneity



Specific challenges of bioproduction, engineering, analysis /quality control



Requiring scientific expertise consolidated in the field

+

Dedicated equipment with adapted protocols

Assisted by AI



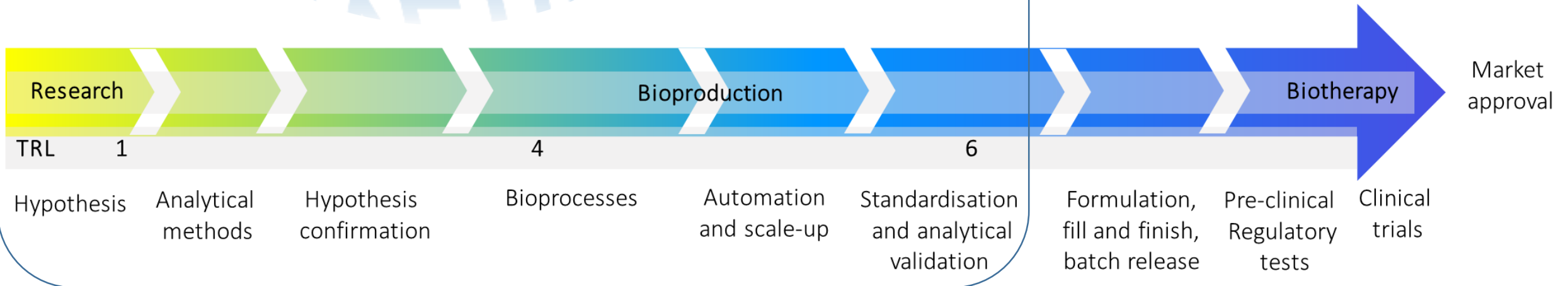
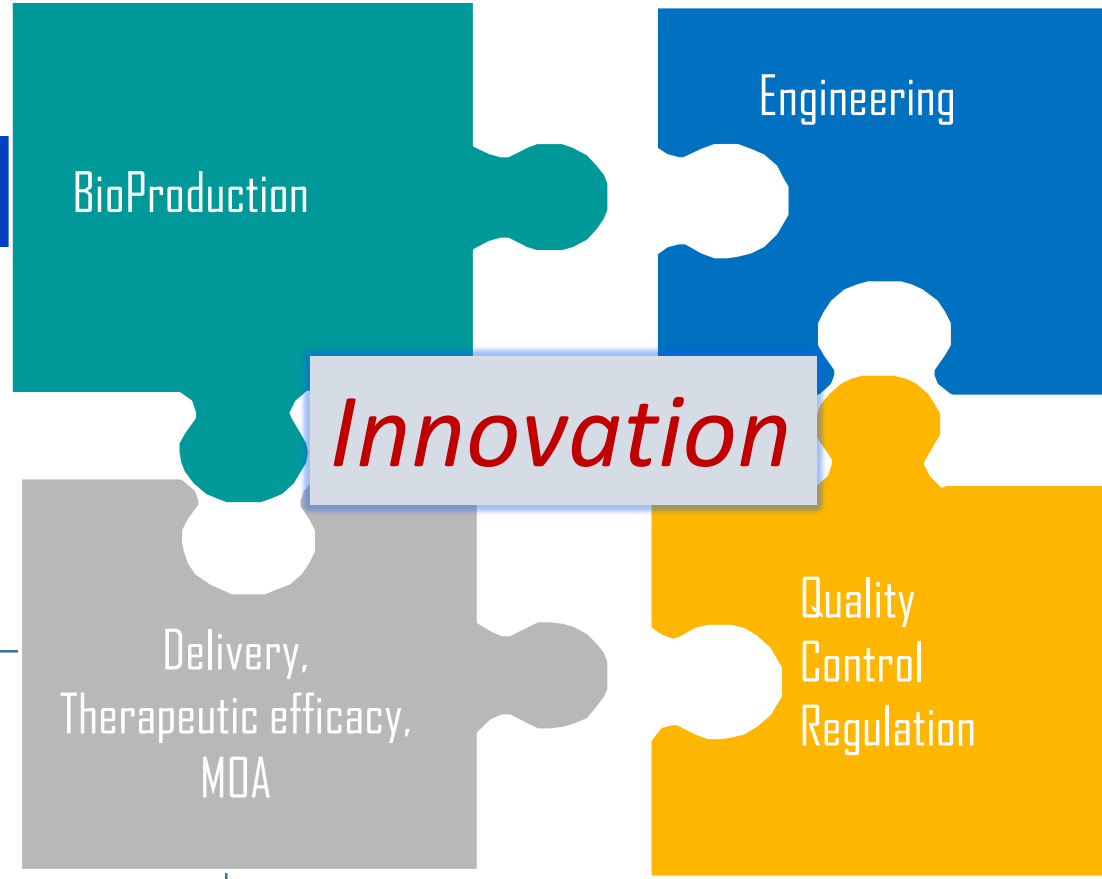
Recombinant protein



Specific needs to meet

Why IVETH Integrator ?

- ✓ 15 years of experience in the field of EVs
- ✓ Equipment inventory unique in Europe
- ✓ A multidisciplinary approach
- ✓ An innovation drive (4 licensed patents, 2 of the 4 French EV biotech start-ups from our technologies)
- ✓ An offer including all the steps prior to clinical batch production/release

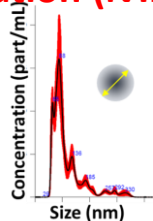


Multidisciplinary / multimodal / multiscale characterization toolbox

Secrétariat général pour l'investissement

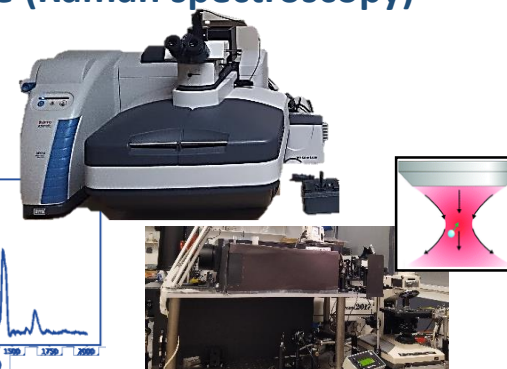
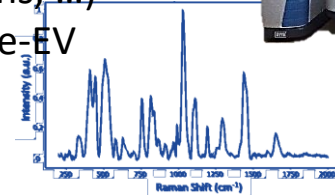
Physical characterization (NTA, ILM)

- Size
- Concentration



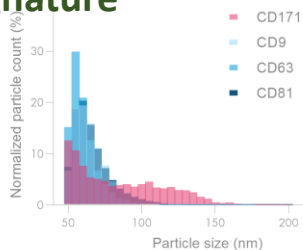
Biomolecular signature (Raman spectroscopy)

- Semi-quantitative analysis (RNA/DNA, lipids, proteins, ...)
- Bulk VS single-EV

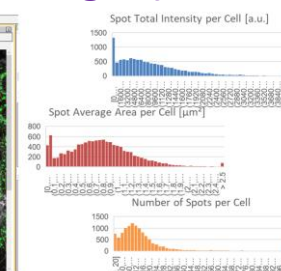
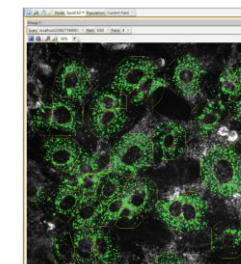


Phenotypic signature

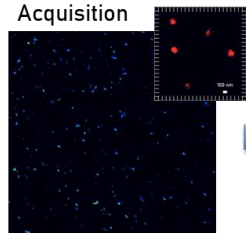
- Specific quantification (biomarkers)



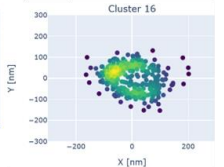
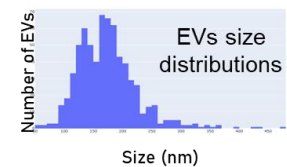
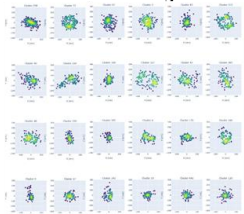
Potency (High content screening, *in ovo* testing, ...)



Acquisition



Statistical Single EV

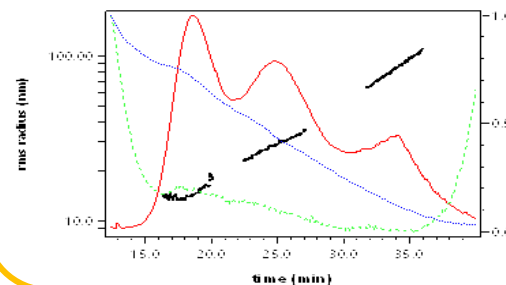


Nanoscopy (SMLM)



EVs morphology

Analytical separation + MALS + UV-Vis + RI + DLS



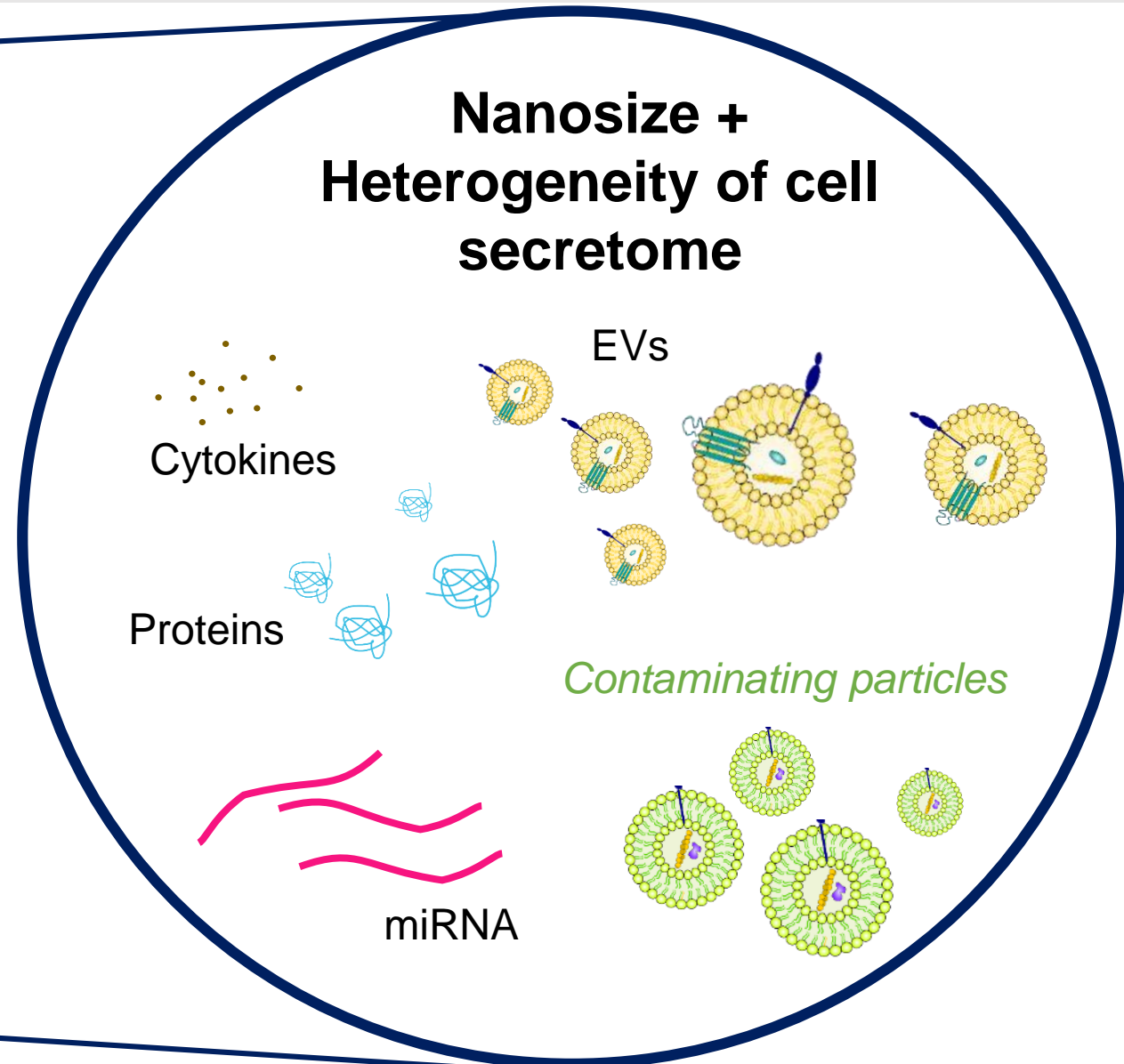
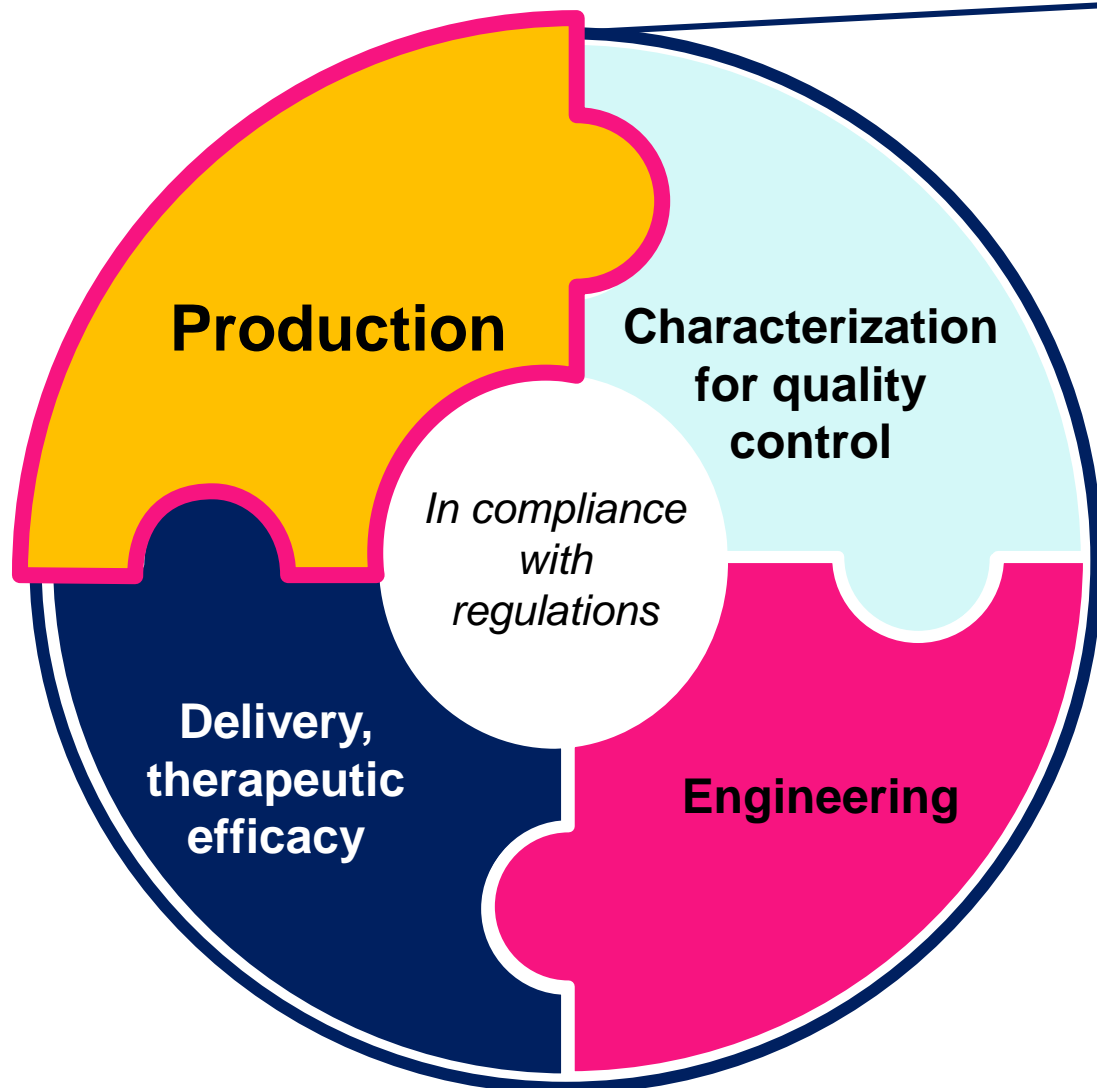
bpi france

ERG\NEO
L'AVENIR EST FAIT D'AUDACE

Inserm
La science pour la santé
From science to health

Région
île de France

Challenges to translate EV-based therapies to clinics



R&D Focuses - bioproduction

Quality control

–
in process control

**Upstream –
Bio-engineering**

**Upstream -
Bioproduction**

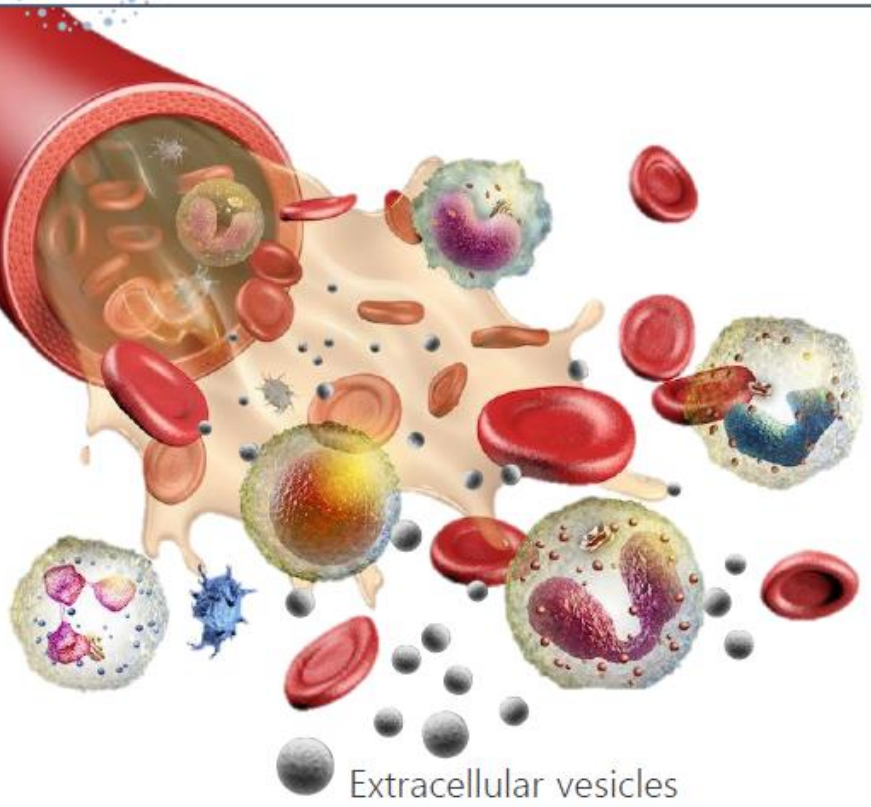
**Downstream -
Isolation**

**Quality control –
critical quality
attributes**

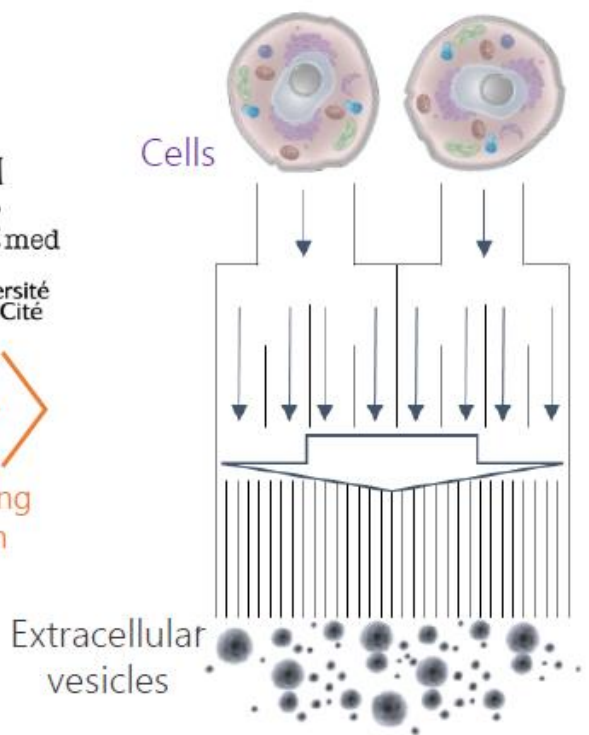
**Artificial intelligence
Multimodal statistical
analyses**



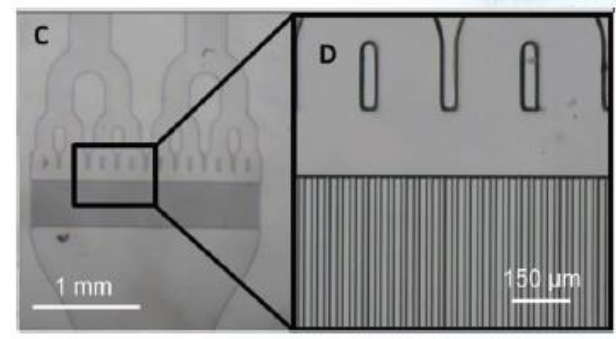
Turbulence stimulation: a bioinspired approach



Shear stress in blood vessels
(a natural process)
↗ Number of circulating EVs



Microfluidic chip to induce a shear stress and stimulate the cells.
↗ in yields but **no scalable**



Piffoux *et al.* Adv Biosystems (2017)



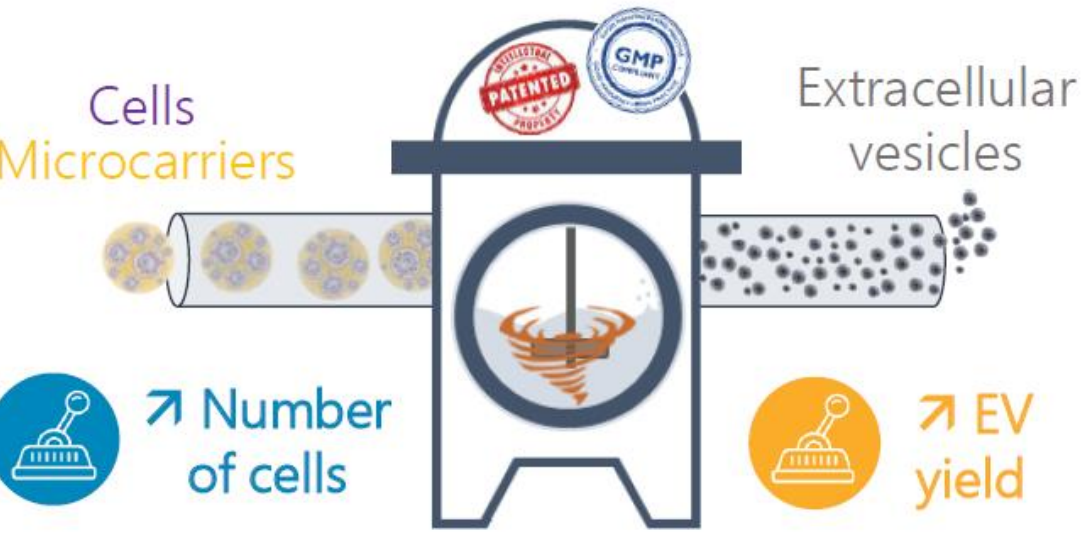
Stimulation by a turbulent flow
in stirred-tank bioreactors!



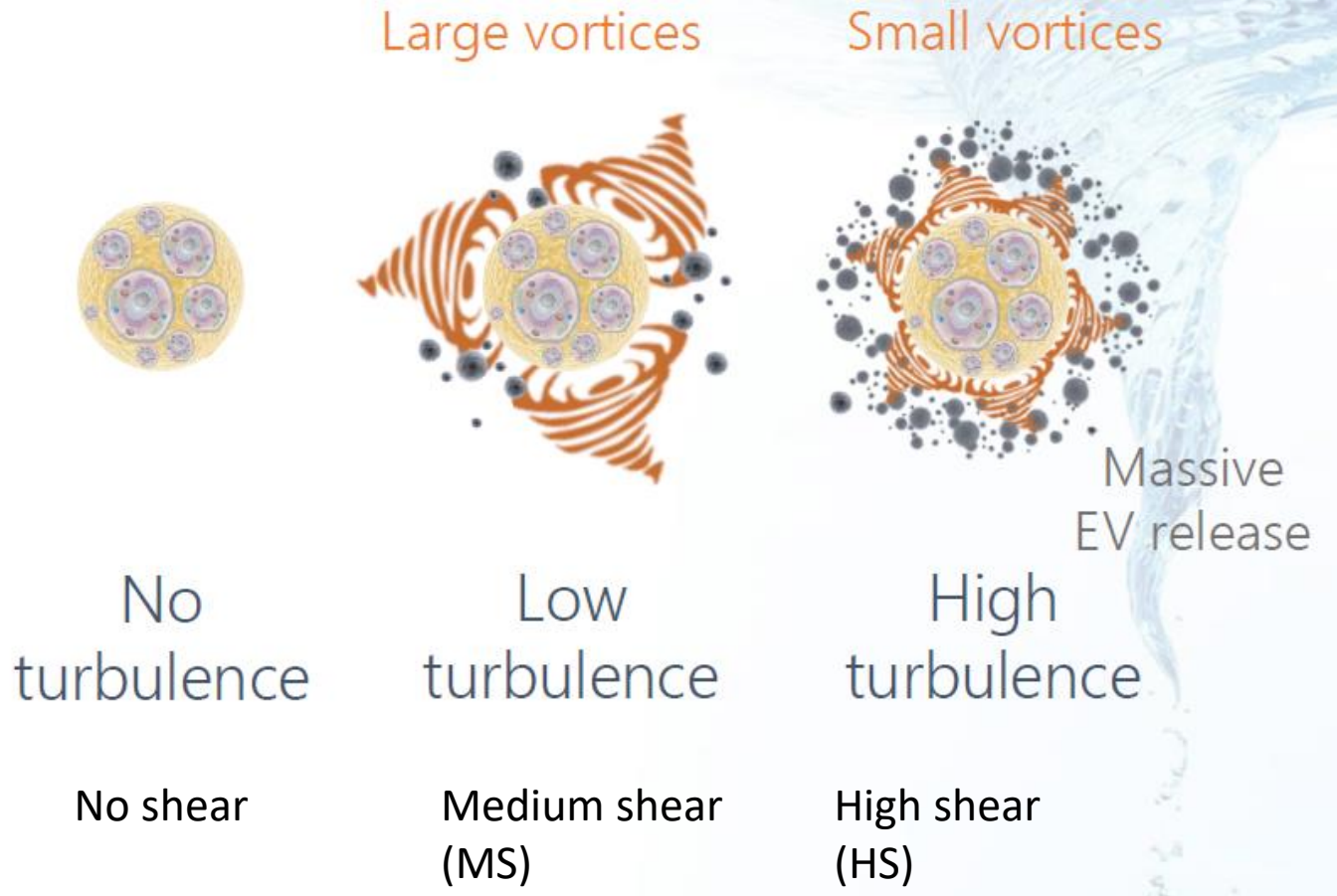
Stimulation by turbulent flow in a bioreactor system



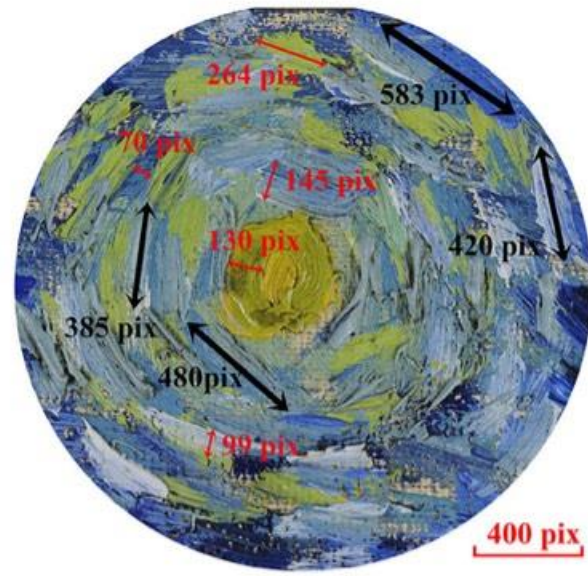
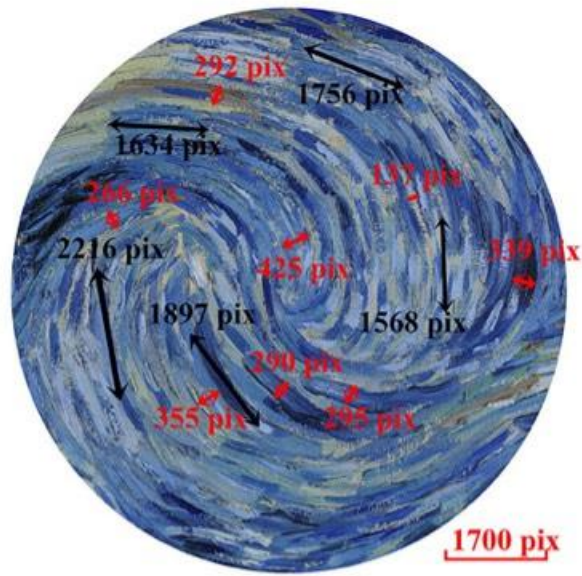
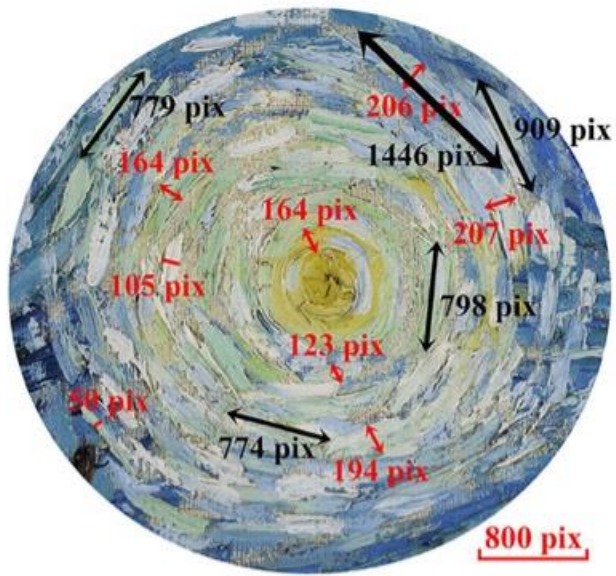
Turbulence stimulation



Well controlled cell mechanical stimulation in bioreactors by a turbulent flow induces massive extracellular vesicle production

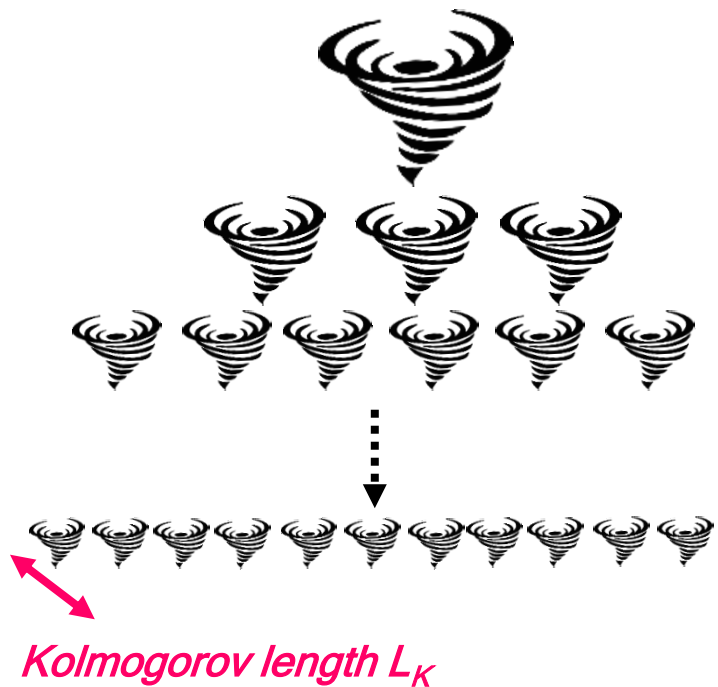






Physics of Fluids 36, 095140
 (2024)
<https://doi.org/10.1063/5.0213627>

How to scale up the process?



← Energy input

↓ Energy cascade

Is the Kolmogorov length a good scaling parameter?

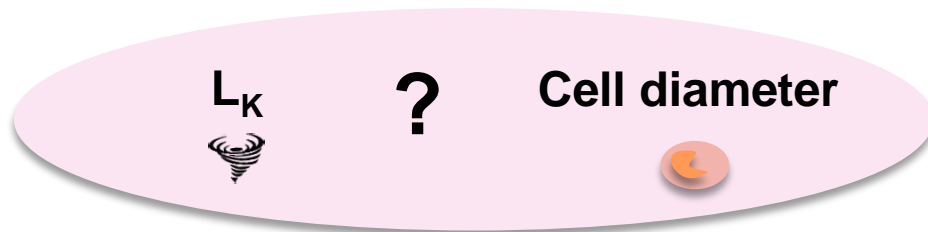
$$L_K = (\nu^3 / \epsilon)^{1/4}$$

ν : Kinematic viscosity

ϵ : Mean dissipation energy rate per unit mass

$$\epsilon = N_p \times N^3 \times D_i^5 / V$$

N_p : Power number
 N : Stirring speed
 D_i : Impeller diameter
 V : Working volume



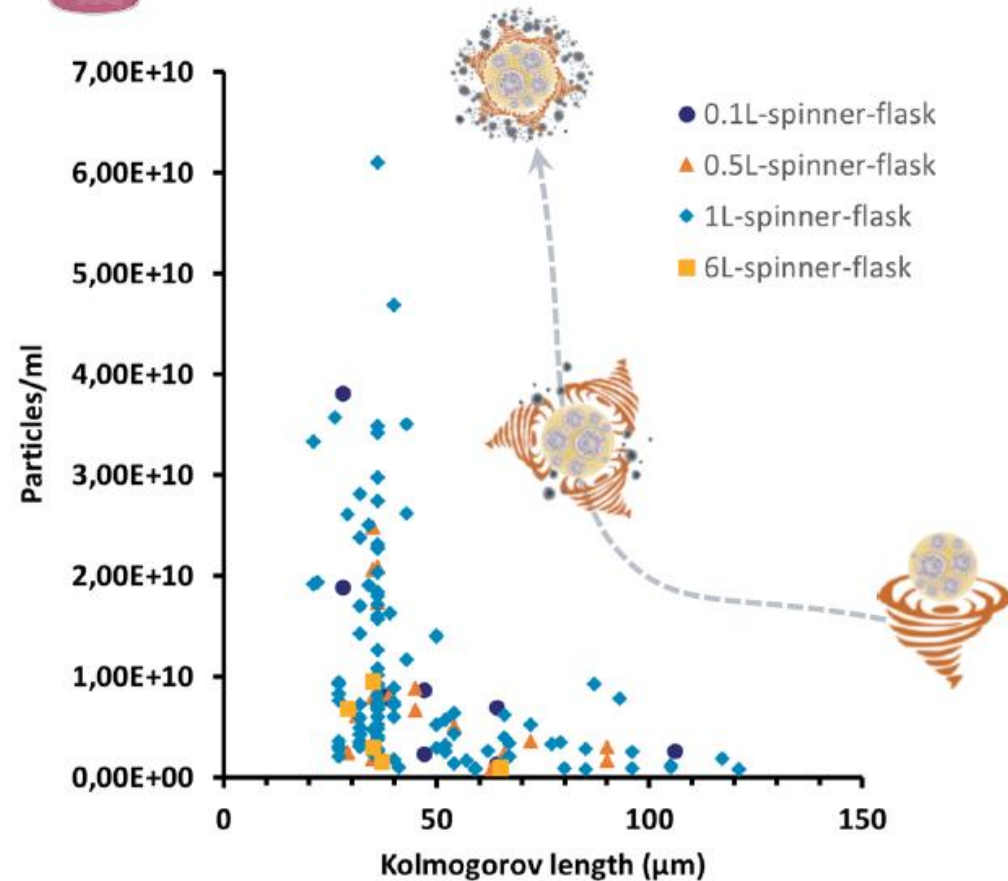
From 100 mL to 1 L ...



Stimulation by turbulent flow in a bioreactor system



Kolmogorov length as scaling parameter



High yield: up to 10 times higher and 20 times faster compared to cell starvation & effective *in vivo* (pig fistulas...)



Turbulent flow highly tunable and easy to implement into existing GMP grade systems (*i.e.* bioreactors)



Scalable: successful scale-up to 10L with human adipose derived stem cells (hASCs)



Process compatible with different cell lines (15 already tested, both in adherence and suspension)

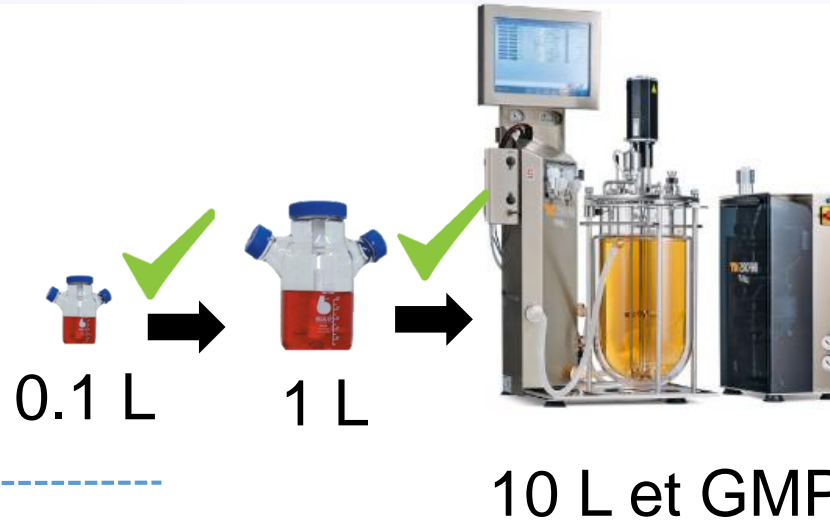
From academic research to start up funding



EVERZOM



Turbulence EV
Bioproduction



Jeanne Volatron CEO



2019 (licence of 4 patents)



19 employees



2,5 M€ + 3 M€

European
Innovation
Council



Scale up to 50 L,
Manufacturing clinical batches 2024

R&D Focuses - bioproduction

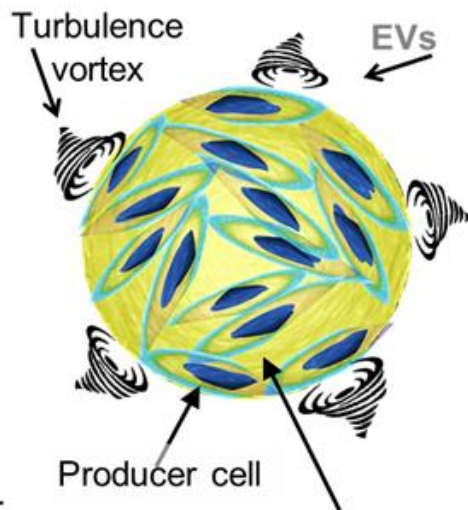
In process -
Quality contr



Oct 2024

Bio-
engineering

EVERZom achieves first bioproduction of clinical grade stem cell-derived exosomes in large scale bioreactors in partnership with the French Blood Establishment (EFS) with unprecedented yields



- *The disruptive, proprietary technology developed by EVERZom offers a yield 100 times higher than traditional methods.*
- *This innovation has just been tech-transferred and validated in a GMP-grade biomanufacturing environment.*
- *This first clinical batch will enable EVERZom to initiate regulatory non-clinical studies of its first drug candidate EVERGel™.*

10L – bioreactor → >10¹³ EVs

GMP bioreactor

3 patent applications
licensed to



digestive fistulas in Crohn's disease

Abnormal digestive organ communications
Crohn's disease

=> **High morbidity, poor healing rates**

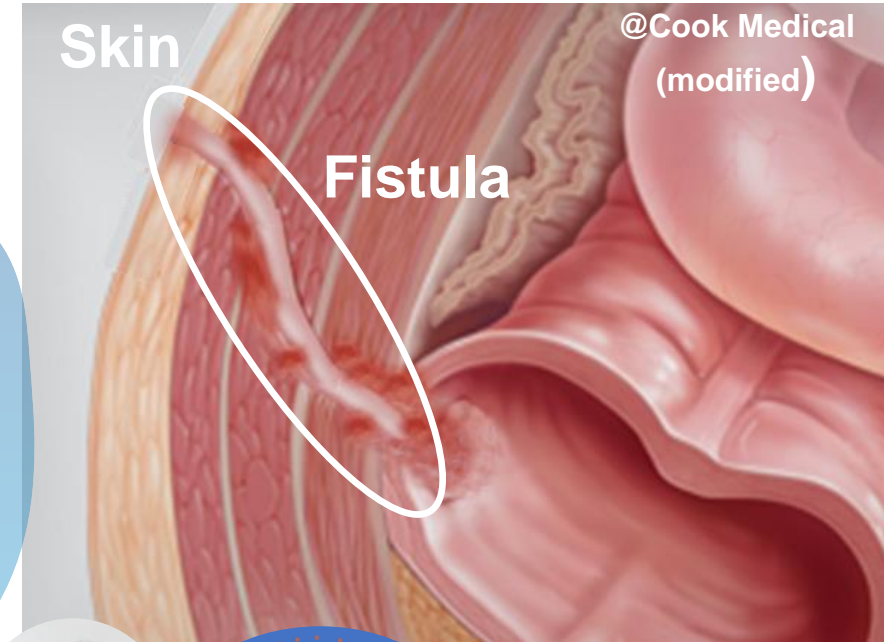
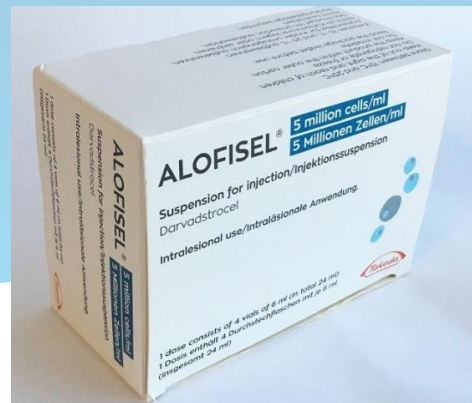
First allogeneic stromal cell therapy approved in Europe

50 K€ / dose of 120 million Adipose stromal cells

Immunomodulation effect

51% of fistula remission

(36% for standard of care)



Amanda Silva ERC

Georgiev et al. *J Gastrointest Surg* 22, 2003 (2018)

Panés et al. *Gastroenterology* 154, 334 (2018)

How to fulfil unmet needs - efficacy, safety, cost, logistics?

Our hypothesis

Extracellular vesicles (EVs) may advantageously replace Stromal Cell Therapy

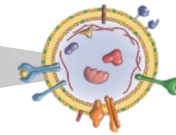
Today

Cell therapy



Tomorrow

EV therapy



No replication

No differentiation

Extended shelf life

Reduced immunogenicity

Administration /delivery roadblock in EV translation to clinics

Challenges

EV Retention in the target

EV administration by intravenous injection



**Fast clearance,
off-target biodistribution**

Pre-clinical study

Gap

Clinical translation

Hypothesis

EV delivery locally by a fistula occluder gel for synergy

Challenges

Administration

Concepts

EV carrier gel

Goals

Retention on the target
Mechanical effect

Pre-clinical study

Clinical translation



Repurposing of a thermoresponsive gel for fistula occlusion: Poloxamer 407 gel

**Authorized vessel
occluder
medical device**



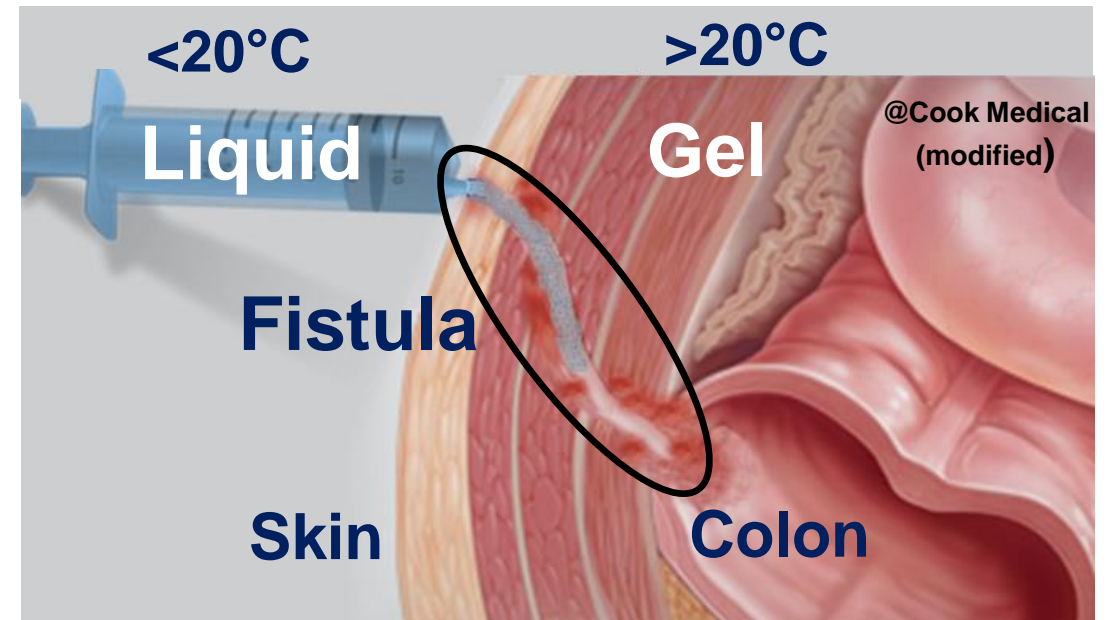
Repurposing of a thermoresponsive gel for fistula occlusion: Poloxamer 407 gel

Authorized vessel occluder medical device

Repurposing

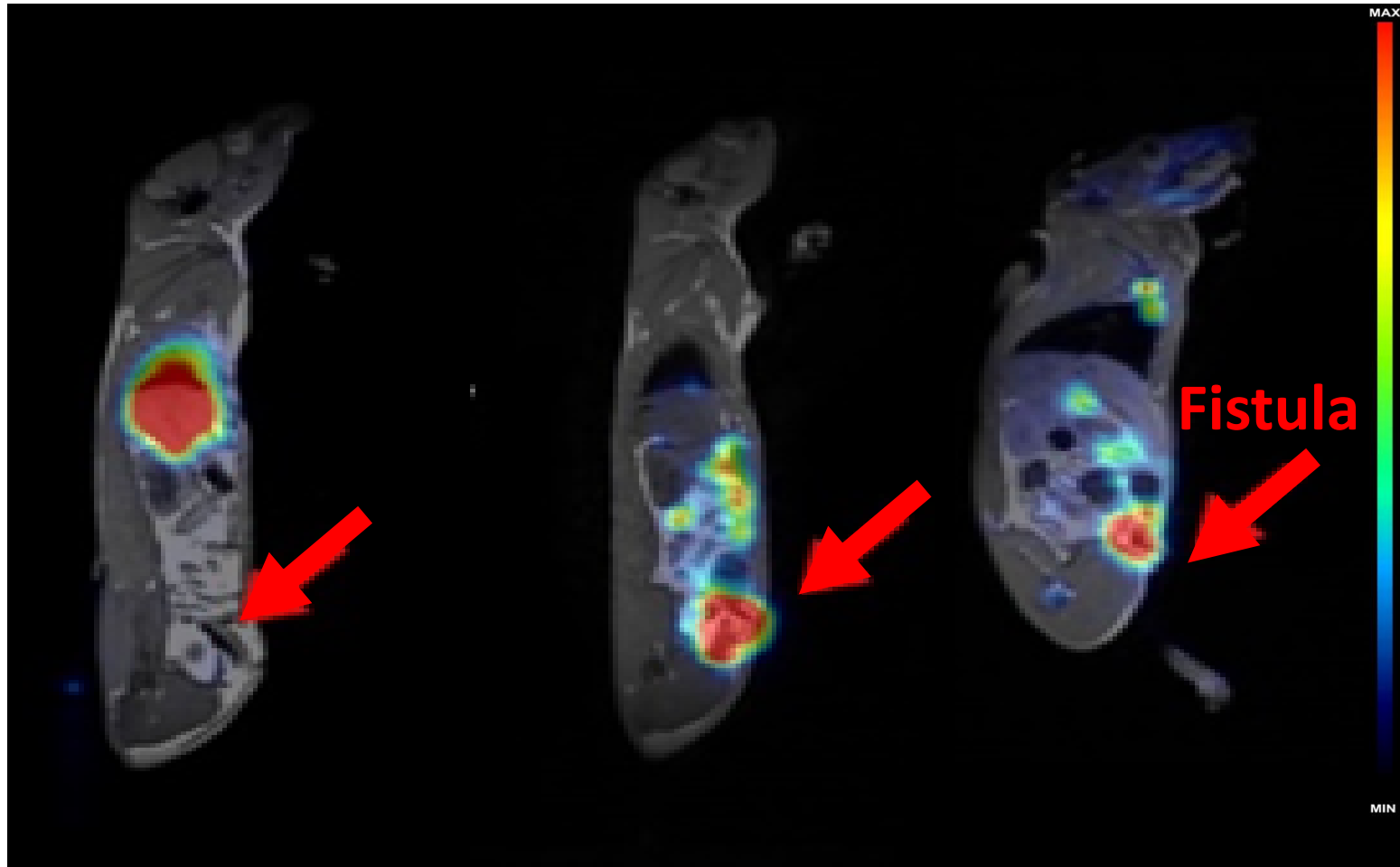


Fistula occluder



A Silva et al. Patent EP161788856

Local administration using the thermoresponsive gel reduces off-target distribution of allogenic MSC EVs: rat colocutaneous fistula model



PET-MRI Images 1h
after the administration of
EVs labelled with
a PET tracer – colocutaneous
fistula model in rats

EV intravenous injection
In saline

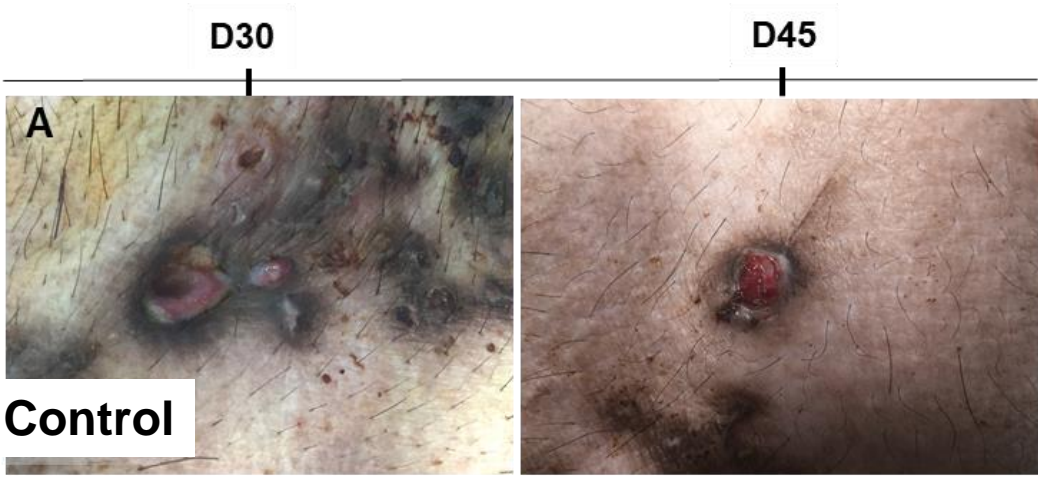
EV local injection
In saline

EV local injection
In the gel

Berger *et al. Nanoscale*,
2021, 13, 218-232

Allogenic adipose stromal cell EVs in gel induced oesophageal fistula closure in pigs

0% closure



Control

67% closure

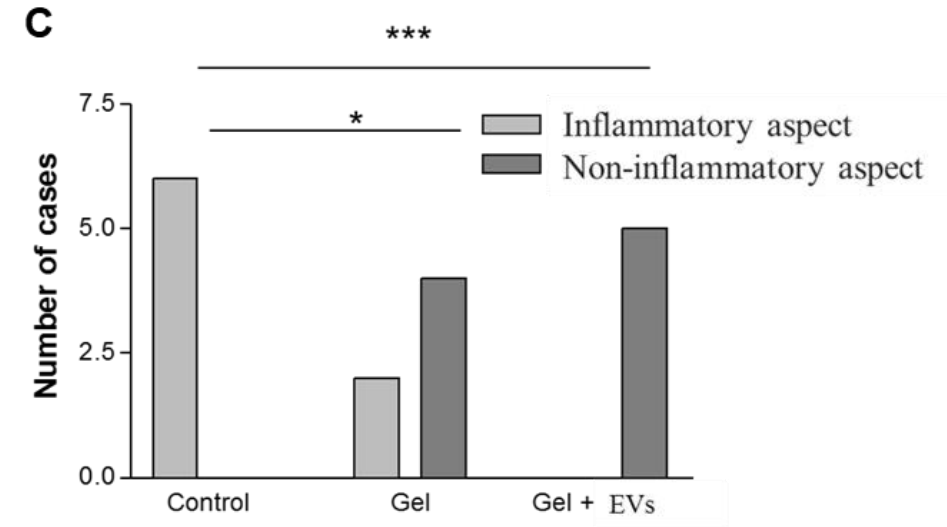
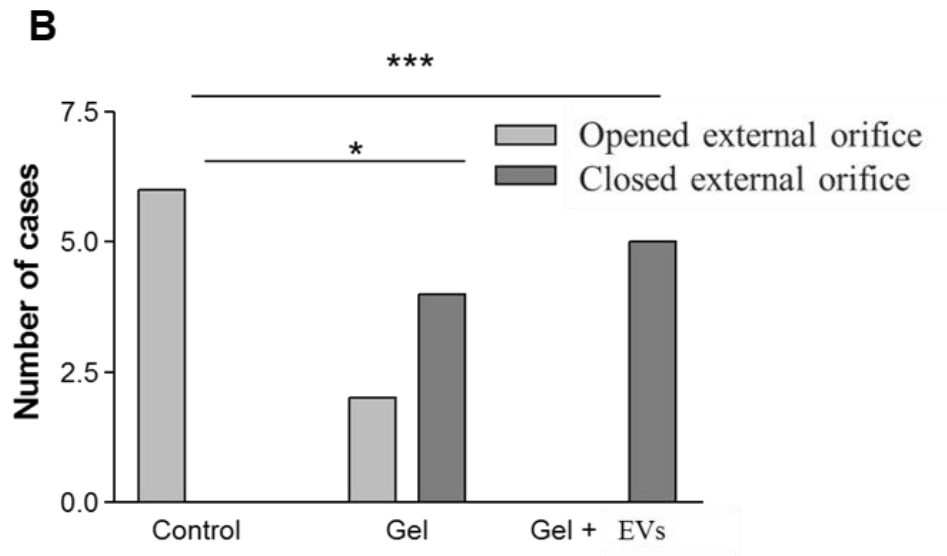


Gel

100% closure

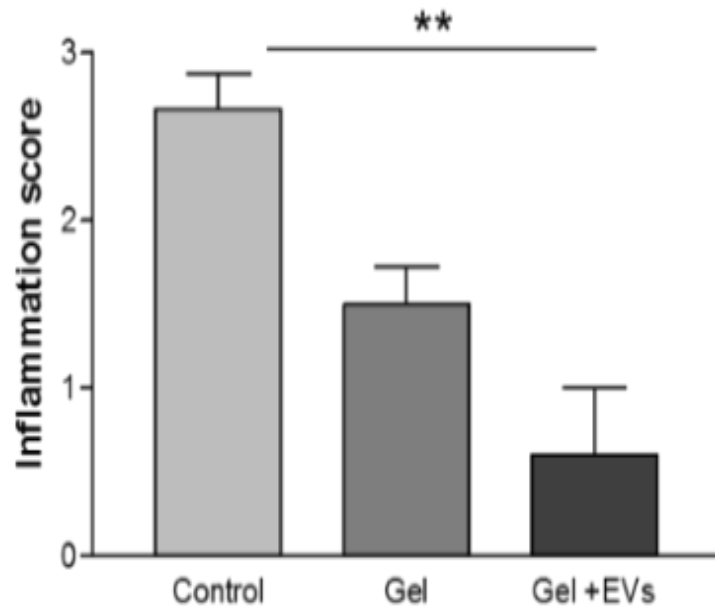


Gel + 10¹² EVs

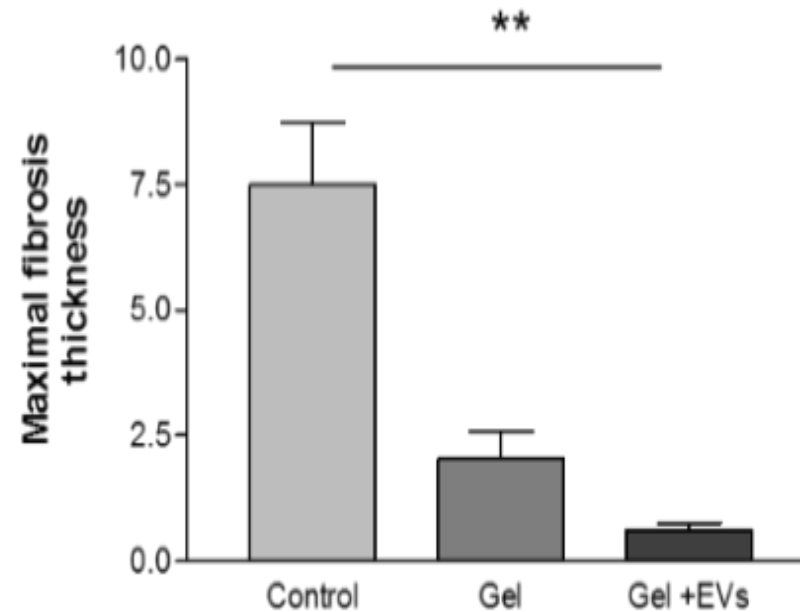


Allogenic adipose stromal cell EVs in gel induced oesophageal fistula closure in pigs

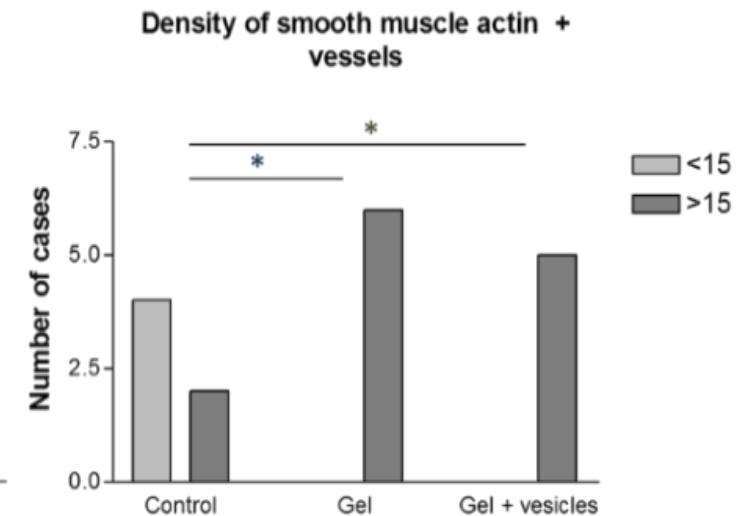
Reducing inflammation



Reducing fibrosis

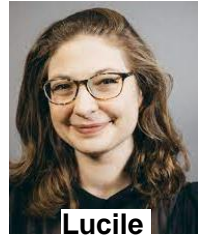


Favoring angiogenesis



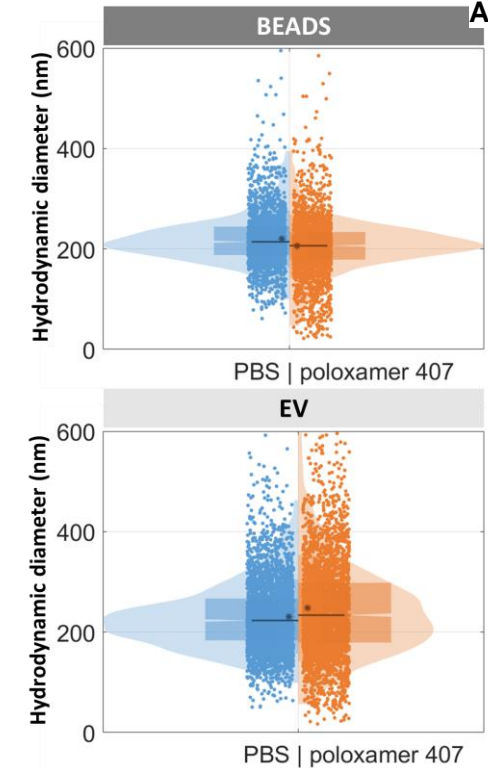
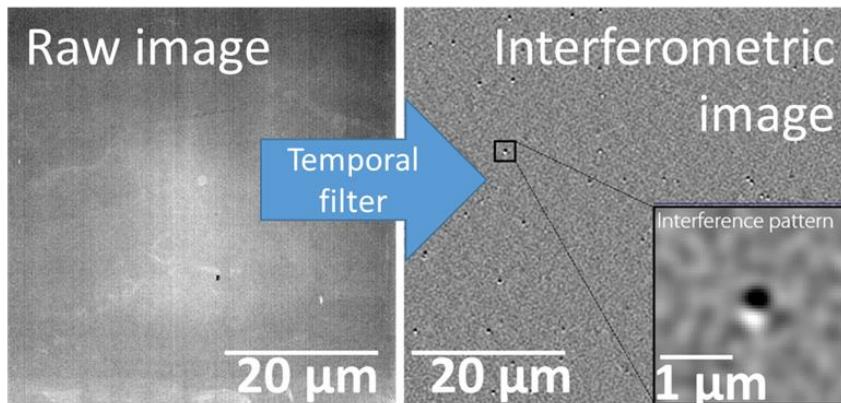
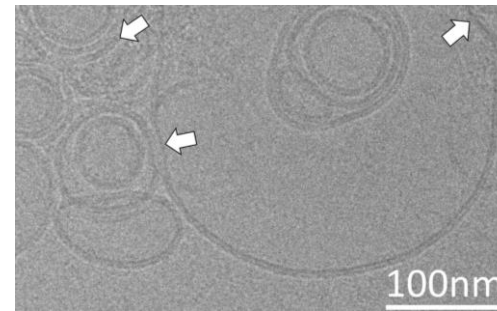
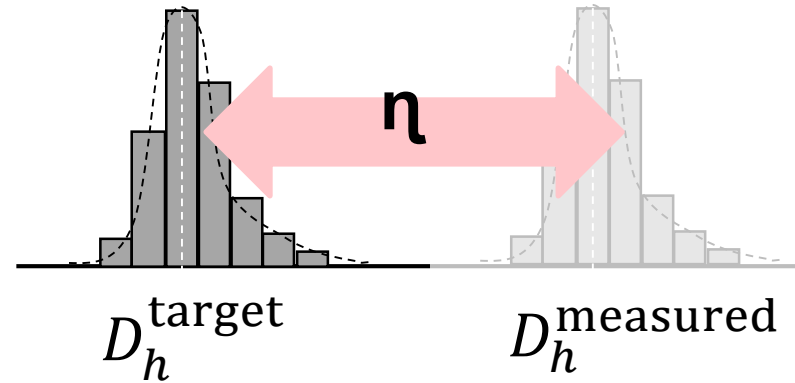
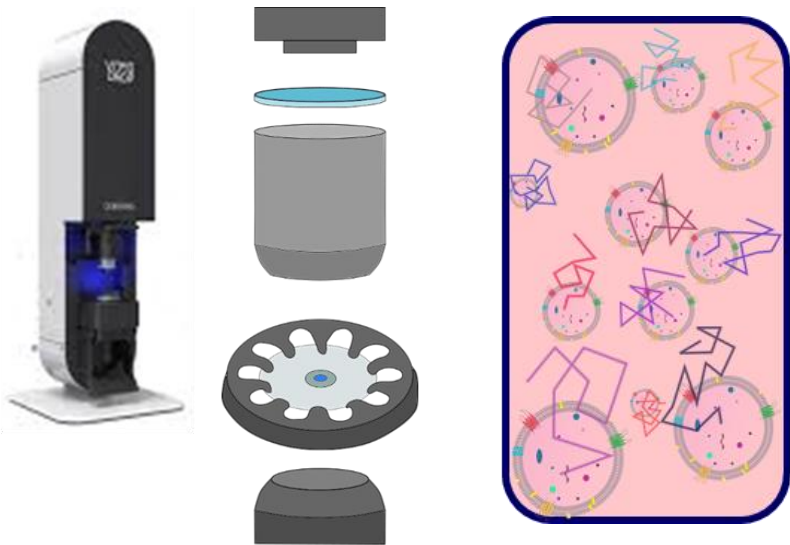


Marie Berger



Lucile Alexandre

myriade



Lucile Alexandre et al. **Investigating Extracellular Vesicles in Viscous Formulations: Interplay of Nanoparticle Tracking and Nanorheology via Interferometric Light Microscopy** Small Science 2024
10.1002/smsc.202400319

Our pipeline for fistula therapy

Third generation product:
Thermo-responsive
hydrogel plus EVs

Pre-clinical
stage

Second generation product:
Thermo-responsive
hydrogel plus contrast agent

Pre-clinical
stage

First generation product:
Thermo-responsive
hydrogel

Pre-clinical
stage

Clinical
stage

Waiting for clinical grade
GMP EV batch from
Everzom (2024)





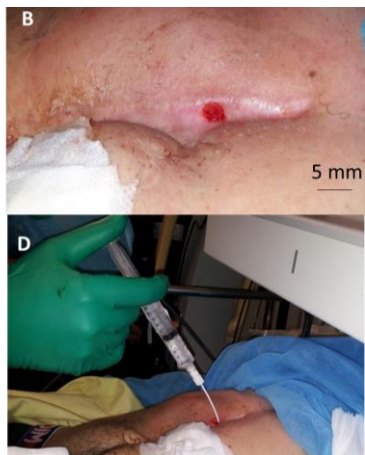
Clinics and Research in Hepatology and
Gastroenterology
Volume 45, Issue 4, July 2021, 101474



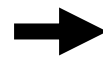
Case report

Enhancing digestive fistula healing by
the off-label use of a thermoresponsive
vessel occluder polymer associated with
esophageal stent placement: A case
report

Arthur Berger^{a, b}, Eric Caudron^c, Guillaume Perrod^{a, b}, Imane Boucenna^d,
Florence Gazeau^d, Claire Wilhelm^d, Anne Berger^a, Olivier Clément^c,
Christophe Cellier^a, Amanda K.A. Silva^{d, 1}, Gabriel Rahmi^{a, b, 1}  



4 mm



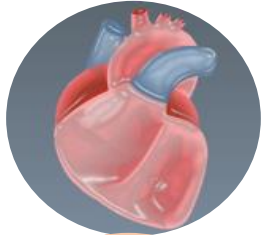
1 mm



Berger et al. Clin Res Hepatol Gastroenterol
2020;S2210-7401(20)30169-8

6 PATIENTS: PARTIAL FISTULA HEALING

Turbulence-triggered EVs: proof of regenerative effect in 6 models



Myocardial infarction model in mice



Iris Marangon
Post-doc



Inflammatory perianal fistula model in rats



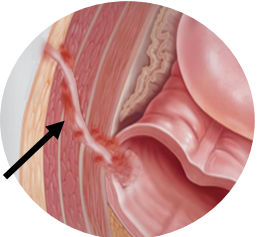
Boris Rosenbaum
(MD), Master student

Post-surgical colo-cutaneous fistula model in rats

Nanoscale,
2021, 13, 218-232



Artur Berger (MD)
PhD student



Post-surgical gastro-cutaneous fistula model
in rats and pigs



Guillaume Pere (MD)
Master student

Esophageal stricture in pigs

Nanoscale,
2021, 13, 14866-78



Elise Coffin (MD)
Master student

Rat model of radiation induced colitis

Journal of Crohn's and Colitis,
2023
Communication Biology,
2024

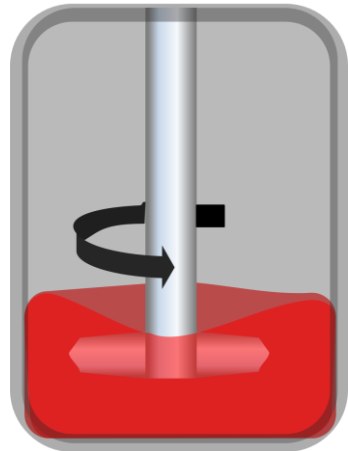


Anna Sebagh
(PhD)



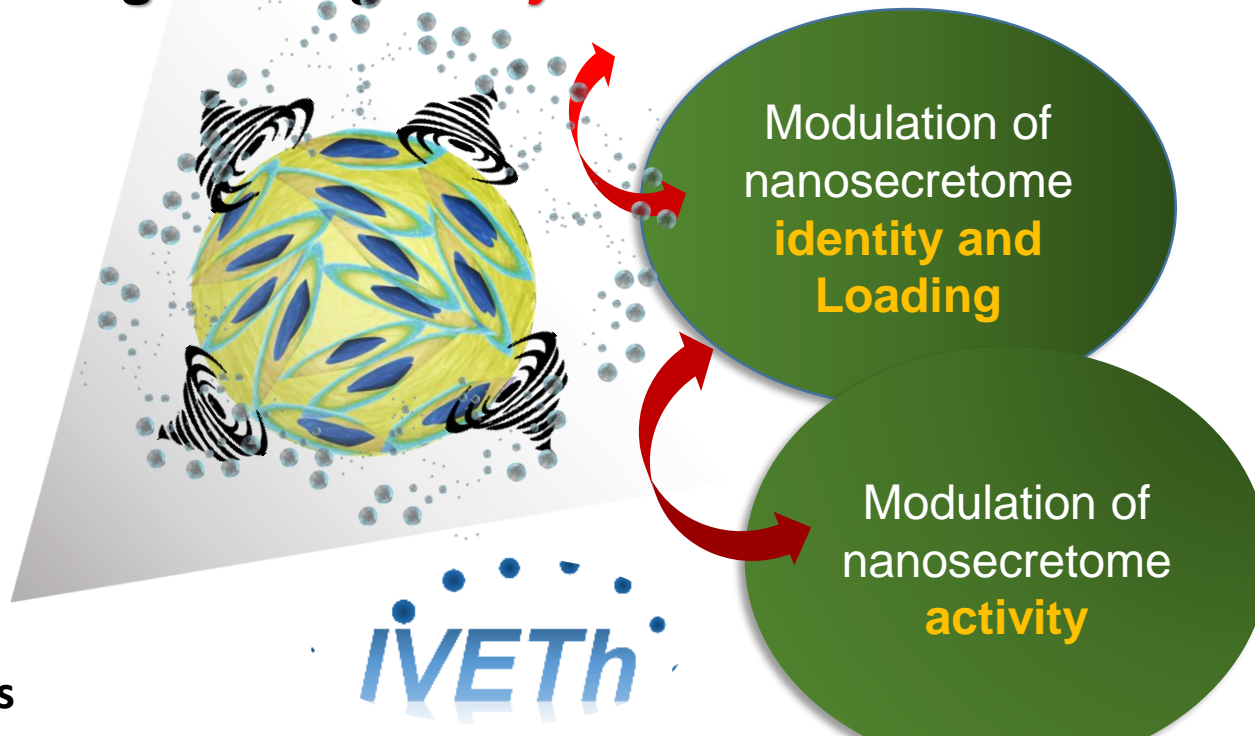
Innovations in Bioproduction of subcellular Biotherapies

Cell engineering + *Physical stimulation*



high-yield scalable
turbulence green
production
in GMP bioreactors

4 patent applications
licensed to



Quantitative Structure-activity relationship

→ Prediction of function
and mode of action
of subfractions

→ Screening
→ Quality control

Optimizing EV nature and function depending on the application

Postdoc

L. Alexandre A. Cronemberger S. Mozafari

Doc

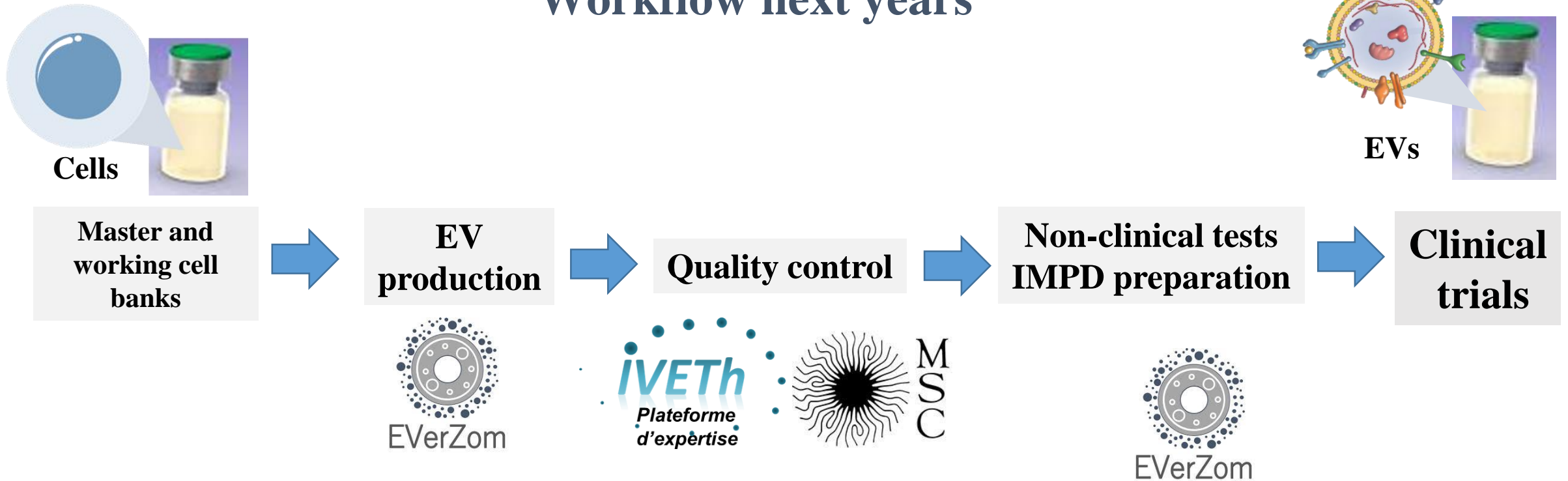
L. Jabbour A. Guichard JB Mattioni

E. Madec (Everzom cifre) Panli Yu S Cam

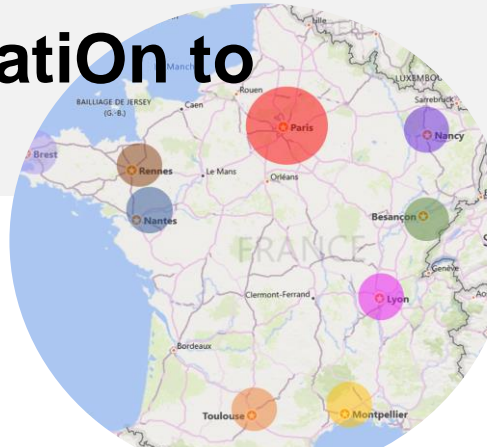
S. Razafindrakoto, IE C. Ribes, Tech E. Surply, Tech

5. Innovation from science to society: spin-off co-founding

Interactions – Workflow next years



4. Regulatory frame - Work group: Extracellular Vesicle translation to clinical perspectives – EVOLVE France



Challenge

No guidelines dedicated for EVs



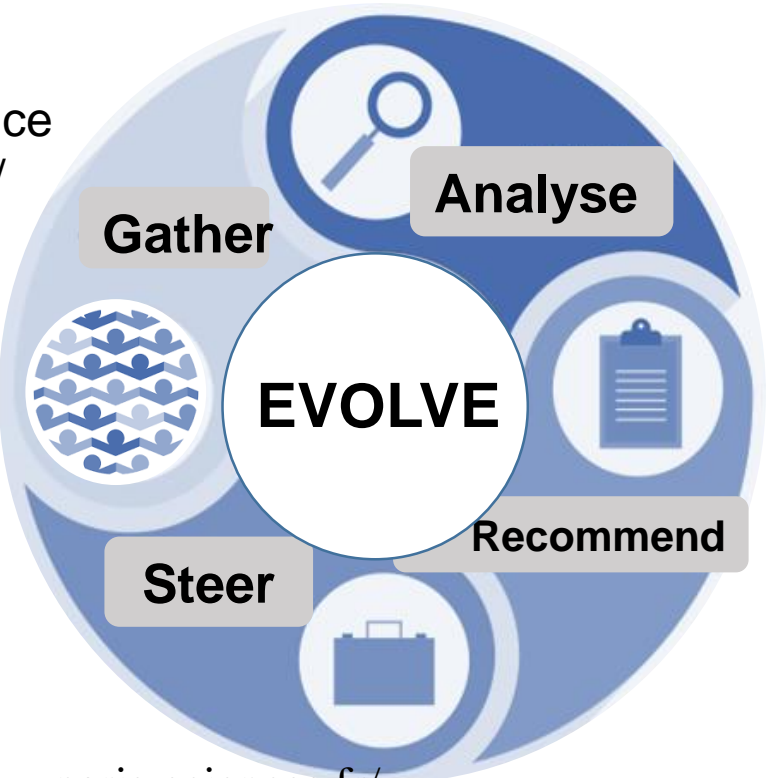
Coordination

A. Silva Brun
S. Banzet



Approach

Collective intelligence
48 Researchers / clinicians



A tentative roadmap for writing an Investigational Medicinal Product Dossier (IMPD) for EVs

32 Recommendations
Tentative quality control strategy



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)
Advanced Drug Delivery Reviews
journal homepage: www.elsevier.com/locate/adr



Development of extracellular vesicle-based medicinal products: A position paper of the group “Extracellular Vesicle translation to clinical perspectives – EVOLVE France”



Amanda K.A. Silva ^{a,*}, Marie Morille ^b, Max Piffoux ^{a,c,d}, Surendar Arumugam ^a, Philippe Mauduit ^{c,e}, Jérôme Larghero ^{f,g}, Arnaud Bianchi ^h, Kelly Aubertin ^a, Olivier Blanc-Brude ⁱ, Danièle Noël ^{j,k}, Emilie Velot ^{h,l}, Célia Ravel ^{m,n}, Céline Elie-Caille ^o, Anna Sebbagh ^a, Chantal Boulanger ⁱ, Claire Wilhelm ^p, Gabriel Rahmi ^{q,r}, Isabelle Raymond-Letron ^{s,t}, Kondareddy Cherukula ^a, Tristan Montier ^{u,v}, Christophe Martinaud ^{c,k,w}, Jean-Marie Bach ^x, Olivier Favre-Bulle ^y, Jolanda Spadavecchia ^z, Christian Jorgensen ^{j,k}, Philippe Menasché ^{i,aa}, Clotilde Aussel ^{c,ab}, Joël Chopineau ^b, Mathilde Mosser ^x, Matti Ullah ^a, Nicolas Sailliet ^{ac}, Nathalie Luciani ^a, Noëlle Mathieu ^{ad}, Pierre-Emmanuel Rautou ^{ae}, Sophie Brouard ^{ac}, Wilfrid Boireau ^o, Sébastien Jauliac ^{af}, Marianne Dedier ^{c,ab}, Jean-Hugues Trouvin ^{ag}, Florence Gazeau ^a, Marina Trouillas ^{c,k,ab}, Juliette Peltzer ^{c,k,ab}, Antoine Monsel ^{ah,ai}, Sébastien Banzet ^{c,k,ab,*}



Développement de biothérapies basées sur la délivrance locale d'ARN thérapeutiques par des vésicules hybrides fonctionnalisées pour la régénération musculo-squelettique

De la production de vésicules extracellulaires isolées de cellule stromales mésenchymateuses dérivées d'iPS à l'application clinique

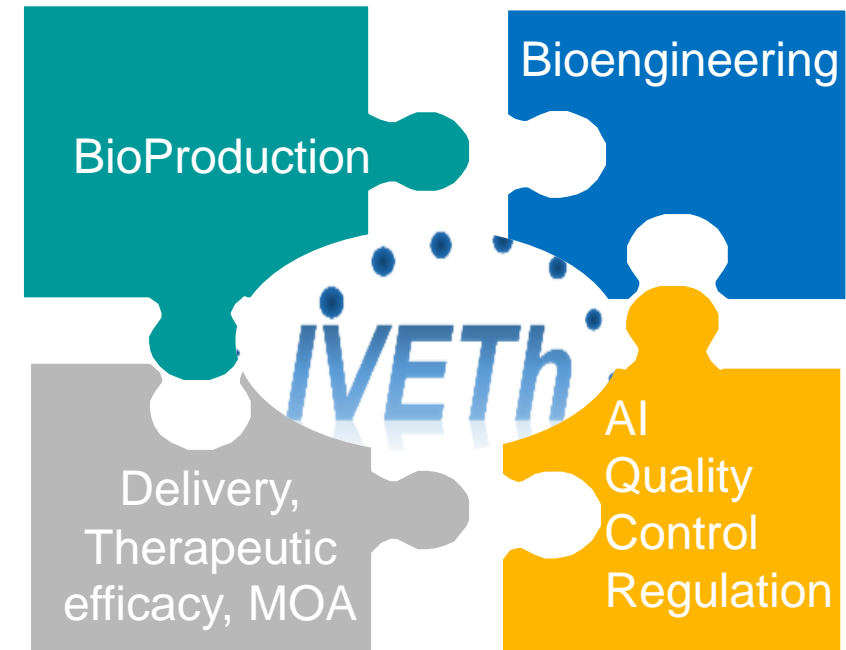


PROGRAMME
DE RECHERCHE
BIOTHÉRAPIES



Intégrateur IVETH : Innovation HUB

Innovation



Approche multidisciplinaire pour stimuler la bioproduction et l'ingénierie de vésicules extracellulaires (EVs) chez les bactéries à Gram+ à visée thérapeutique anti-inflammatoire



**Florence
GAZEAU, PhD**
Co-founder and
Executive leader



**Amanda
SILVA BRUN
PharmD, PhD**
Co-founder and
Executive leader



**Kelly
AUBERTIN, PhD**
Chief Scientific
Officer



**Fahima
DI FEDERICO**
Head of business
development



**Sarah
RAZAFINDRAKOTO**
Chief Technical
Officer



**Olivier BLANC-
BRUDE, PhD**
Chief clinical
development



A4F
Sylvain Cam | UP Cité



Cell insight
Dmitry Ayollo, PhD | UP Cité



AFM
Jean-Marc Di Meglio, PhD | UP Cité



Prestations et soutien technique
Christopher Ribes | UP Cité



Hugo Salmon PhD. | UPCité



Bioproduction
André Andrade, PhD. | CNRS



Nanoscopie
Nicolas Kuzsla | UP Cité



Estelle Surply | CNRS



Salwa Abid | CNRS



Raman
Sergei Kruglik, PhD | Sorbonne Université



Stéphanie Mangenot, PhD. | UPCité



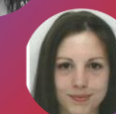
Artificial intelligence
Safa BOUDABOUS | CNRS



Gene Editing / Screening
Julia Dancourt, PhD | UP Cité



Lucile Alexandre, PhD. | CNRS



Alice NICOLAÏ | CNRS

MSC-Med Team



Consortium IVETH



S. Banzet

P. Mauduit

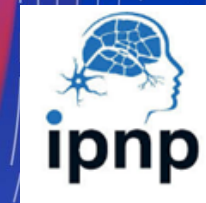


PE Rautou



CENTRE DE RECHERCHE
EN IMMUNOLOGIE

D. Taresté



C. Boulanger

C. Théry –
C. Guérin



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