Industrial valorization of science:

Examples and discussion

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PhD, Resident in oncology

Co-founder and Scientific advisor of Everzom, Evora bioscience and Therafast Bio

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Conflict of interest declaration

- Co-founder and Scientific advisor of Everzom, Evora bioscience and Therafast Bio
- Shareholder : Everzom, Evora bioscience and Therafast Bio

Introduction note

- This discussion will be very critical, but try to be as factual as possible

Background

- 2011 : Medicine
- 2014 : M2
- 2015-2017: PhD in biology/biophysics
- 2018 – 2021 : Medical « externat »
- 2021-2027 : Resident in oncology

+ M2 in statistics

Academic research

2018 :Therafast bio

2019: Everzom

2020: Evora

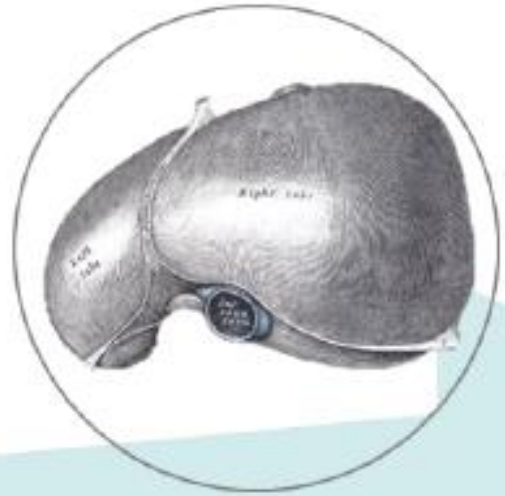
Plan

- Introduction / context on EVs
- Everzom Example and discussion
- Evora Bioscience Example and discussion
- Bonus : Therafast Bio Example

Introduction

What are Extracellular Vesicles (EVs)?

1/10 000 of a millimeter
10x smaller than a bacteria



Organites
1 µm

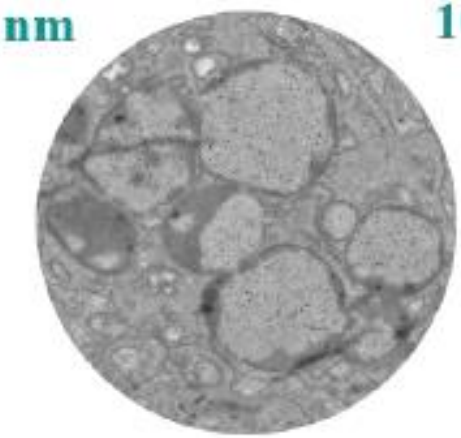
Tissues
5 mm

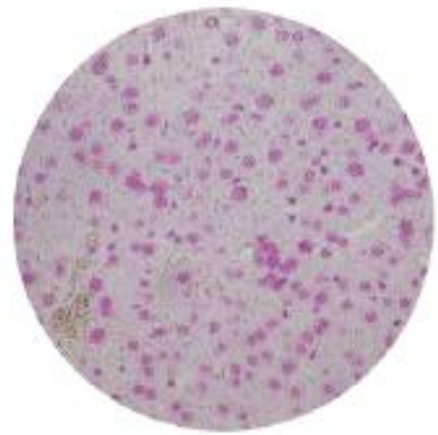
Organism
1 m

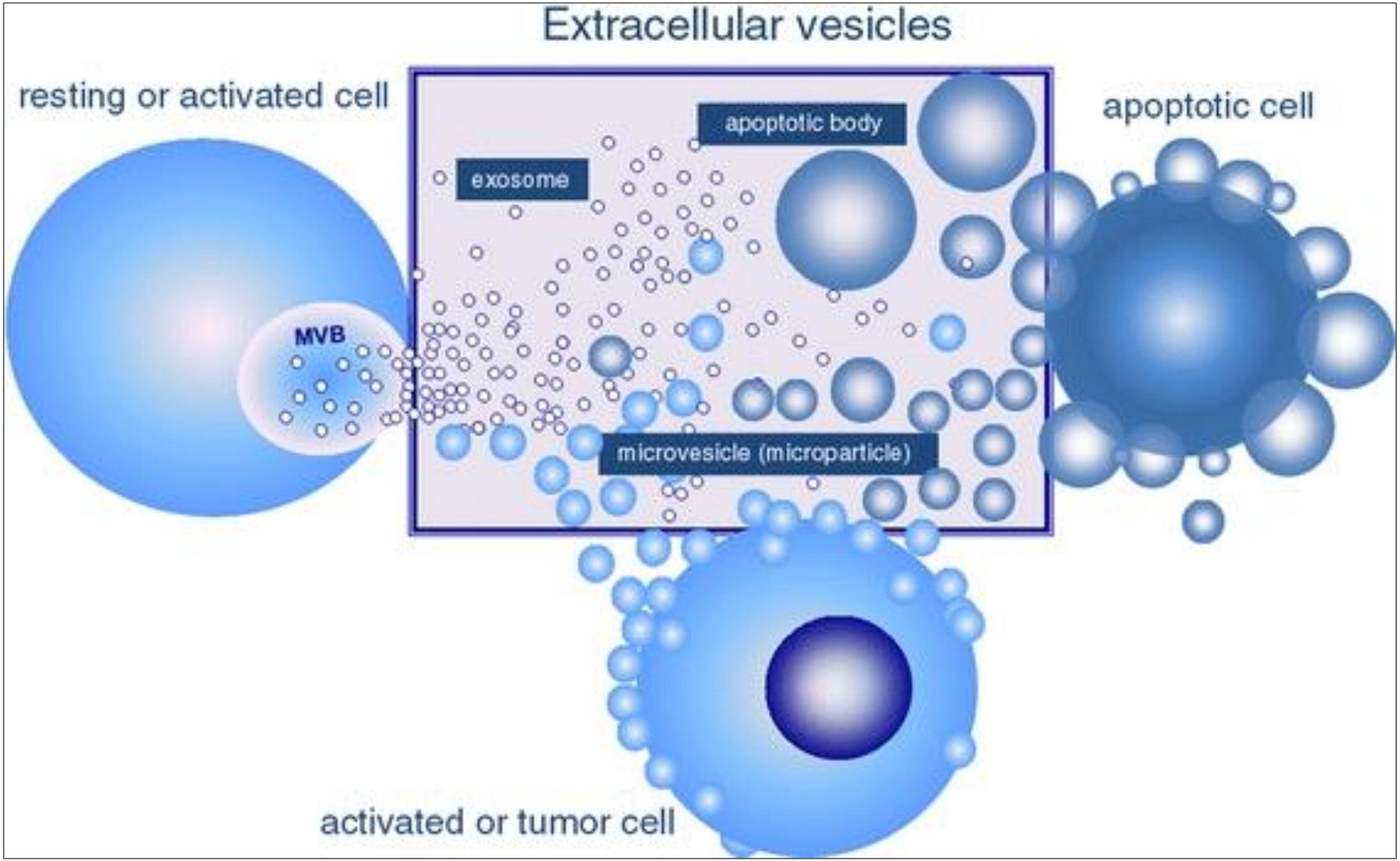
EVs
100 nm

Cells
10 µm

Organs
10 cm






Extracellular vesicles
resting or activated cell
apoptotic body
exosome
apoptotic cell
MVB
microvesicle (microparticle)
activated or tumor cell

EVs as an alternative to cell therapy ?

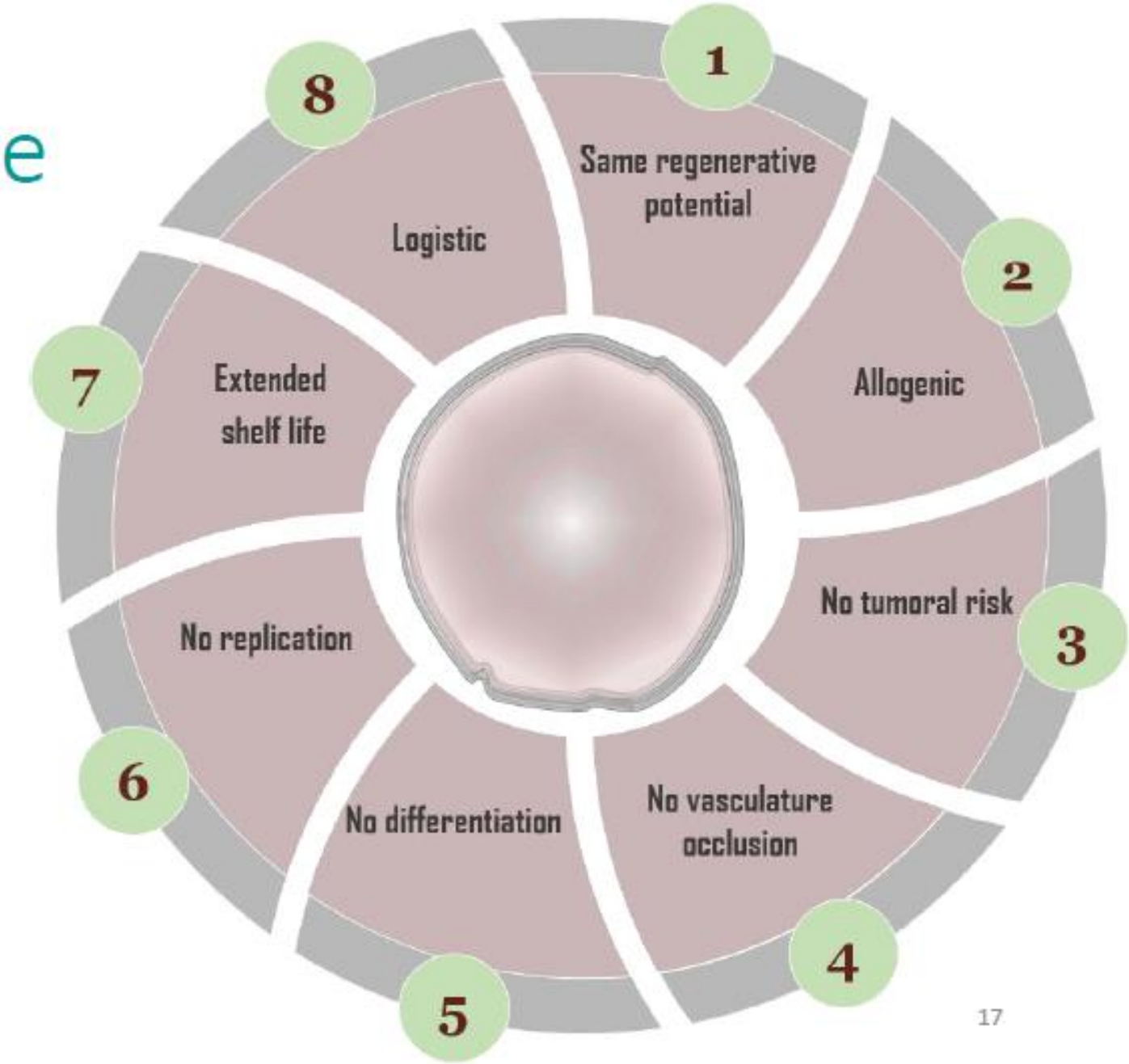
Off the shelf
(one batch for many patients)

Easier

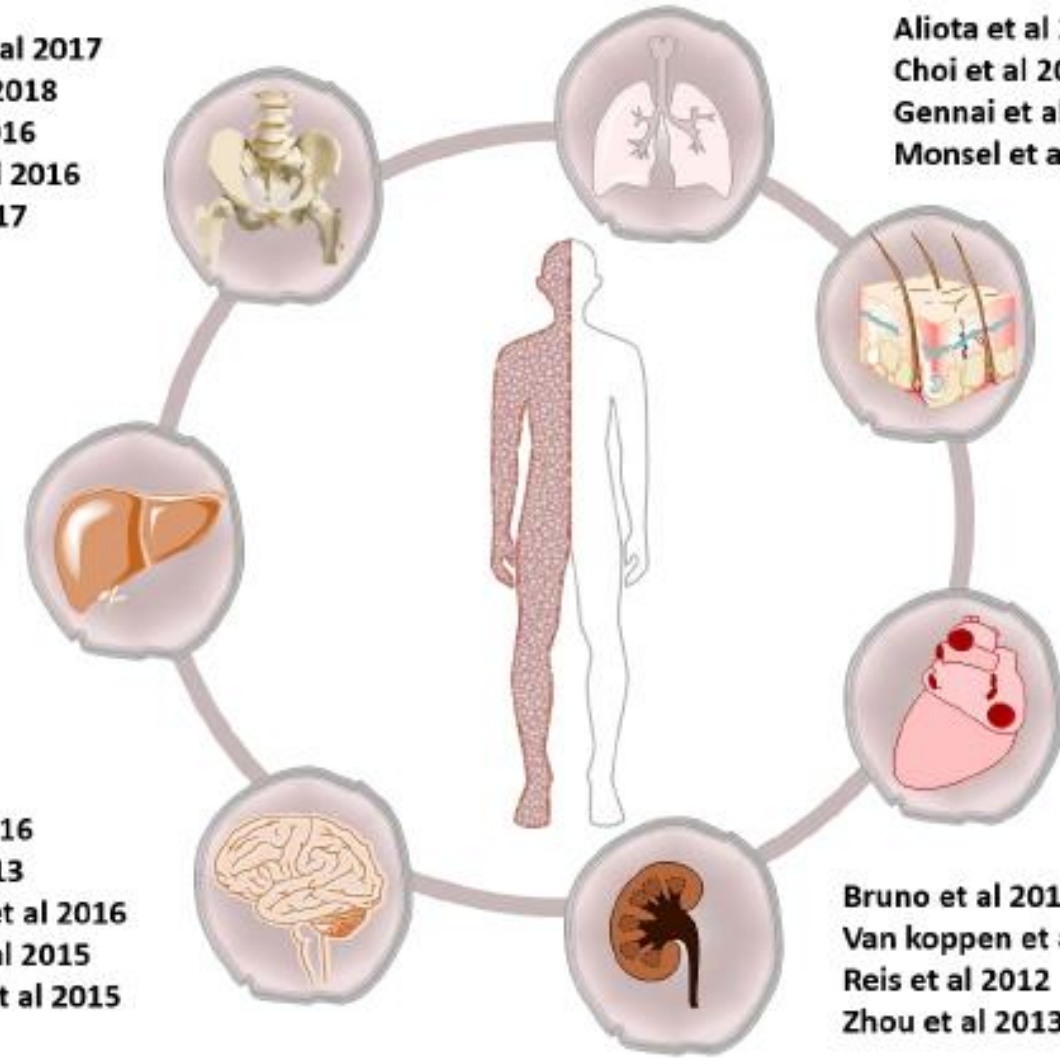
Cheaper

Safer

Democratizing cell therapy



EVs are versatile in regenerative medicine



Cosenza et al 2017
Vonk et al 2018
Qin et al 2016
Zhang et al 2016
Liu et al 2017

Lee et al 2012
Aliota et al 2016
Choi et al 2014
Gennai et al 2015
Monsel et al 2015

Zhang B et al 2015
Zhang J et al 2015
Fang et al 2016

Li et al 2013
Tan et al 2014

Timmers et al 2007
Lai et al 2010
Yu et al 2015
Arslan et al 2013
Bian et al 2014
Teng et al 2015

Kim et al 2016
Xin et al 2013
Ophelders et al 2016
Zhang Y et al 2015
Doeppner et al 2015

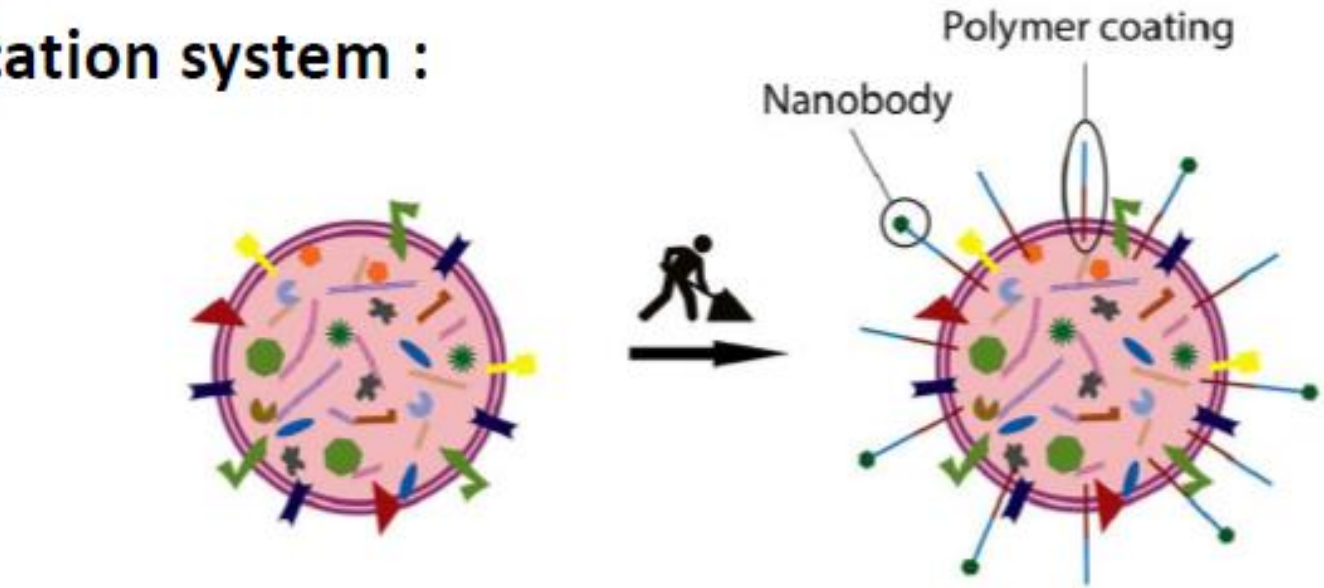
Bruno et al 2012
Van koppen et al 2012
Reis et al 2012
Zhou et al 2013

Common mechanism ?
Inflamation resolution ?

Extracellular Vesicles in Drug Delivery

Hijacking nature's communication system :

- Targeting properties ?
- Naturally non toxic ?
- Protection of the cargo ?
- Intrinsic biological properties ?



Adapted From Sanders koojimans et Al

Overview of ongoing clinical trials with EVs.

Rank	Title	Status	Conditions	Interventions	Phases	Country	URL
1	Expanded Access Protocol on Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicle Infusion Treatment for Patients With COVID-19 Associated ARDS	Available	Covid19|ARDS|Hypoxia|Cytokine Storm	Biological: Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicles Infusion Treatment	Not Applicable		https://ClinicalTrials.gov/show/NCT04657458
2	Autologous Serum-derived EV for Venous Trophic Lesions Not Responsive to Conventional Treatments	Recruiting	Ulcer Venous	Other: Autologous extracellular vesicles from serum	Not Applicable	Italy	https://ClinicalTrials.gov/show/NCT04652531
3	Safety and Efficiency of Method of Exosome Inhalation in COVID-19 Associated Pneumonia	Enrolling by invitation	Covid19|SARS-CoV-2 PNEUMONIA|COVID-19	Drug: EXO 1 inhalation|Drug: EXO 2 inhalation|Drug: Placebo inhalation	Phase 2	Russian Federation	https://ClinicalTrials.gov/show/NCT04602442
4	A Clinical Study of Mesenchymal Stem Cell Exosomes Nebulizer for the Treatment of ARDS	Not yet recruiting	Acute Respiratory Distress Syndrome	Biological: low dose hMSC-Exos|Biological: medium dose hMSC-Exos|Biological: high dose hMSC-Exos|Biological: Dosage 1of hMSC-Exos|Biological: Dosage 2 of hMSC-Exos|Biological: No hMSC-derived exosomes	Phase 1|Phase 2	China	https://ClinicalTrials.gov/show/NCT04602104
5	A Clinical Study of Mesenchymal Progenitor Cell Exosomes Nebulizer for the Treatment of Pulmonary Infection	Recruiting	Drug-resistant	Biological: Dosage 1 of MPCs-derived exosomes|Biological: Dosage 2 of MPCs-derived exosomes|Biological: No MPCs-derived exosomes	Phase 1|Phase 2	China	https://ClinicalTrials.gov/show/NCT04544215
6	Extracellular Vesicle Infusion Therapy for Severe COVID-19	Not yet recruiting	Covid19|ARDS|Pneumonia, Viral	Biological: DB-001	Phase 2		https://ClinicalTrials.gov/show/NCT04493242
7	Evaluation of Safety and Efficiency of Method of Exosome Inhalation in SARS-CoV-2 Associated Pneumonia.	Completed	Covid19|SARS-CoV-2 PNEUMONIA|COVID-19	Drug: EXO 1 inhalation|Drug: EXO 2 inhalation|Drug: Placebo inhalation	Phase 1|Phase 2	Russian Federation	https://ClinicalTrials.gov/show/NCT04491240
8	COVID-19 Specific T Cell Derived Exosomes (CSTC-Exo)	Active, not recruiting	Corona Virus Infection|Pneumonia	Biological: COVID-19 Specific T Cell derived exosomes (CSTC-Exo)	Phase 1	Turkey	https://ClinicalTrials.gov/show/NCT04389385
9	the Safety and the Efficacy Evaluation of Allogenic Adipose MSC-Exos in Patients With Alzheimer's Disease	Recruiting	Alzheimer Disease	Biological: low dosage MSCs-Exos administrated for nasal drip|Biological: mild dosage MSCs-Exos administrated for nasal drip|Biological: high dosage MSCs-Exos administrated for nasal drip	Phase 1|Phase 2	China	https://ClinicalTrials.gov/show/NCT04388982
10	Exosome of Mesenchymal Stem Cells for Multiple Organ Dysfuntion Syndrome After Surgical Repaire of Acute Type A Aortic Dissection	Not yet recruiting	Multiple Organ Failure	Biological: Exosome of Mesenchymal stromal cells	Not Applicable	China	https://ClinicalTrials.gov/show/NCT04356300
11	Safety Evaluation of Intracoronary Infusion of Extracellular Vesicles in Patients With AMI	Not yet recruiting	Heart Attack	Drug: PEP(extracellular vesicles) in Acute Myocardial Infarction	Phase 1	United States	https://ClinicalTrials.gov/show/NCT04327635
12	A Tolerance Clinical Study on Aerosol Inhalation of Mesenchymal Stem Cells Exosomes In Healthy Volunteers	Recruiting	Healthy	Biological: 1X level of MSCs-Exo|Biological: 2X level of MSCs-Exo|Biological: 4X level of MSCs-Exo|Biological: 6X level of MSCs-Exo|Biological: 8X level of MSCs-Exo|Biological: 10X level of MSCs-Exo	Phase 1	China	https://ClinicalTrials.gov/show/NCT04313647
13	Efficacy of Platelet- and Extracellular Vesicle-rich Plasma in Chronic Postsurgical Temporal Bone Inflammations	Completed	Otitis Media Chronic|Temporal Bone	Drug: Platelet- and extracellular vesicle-rich plasma|Drug: Standard conservative treatment	Not Applicable	Slovenia	https://ClinicalTrials.gov/show/NCT04281901
14	A Pilot Clinical Study on Inhalation of Mesenchymal Stem Cells Exosomes Treating Severe Novel Coronavirus Pneumonia	Completed	Coronavirus	Biological: Mesenchymal stromal cells-derived exosomes	Phase 1	China	https://ClinicalTrials.gov/show/NCT04276987
15	Evaluation of Adipose Derived Stem Cells Exo.in Treatment of Periodontitis	Recruiting	Periodontitis	Biological: adipose derived stem cells exosomes	Early Phase 1	Egypt	https://ClinicalTrials.gov/show/NCT04270006
16	Effect of UMSCs Derived Exosomes on Dry Eye in Patients With cGVHD	Recruiting	Dry Eye	Drug: Umbilical Mesenchymal Stem Cells derived Exosomes	Phase 1|Phase 2	China	https://ClinicalTrials.gov/show/NCT04213248
17	The Use of Exosomes In Craniofacial Neuralgia	Enrolling by	Neuralgia	Other: Neonatal stem cells Exosomes	Not Applicable	United States	https://ClinicalTrials.gov/show/NCT04202783
18	Focused Ultrasound and Exosomes to Treat Depression, Anxiety, and Dementias	Enrolling by invitation	Refractory Depression|Anxiety Disorders|Neurodegenerative Diseases	Other: Stem cells xosomes	Not Applicable	United States	https://ClinicalTrials.gov/show/NCT04202770
19	MSC EVs in Dystrophic Epidermolysis Bullosa	Not yet recruiting	Dystrophic Epidermolysis Bullosa	Drug: AGLE 102 (Mesenchymal Stromal Cells-derived extracellular vesicles)	Phase 1|Phase 2		https://ClinicalTrials.gov/show/NCT04173650
20	iExosomes in Treating Participants With Metastatic Pancreas Cancer With KrasG12D Mutation	Not yet recruiting	KRAS NP_004976.2: p.G12D|Metastatic Pancreatic Adenocarcinoma | Pancreatic Ductal Adenocarcinoma |Stage IV Pancreatic Cancer AJCC v8	Drug: Mesenchymal Stromal Cells-derived Exosomes with KRAS G12D siRNA	Phase 1	United States	https://ClinicalTrials.gov/show/NCT03608631
21	Plant Exosomes and Patients Diagnosed With Polycystic Ovary Syndrome (PCOS) 17	Recruiting	Polycystic Ovary Syndrome	Other: Ginger exosomes|Other: Aloe exosomes|Other: Placebo	Not Applicable	United States	https://ClinicalTrials.gov/show/NCT03493984
22	MSC-Exos Promote Healing of MHs	Recruiting	Macular Holes	Biological: exosomes derived from mesenchymal stem cells (MSC-Exo)	Early Phase 1	China	https://ClinicalTrials.gov/show/NCT03437759
23	Allogenic Mesenchymal Stem Cell Derived Exosome in Patients With Acute Ischemic Stroke	Recruiting	Cerebrovascular Disorders	Allogenic mesenchymal stem cells derived exosome enriched by miR-124	Phase 1|Phase 2	Iran	https://ClinicalTrials.gov/show/NCT03384433
24	Effect of Plasma Derived Exosomes on Cutaneous Wound Healing	Enrolling by invitation	Ulcer	Other: plasma-derived exosomes	Early Phase 1	Japan	https://ClinicalTrials.gov/show/NCT02565264
25	Effect of Microvesicles and Exosomes Therapy on β-cell Mass in Type I Diabetes Mellitus (T1DM)	Unknown status	Diabetes Mellitus Type 1	Biological: mesenchymal stem cells exosomes.	Phase 2|Phase 3	Egypt	https://ClinicalTrials.gov/show/NCT02138331
26	Edible Plant Exosome Ability to Prevent Oral Mucositis Associated With Chemoradiation Treatment of Head and Neck Cancer	Active, not recruiting	Head and Neck Cancer|Oral Mucositis	Dietary Supplement: Grape extract|Drug: Lortab, Fentanyl patch, mouthwash	Phase 1	United States	https://ClinicalTrials.gov/show/NCT01668849
27	Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue	Active, not recruiting	Colon Cancer	Dietary Supplement: curcumin|Dietary Supplement: Curcumin conjugated with plant exosomes|Other: No intervention	Phase 1	United States	https://ClinicalTrials.gov/show/NCT01294072
28	Trial of a Vaccination With Tumor Antigen-loaded Dendritic Cell-derived Exosomes	Completed	Non Small Cell Lung Cancer	Biological: Dex2 Tumor Antigen-loaded Dendritic Cell-derived Exosomes	Phase 2	France	https://ClinicalTrials.gov/show/NCT01159288

Ongoing clinical trials

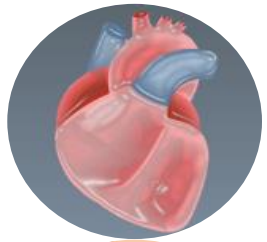
⇒Phase I ++

⇒COVID ++

⇒Cancer

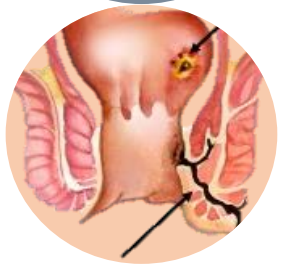
⇒ulcers

Turbulence-triggered EVs: proof of regenerative effect in 5 models



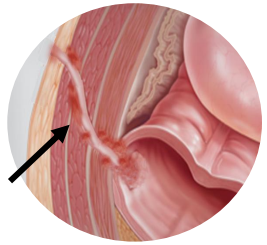
Chronic heart failure model in mice

Iris Marangon
Post-doc



Inflammatory **perianal fistula** model in rats

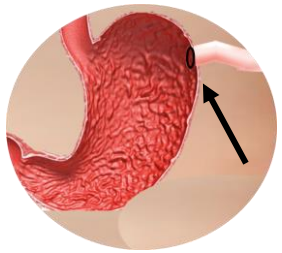
Boris Rosenbaum (MD), Master student



Post-surgical **colo-cutaneous fistula** model in rats

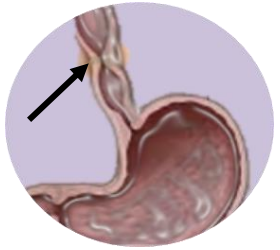
Nanoscale, 2021, 13, 218-232

Artur Berger (MD)
PhD student



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

Guillaume Pere (MD)
Master student



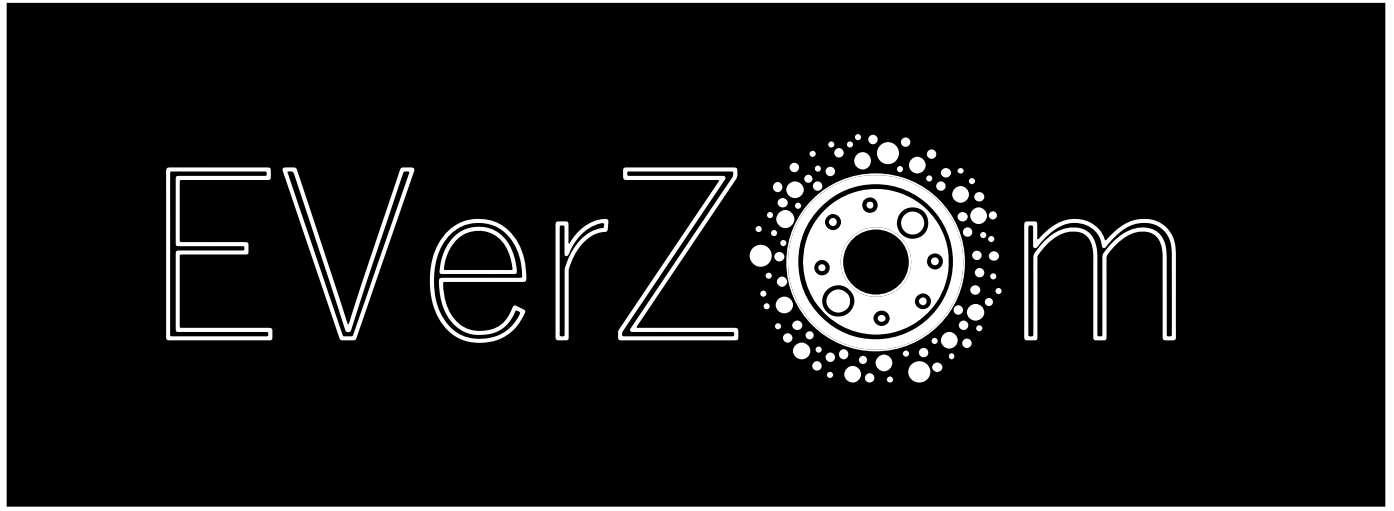
Esophageal stricture in **pigs**

Nanoscale, 2021, 13, 14866-78

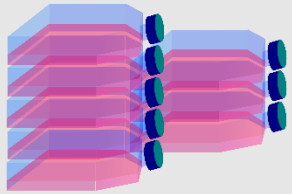
Elise Coffin (MD)
Master student

Everzom

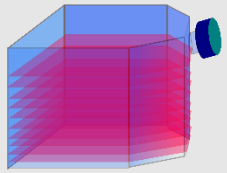
- A CDMO company that aims at producing EVs at large scale for clinical translation



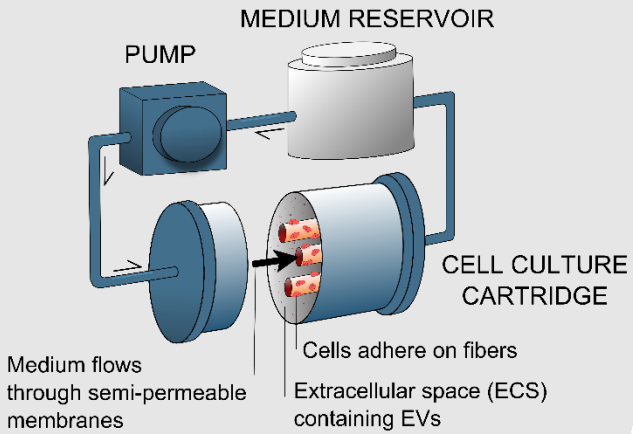
SCALING OUT



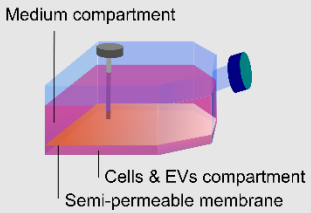
2D flasks



Hyperflasks

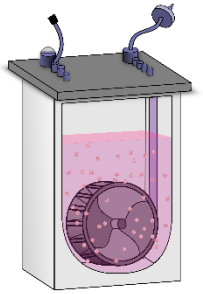


Hollow fiber bioreactor

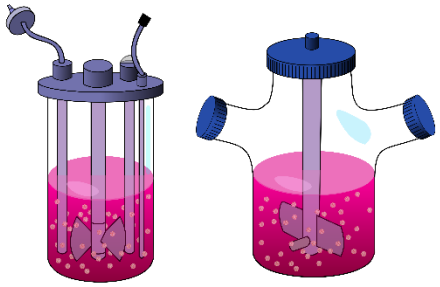


Integra CELLine

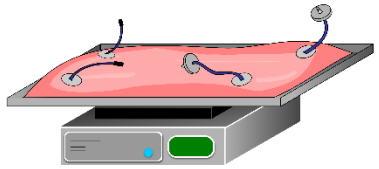
SCALING UP



Vertical wheel™ bioreactor



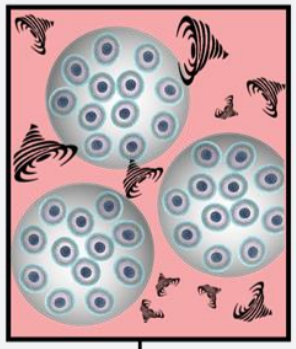
Stirred-tank bioreactor

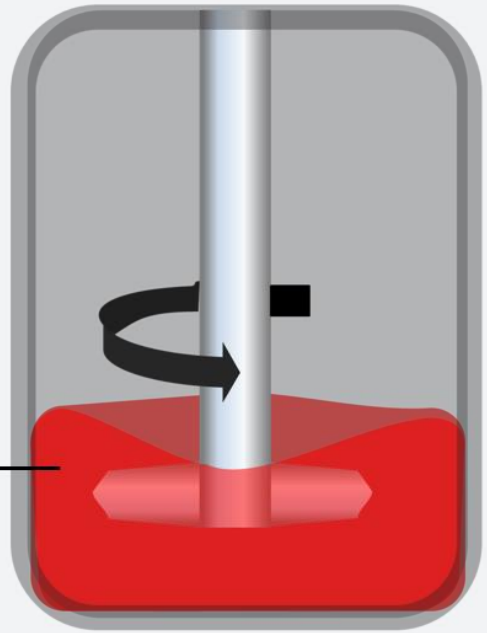


WAVE bioreactor

GMPc production techniques / production metrics

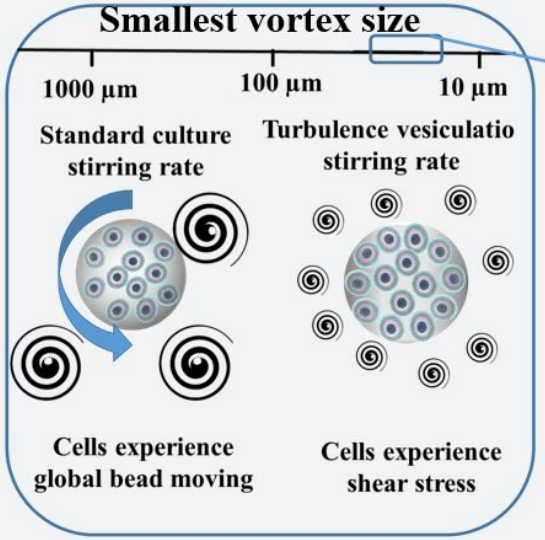
The higher the stirring, the smallest the obtained vortices

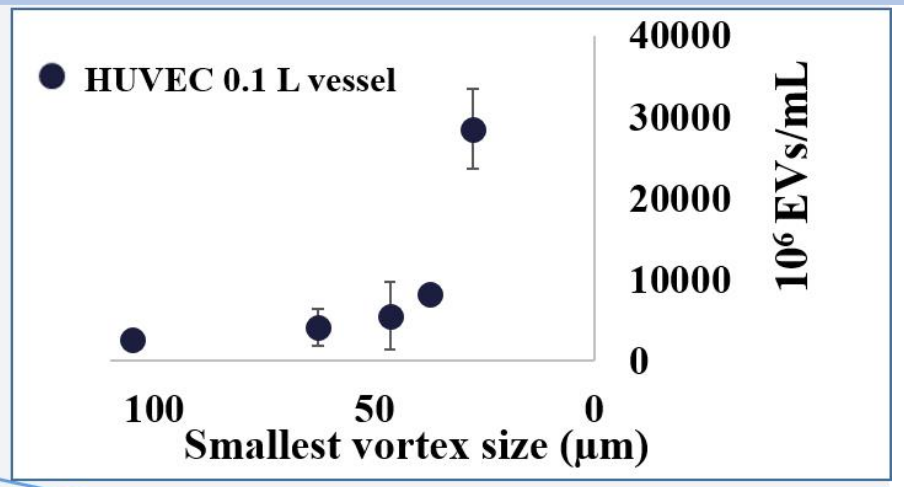


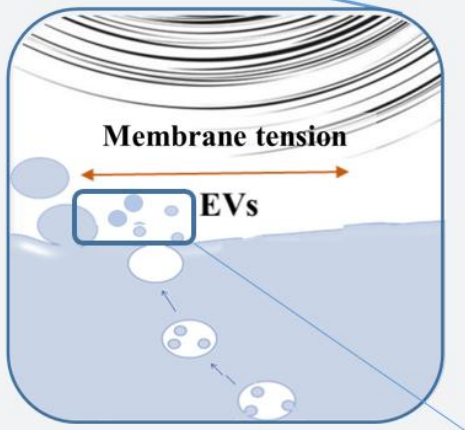


Cells cultured on microbeads under stirring

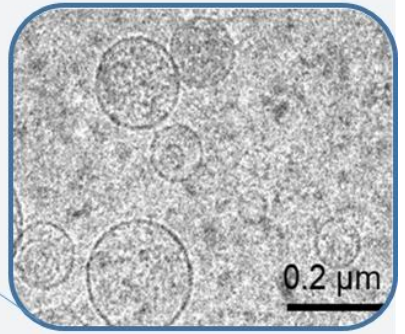
Energy is dissipated in the <u>smallest vortices</u> by friction







FeasEVble team
FR1756183



Integrated

High-yield

Scalable

Fast-forward /serum-free

Reduced operational steps

GMPc production techniques / production metrics

How to scale-up?
By keeping the smallest vortex size constant

Smallest vortex size (L) depends on stirring rate, bioreactor volume and impeller diameter

Kolmogorov equation

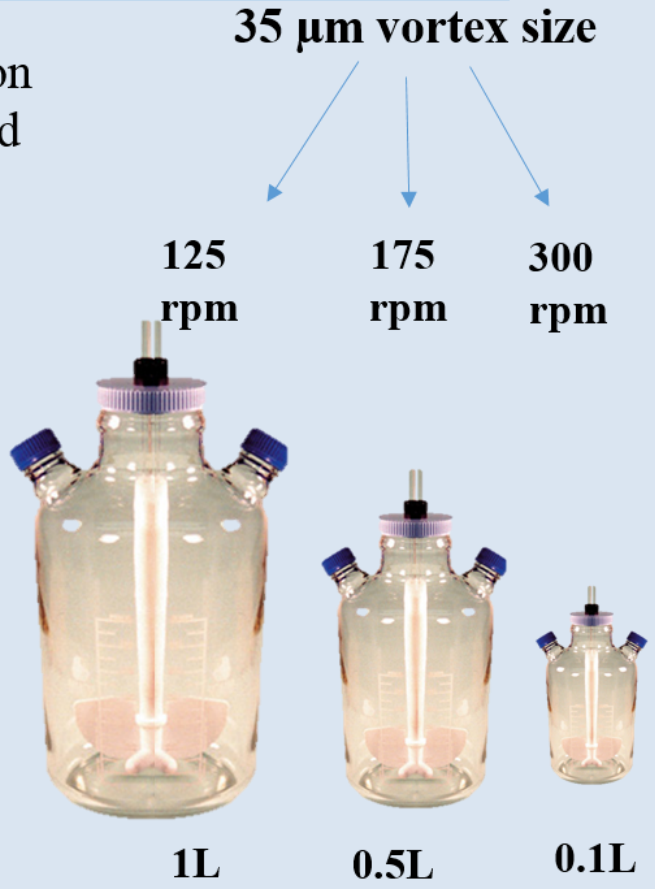
$$L = \left(\frac{\nu^3}{\varepsilon}\right)^{1/4}$$

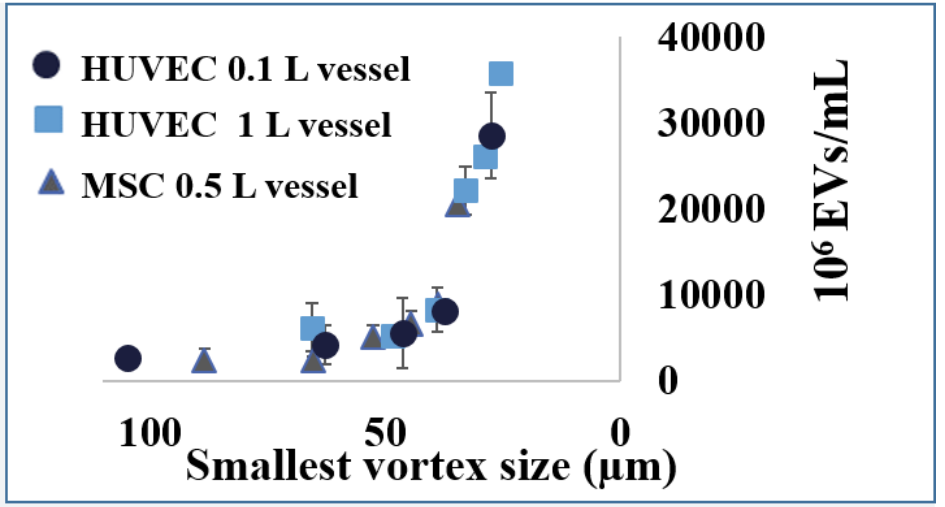
$$\varepsilon = N_p D_i^5 N^3 / V_d$$

Impeller diameter

Stirring rate

Volume





FeasEVble team
FR1756183

Also works on cells in suspension

10 fold increase in yield (EV/cell)

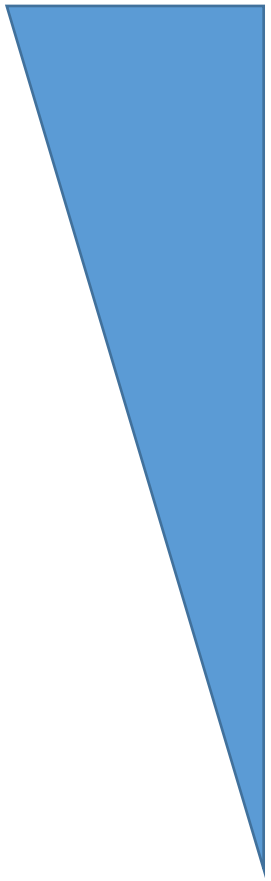
Main driver of cost in EV production is cell culture

=> Largely reduce the cost

GMPc production techniques / production metrics

- How to characterize EVs ?
- How to quantify production ?

IVETH Paris Descartes

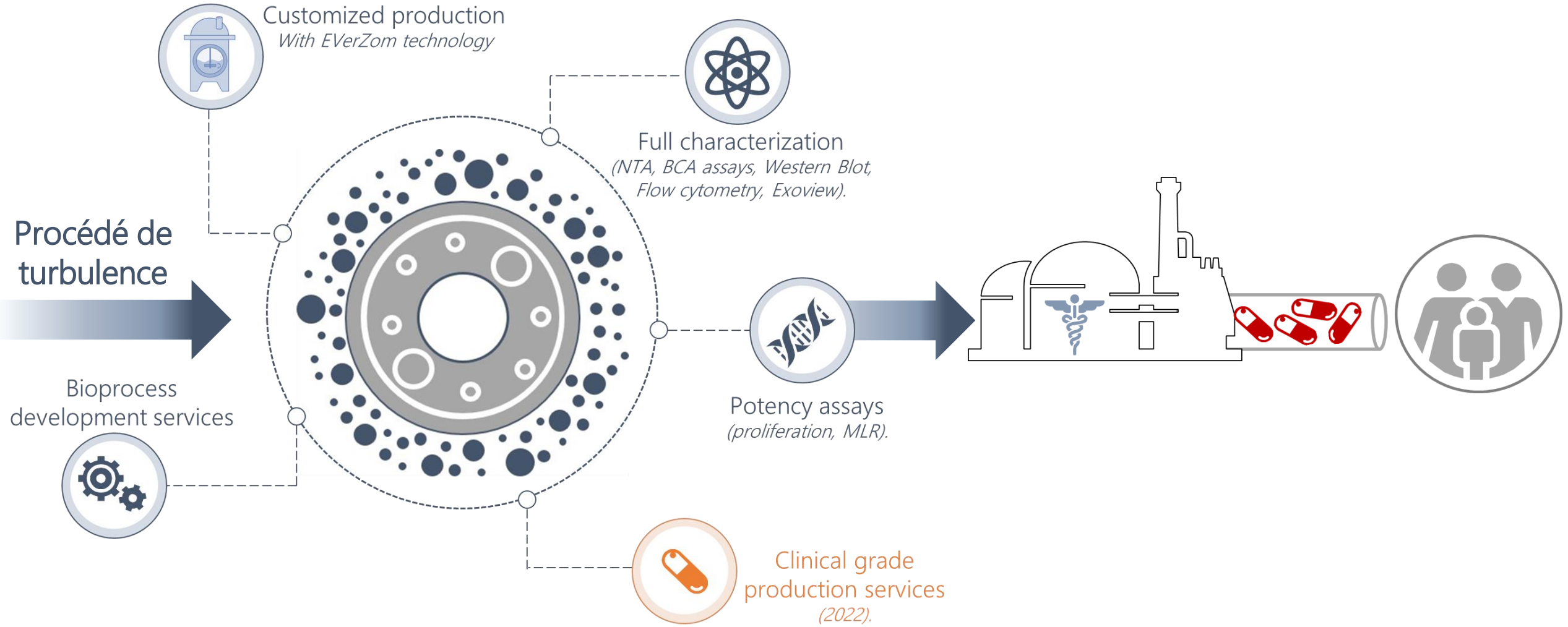


- 3 types of NTA
- DLS
- qNano
- Ultracentrifugation
- Accès microscopie électronique, cytometry, imaging cytometry
- Protein dosage
- RNAseq / proteomics
- Exoview
- Videodrop
- Raman / SERS
- TFF
- A4F
- Etc…

So what would you do next ?

- What is the objective ?
- Creating a company ? Staying academic ? Being both ?
- Business Model ?
- Intellectual property ?
- Team ?
- Regulatory environement ?
- Shares ?
- Location ?
- Relations with academia ?
- Technology transfer ?
- Funding : Grant ? Private money ? Business angels ? Fund ?
- Public relations and networking ?

EVerZom: Plateforme de bioproduction de VEs



Plateforme EVerZom → Biotechs / académiques / BP → Patients

GMPc production techniques / production metrics

Our suggested tests for the critical quality attributes and other required tests	Development phase	Clinical batch production			
		In-process control	Drug substance control	Stability test (drug substance and finished product)	Finished product control
QUANTITY ATTRIBUTE					
Particle quantification by NTA	M		AC	AC	AC
Total protein quantification by colorimetric assays	M	M	AC	AC	AC
IDENTITY ATTRIBUTE					
Size and structure by TEM-based methods	M				
Hydrodynamic diameter analysis by NTA	M	M	AC	AC	AC
Immunochemical characterization by Elisa, MACSPlex Exosome Kit, Exoview, small particle cytometry or nanoflow cytometry	M		AC	AC	AC
DNA content (with/without DNase treatment)	M		AC		AC
RNA content (optionally with/without RNase treatment)	M		AC		AC
PURITY ATTRIBUTE					
Ratio of particle counts/micrograms of proteins	M	M	AC	AC	AC
IMPURITY / CONTAMINANTS					
Albumin or fibrinogen quantification (if EV secretion step in complete medium)	M		UL		UL
DNA (optionally RNA) quantification with and without DNase (optionally RNase) treatment, as indicated above					
Priming molecule concentration (if relevant)					
Endotoxin, sterility and mycoplasma test (according to the Eur. Pharm.) and virus testing (*in vitro* and/or *in vivo*)			AC		AC
BIOLOGICAL ACTIVITY					
Potency tests *in vitro*	M		M	M	M
Potency tests *in vivo* (if any)	M				M
OTHERS					
Appearance and description: physical state (eg., solid, liquid), color, etc.	M			AC	AC
General tests: pH and osmolarity	M			AC	AC

Regulatory setting

EVs are nanoparticles => Nanoparticle regulation ?

EVs are cell products => Biologic regulation ?

EVs contain nucleic acid => ATMP regulation ?

⇒**It depends on your mechanism of action**

« engineering with nucleic acid » => ATMP

« natural Evs » => Biologic

⇒**It depends from one country to another !**

France ATMP => biologic

Europe => rather biologic

US => rather ATMP

Regulatory setting

c) Characterization of single vesicles: use two different but complementary techniques, for example:
i. electron or atomic force microscopy (and show both close-up and wide-field)
ii. single particle analyzers (not electron microscope-based)

No specific guidelines

(would you want it ?)

MISEV 2018

as a regulatory guideline ?

Not really adapted

(topology, no discussion on reproductibility, etc)

d)

Still valid, but has evolved with a rapidly increasing number of techniques used to analyze EVs.

i. Techniques providing images of single EVs at high resolution, such as electron microscopy and related techniques, scanning-probe microscopy (SPM) including atomic-Force microscopy (AFM), or super-resolution microscopy: these techniques are not interchangeable in the information they provide. When reporting results, both close-up and wide-field images must be provided.

ii. Single particle analysis techniques that estimate biophysical features of EVs from other techniques than high-resolution images: size measured by resistive pulse sensing (electric field displacement), or light scattering properties [nanoparticle tracking analysis (NTA), high resolution flow cytometry, multi-angle light scattering coupled to asymmetric flow field-flow fractionation (AF4)]; or fluorescence properties [fluorescence correlation spectroscopy (FCS), high-resolution flow cytometry]. Chemical composition measured by Raman spectroscopy.

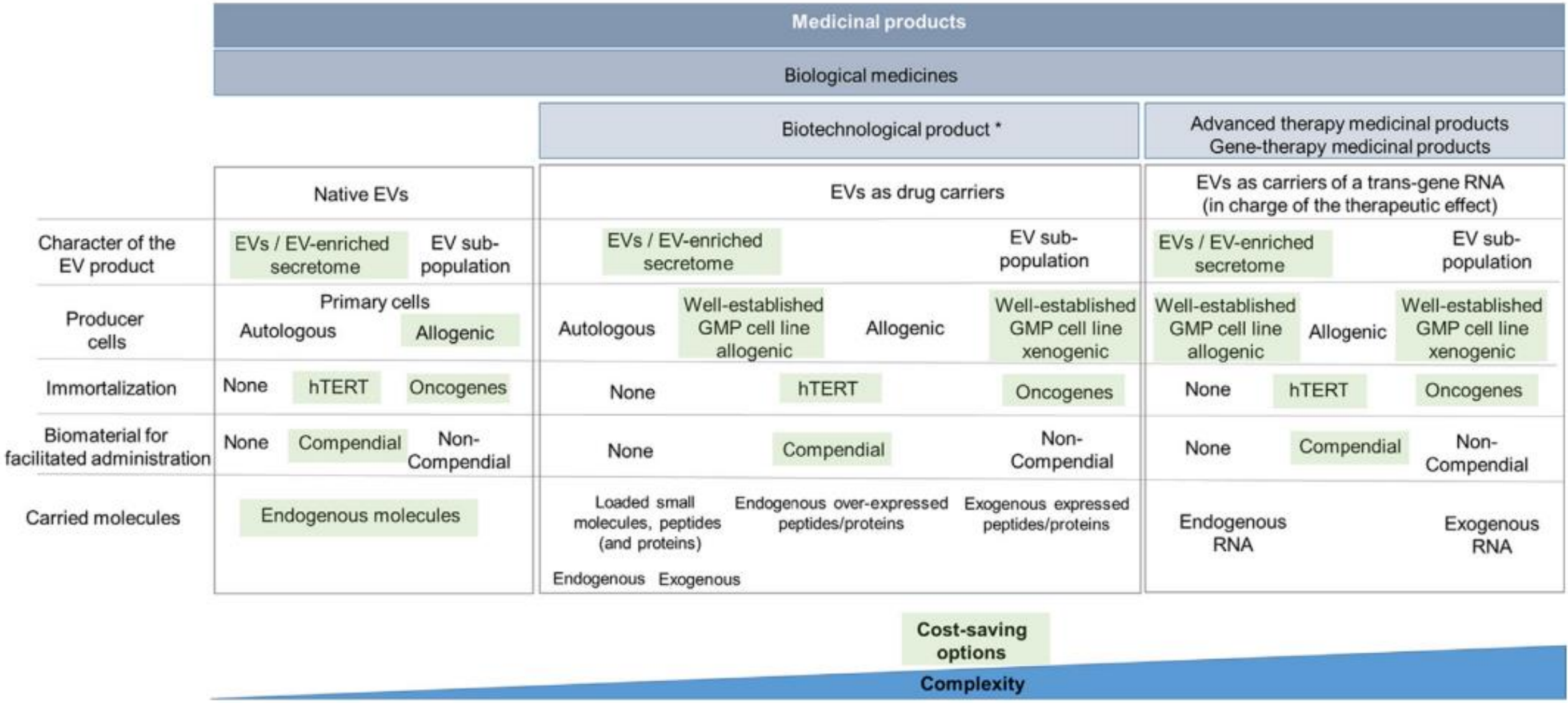
Other techniques are being developed that may combine these two categories but have not yet been widely used (see 4c p.20).

Whatever technique is used, all experimental details for both acquisition and analysis must be reported.

Note that not all techniques are equally adapted to all EVs: large EVs (> 400 nm) and very small EVs (< 50 nm) are not well quantified by all NTA; small EVs are not easy to detect by most common flow cytometers. Some large EVs (and aggregates of small EVs) can be imaged by light/fluorescence microscopy. EVs smaller than the refraction limit or resolution of a microscope can still be detected by fluorescence, but no structural information can be obtained, and a single EV cannot be distinguished from a small EV cluster purely based on structural details.

MISEV2018 additional characterization. We now recommend that the topology of EV-associated components be assessed, that is, whether a component is luminal or on/at the surface of EVs, **at least for those required for a given EV-associated function**. Topology may be particularly important for certain classes of biomolecules. Protease and nuclease digestions, detergent permeabilization, and antibodies to outer epitopes (should bind) or inner epitopes (should not bind) can be used to probe topology.

Regulatory setting



Regulatory setting

CTD Module 3 content	Our selection of general relevant guidelines for EV-based products
"DRUG SUBSTANCE	
General information - Nomenclature - Structure - General Properties	EMA/CAT/852602/2018* [20] EMA/CHMP/BWP/534898/2008 [52] ICH Topic M4Q [50]
Manufacture - Manufacturer (name, address, and responsibilities) - Description of Manufacturing Process and Process Controls (flow diagram) - Control of Materials - Controls of Critical Steps and Intermediates - Process Validation and/or Evaluation - Manufacturing Process Development	EMA/CAT/852602/2018* [20] EMA/CHMP/BWP/534898/2008 [52] ICH Q5D [53] CPMP/BWP/3088/99 [54] EMA/CHMP/BWP/814397/2011 [55] EMEA/CHMP/BWP/398498/05 [56] EMEA/410/01 [57] EMA/CHMP/BWP/706271/2010 [58] GMP guidelines annex 13 [59] ICH Q9 [51] EMEA/CHMP/SWP/28367/07 [60] ICH Q5E [61]
Characterization - Elucidation of Structure and other Characteristics - Impurities	ICH Topic Q6B [62] EMA/CHMP/BWP/534898/2008 [52] EMEA/CHMP/BWP/398498/05 [56] ICH Topic Q5A (R1) [63]
Control of Drug Substance - Specification - Analytical Procedures - Validation of Analytical Procedures - Batch Analyses - Justification of Specification	ICH Topic Q6B [62] EMA/CHMP/BWP/534898/2008 [52] ICH Q2A [64] ICH Q2B [65] EMA/CAT/852602/2018 [20]
Reference Standards or Materials	EMA/CHMP/BWP/534898/2008 [52]
Container Closure System	EMA/CHMP/BWP/534898/2008 [52]
Stability - Stability Summary and Conclusions - Post-approval Stability Protocol and Stability Commitment - Stability Data	EMA/CHMP/BWP/534898/2008 [52] ICH Q5C [66]

Regulatory setting

DRUG PRODUCT	
Description and Composition of the Drug Product	EMA/CAT/852602/2018* [20] EMA/CHMP/BWP/534898/2008 [52] ICH Topic M4Q [50]
Pharmaceutical Development (manufacturing process, container closure system, microbiological attributes and usage instructions) - Components of the Drug Product - Drug Product (formulation development; overage justification if any; physicochemical and biological properties; manufacturing process development; container closure system; microbiological attributes; compatibility)	EMA/CAT/852602/2018* [20] EMA/CHMP/BWP/534898/2008 [52] ICH Topic M4Q [50]
Manufacture - manufacturer; - batch formula, - description of manufacturing process and process controls; - controls of critical steps and intermediates); - process validation and/or evaluation	EMA/CHMP/BWP/534898/2008 [52] GMP guidelines annex 13 [59] EMA/CAT/852602/2018* [20] ICH Topic Q6B [62]
Control of Excipients - Specifications - Analytical Procedures - Validation of Analytical Procedures - Justification of Specifications - Excipients of Human or Animal Origin - Novel Excipients	EMA/CHMP/BWP/534898/2008 [52] EMEA/CHMP/BWP/398498/05 [56] EMEA/410/01 [57] EMA/CHMP/BWP/706271/2010 [58] EMA/CAT/852602/2018* [20]
Control of Drug Product - Specification(s) - Analytical Procedures - Validation of Analytical Procedures - Batch Analyses - Characterization of Impurities - Justification of Specification(s)	EMA/CHMP/BWP/534898/2008 [52] EMA/CAT/852602/2018* [20] ICH Topic Q6B [62] ICH Q2A [64] ICH Q2B [65]
Reference Standards or Materials	EMA/CHMP/BWP/534898/2008 [52]
Container Closure System	EMA/CHMP/BWP/534898/2008 [52]
Stability - Stability Summary and Conclusion - Post-approval Stability Protocol and Stability Commitment - Stability Data	EMA/CHMP/BWP/534898/2008 [52] ICH Q5C [66]
APPENDICES	
A.1 Facilities and Equipment	Considered "Not applicable" for biological investigational medicinal products in clinical trials according to EMA/CHMP/BWP/534898/2008 [52]
A.2 Adventitious Agents Safety Evaluation	ICH Topic Q 5 A (R1) [63] EMEA/410/01 [57]

CTD Module 3 content	Our selection of general relevant guidelines for EV-based products
A.3 Excipients (novel excipients)	EMEA/CHMP/BWP/398498/05) [56] EMA/CHMP/BWP/534898/2008 [52]
A.4 Solvents for reconstitution and diluents"	This appendice is not in the ICH Topic M4 Q. However, it is recommended by EMA/CHMP/BWP/534898/2008 [52]

Regulatory setting

Our suggested tests for the critical quality attributes and other required tests	Development phase	Clinical batch production			
		In-process control	Drug substance control	Stability test (drug substance and finished product)	Finished product control
QUANTITY ATTRIBUTE					
Particle quantification by NTA	M		AC	AC	AC
Total protein quantification by colorimetric assays	M	M	AC	AC	AC
IDENTITY ATTRIBUTE					
Size and structure by TEM-based methods	M				
Hydrodynamic diameter analysis by NTA	M	M	AC	AC	AC
Immunochemical characterization by Elisa, MACSPlex Exosome Kit, Exoview, small particle cytometry or nanoflow cytometry	M		AC	AC	AC
DNA content (with/without DNase treatment)	M		AC		AC
RNA content (optionally with/without RNase treatment)	M		AC		AC
PURITY ATTRIBUTE					
Ratio of particle counts/micrograms of proteins	M	M	AC	AC	AC
IMPURITY / CONTAMINANTS					
Albumin or fibrinogen quantification (if EV secretion step in complete medium)	M		UL		UL
DNA (optionally RNA) quantification with and without DNase (optionally RNase) treatment, as indicated above					
Priming molecule concentration (if relevant)					
Endotoxin, sterility and mycoplasma test (according to the Eur. Pharm.) and virus testing (*in vitro* and/or *in vivo*)			AC		AC
BIOLOGICAL ACTIVITY					
Potency tests *in vitro*	M		M	M	M
Potency tests *in vivo* (if any)	M				M
OTHERS					
Appearance and description: physical state (eg., solid, liquid), color, etc.	M			AC	AC
General tests: pH and osmolarity	M			AC	AC

Production costs and indications for clinical trials

Production cost and indications

- An heterologous cell therapy product cost about 100k€ /patient
- A typical MSC production cost about 10 k€
- EVs are derived from cells, they at least cost the same price if dose needed are similar
- Everzom estimate a GMPc production (with margins) of 500-20 000 € per 10^{13} EVs depending on cell type
- Production cost usually represents about 10-20% of final price in complex products

Final (minimal) price is mostly dependant on dose/indications

Ocular => 10^{11} EVs ?=> low production cost

Systemic => 10^{13} EVs ? => High production cost

Unique versus repeated injections ?

Potential reimbursement ? €/QALY ?

Evora bioscience

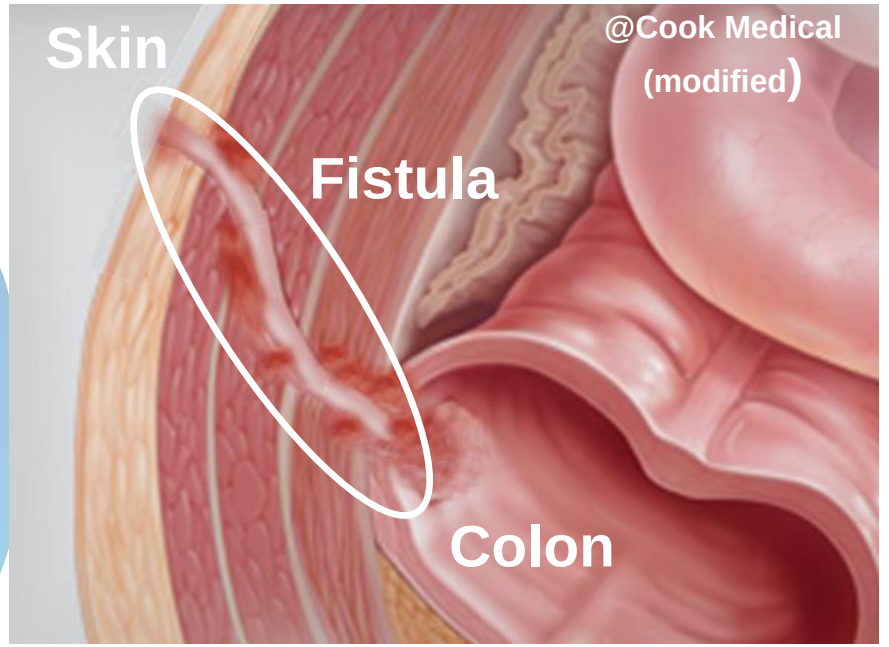
A therapeutic start-up that aims at using EVs to treat gastro-intestinal fistula

Digestive fistulas

Abnormal digestive organ communications

Secondary to surgery, Crohn's disease, cancer, trauma

=> ~1.5 M patients, high morbidity, poor healing rates



Georgiev et al. *J Gastrointest Surg 22, 2003 (2018)*
Panés et al. *Gastroenterology 154, 334 (2018)*

Digestive fistulas

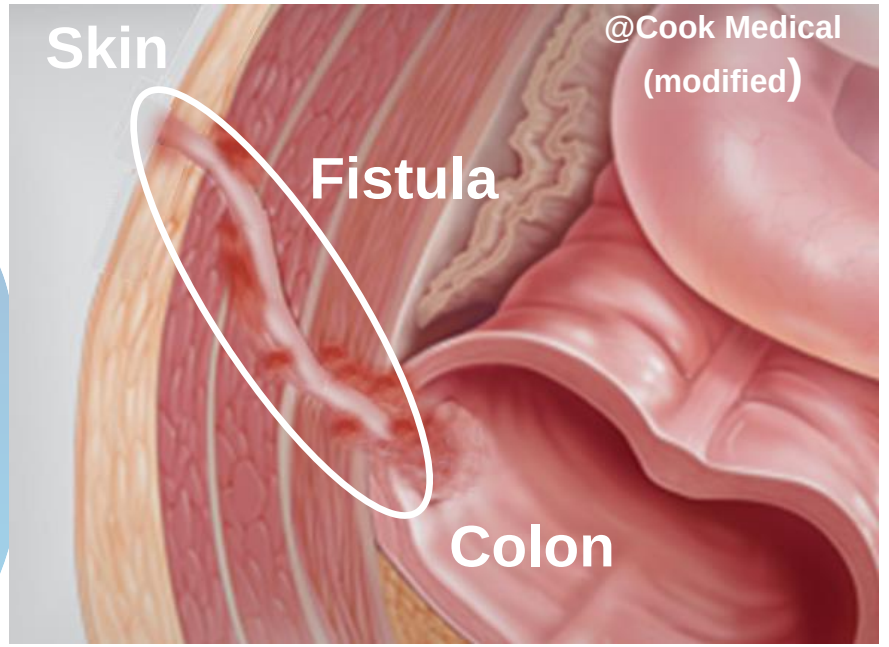
Abnormal digestive organ communications

Secondary to surgery, Crohn's disease, cancer, trauma

=> ~1.5 M patients, high morbidity, poor healing rates

~50 K€ / dose of 120 million cells
Immunomodulation effect
51% of fistula remission
(36% for standard of care)

UNMET NEED



Georgiev et al. *J Gastrointest Surg 22, 2003 (2018)*
Panés et al. *Gastroenterology 154, 334 (2018)*

Roadblocks in Fistula

Challenges

Administration

Keep EVs on the target

EV administration by intravenous injection

Fast clearance,
off-target biodistribution

Gap

Pre-clinical study

Clinical translation

Hypothesis
EV delivery locally by a gel for synergy

Challenges — **Administration**

Concepts — **EV carrier gel**

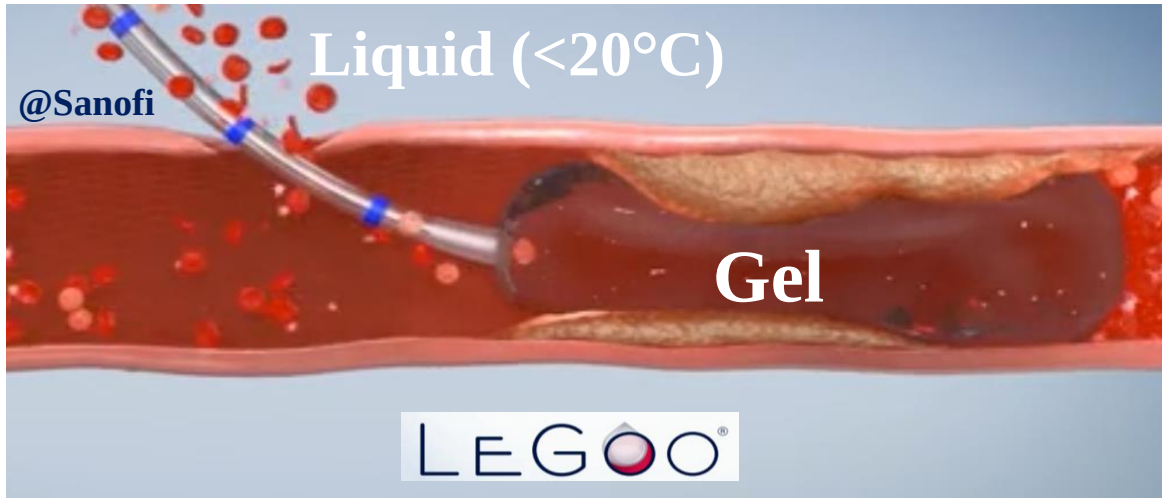
Goals — **Keep EVs on the target**
Mechanical effect

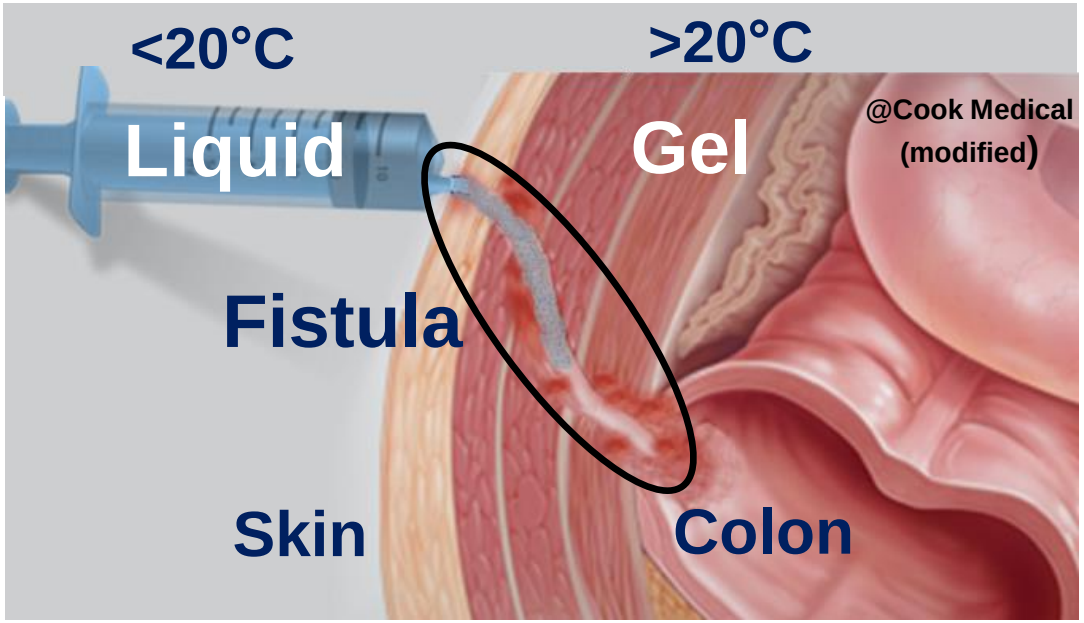
Pre-clinical study

Clinical translation

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

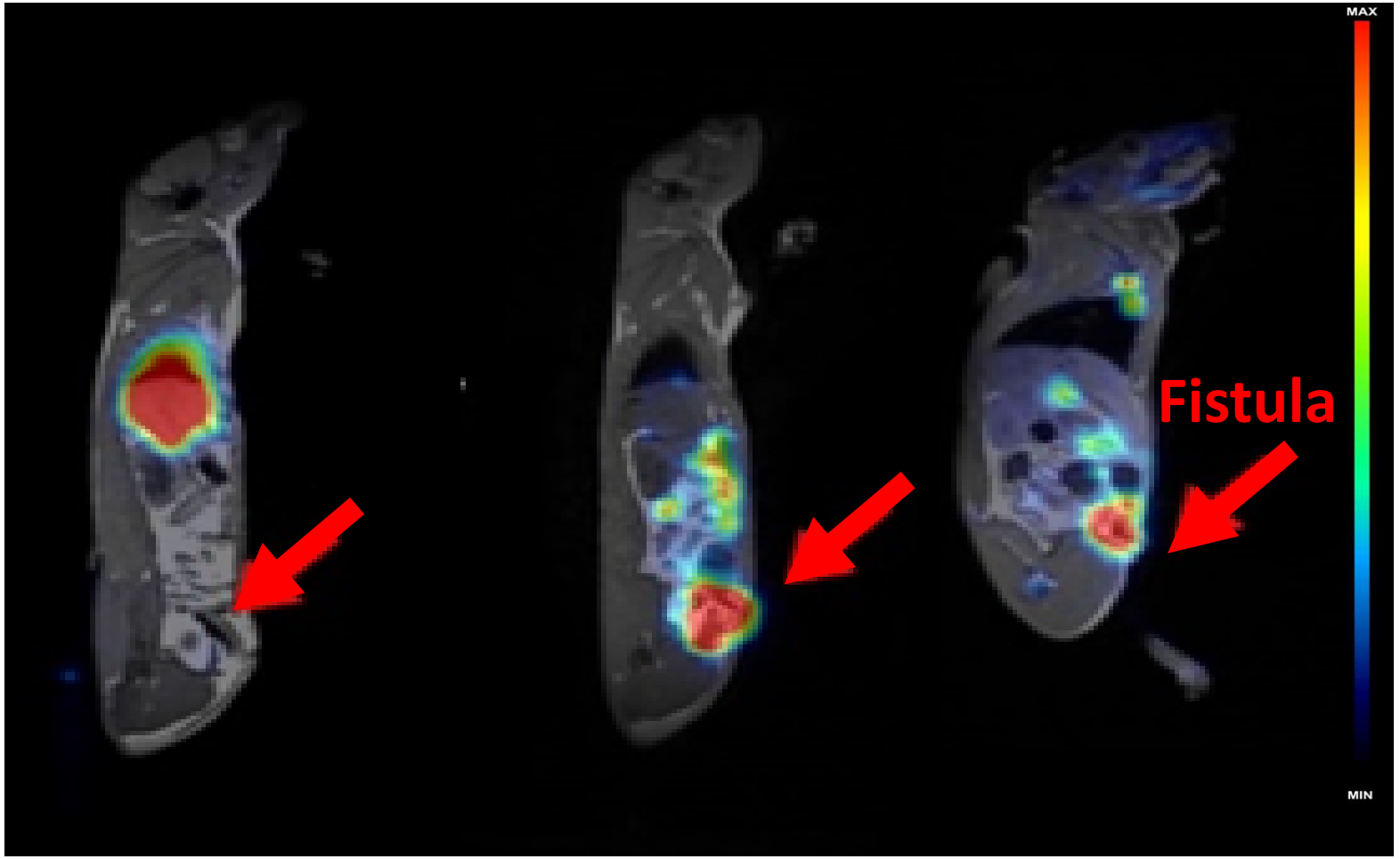
Authorized vessel occluder medical device → **Repurposing** → **Fistula occluder**





A Silva *et al.* Patent EP161788856

Local administration in the Gel keep EVs on site



PET-MRI Images 1h after the administration of murine starvation MSC Evs labelled with a PET tracer

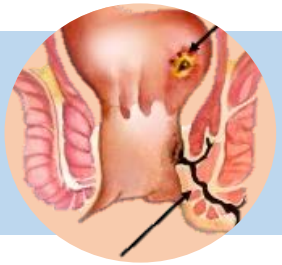
colocutaneous fistula model in rats

EV intravenous injection In saline

EV local injection In saline

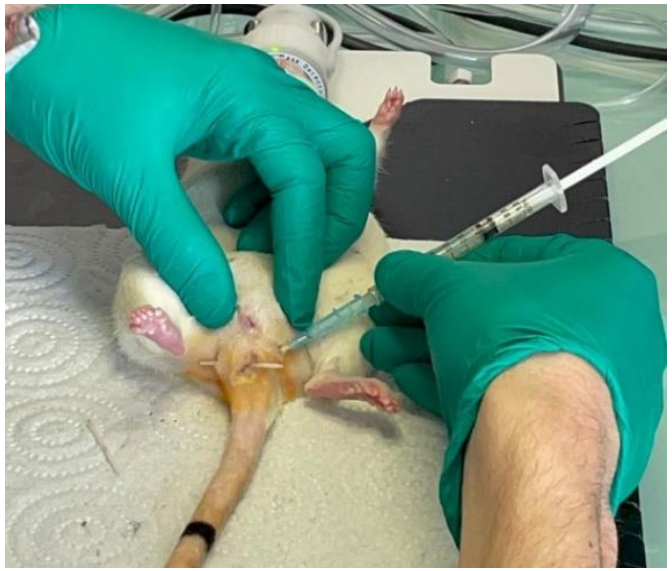
EV local injection In the gel

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



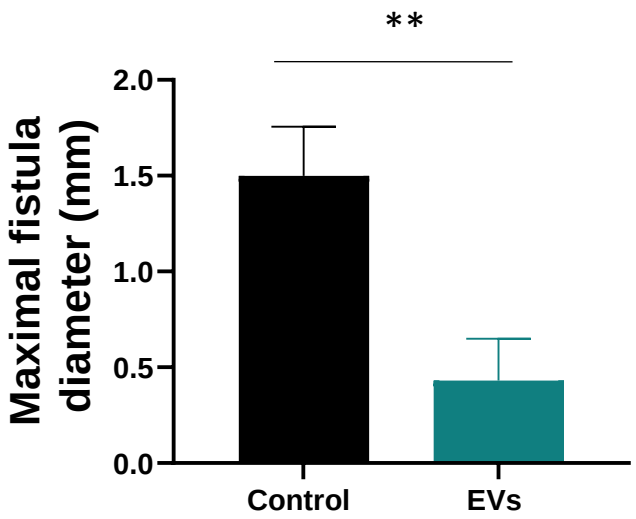
Regenerative effect of human turbulence ADSC EVs (2 x 10^{11}) on inflammatory perianal fistula model in rats

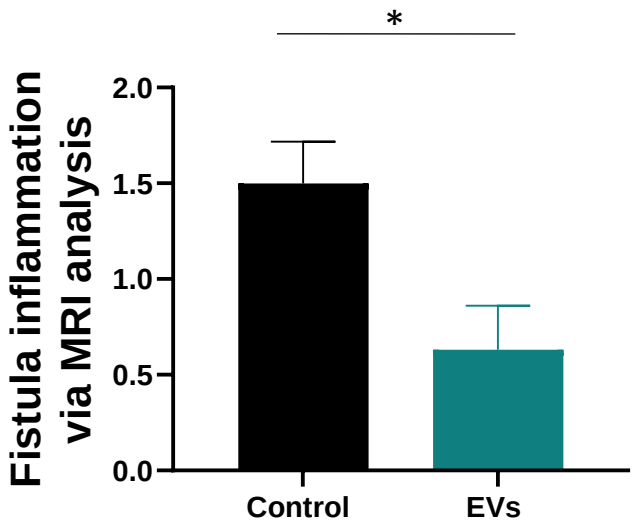
Boris Rosenbaum
(MD), Master student



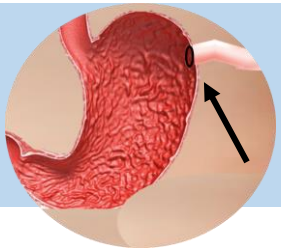
Animal model:
Inflammation by trinitrobenzene sulfonic acid + trans-rectal suture

Reduction of fistula orifice and inflammation



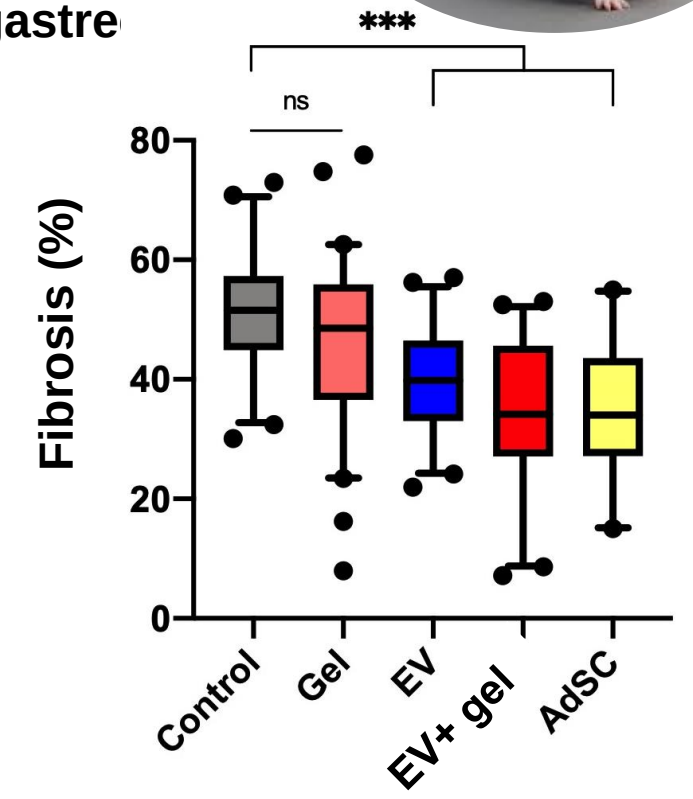


Efficient in perianal fistula in rat

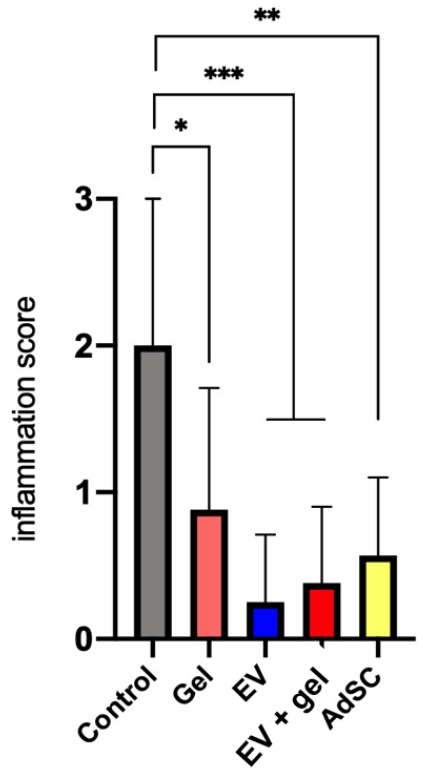


Regenerative effect of human turbulence ADSC EVs (2 x 10^{11}) on post-surgical gastro-cutaneous fistula model in rats

Animal model: gastro-cutaneous fistula after sleeve gastre

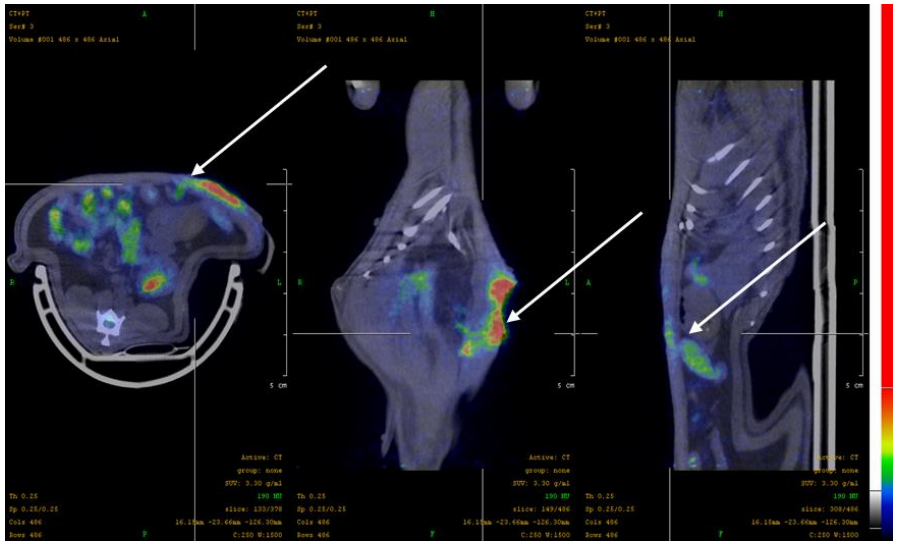


Reduction of fistula fibrosis and inflammation

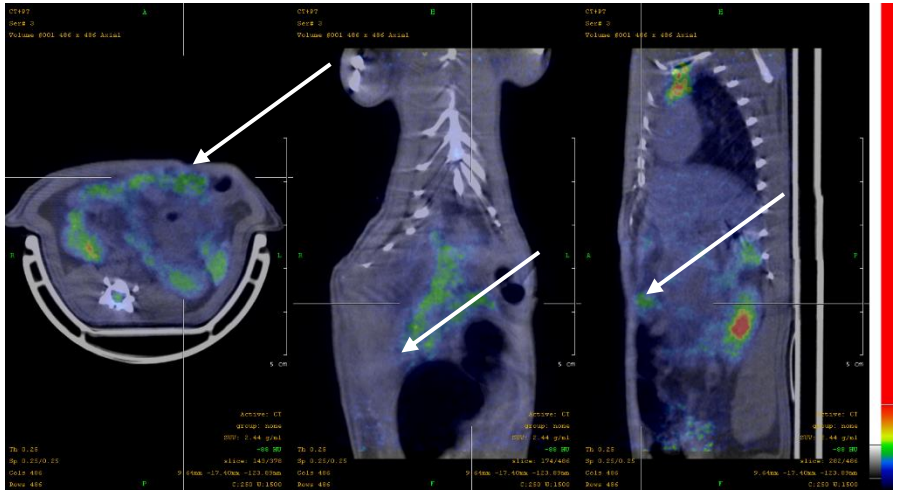


PET analysis [18 F]-fluoro-2-deoxy-d-glucose (FDG) detecting inflammation

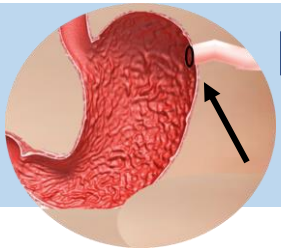
Control



EVs



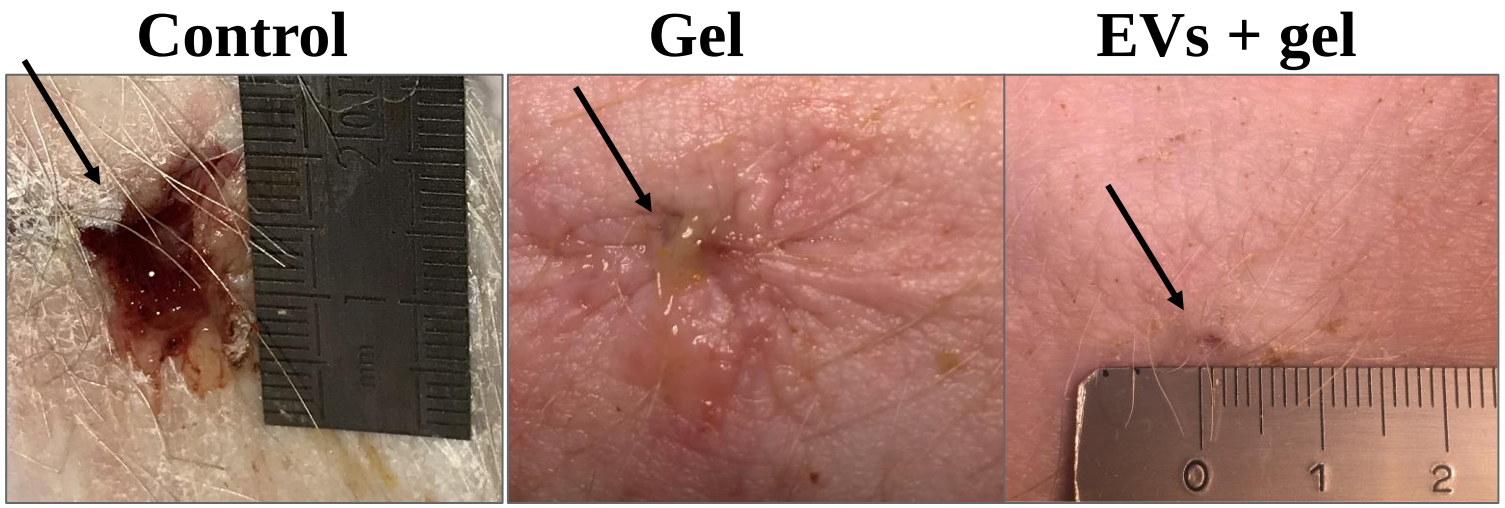
Efficient in gastro-cutaneous fistula in rats

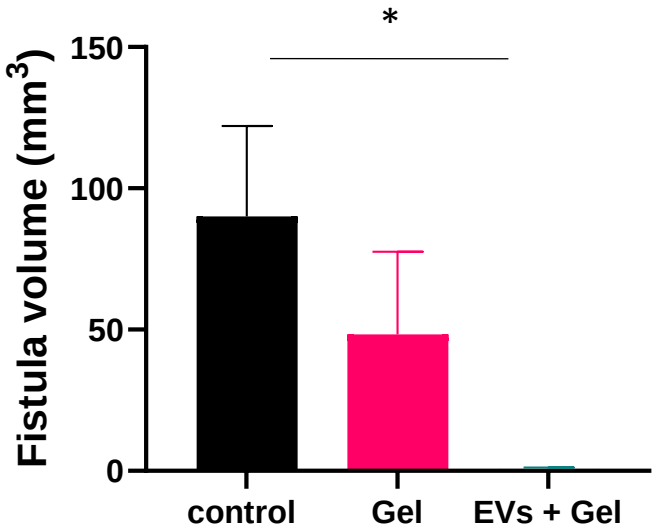


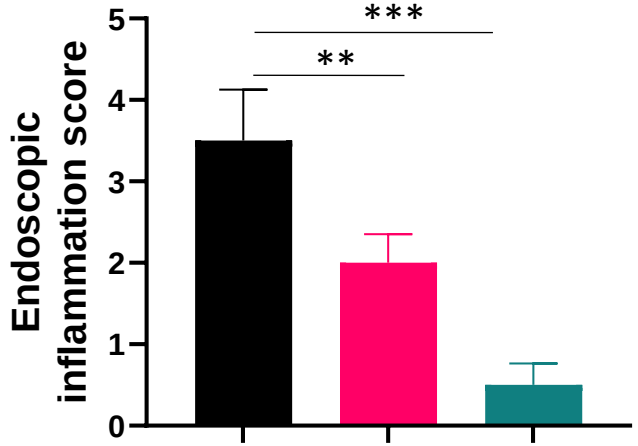
Regenerative effect of human turbulence ADSC EVs (2 x 10^{12}) on post-surgical gastro-cutaneous fistula model in pigs

Animal model: gastro-cutaneous fistula after sleeve gastrectomy

Reduction of fistula orifice and inflammation

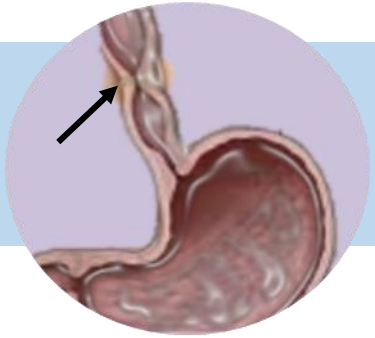






Efficient in gastro-cutaneous fistula in pigs

evora BIOSCIENCES

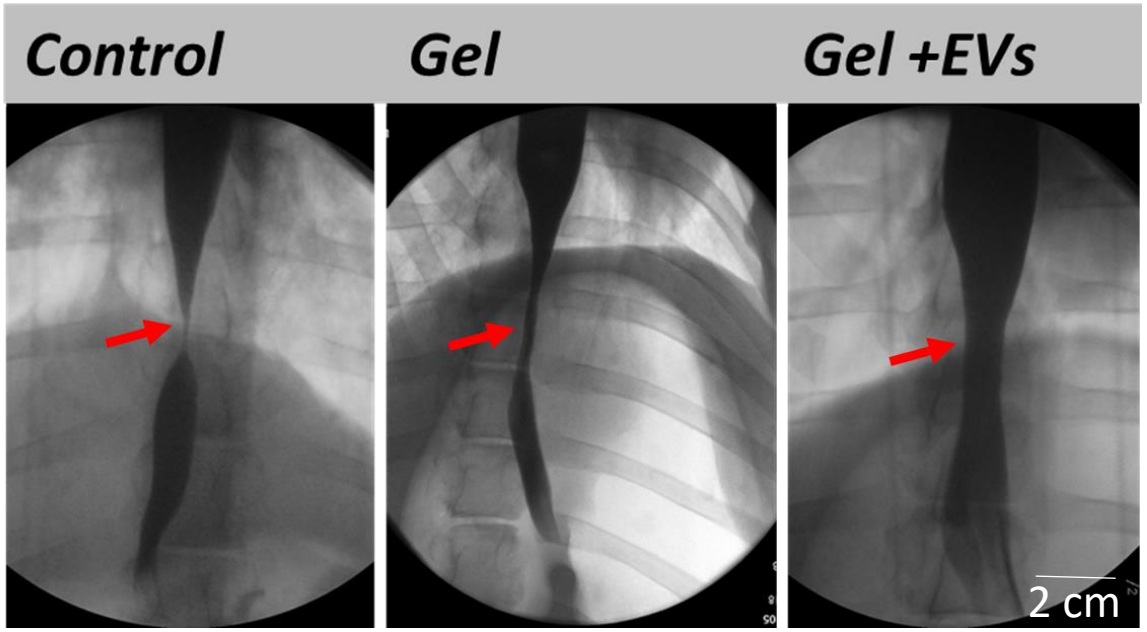


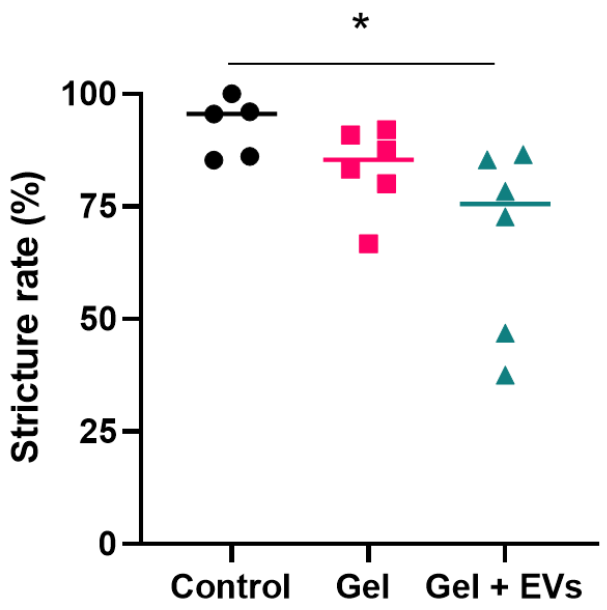
Regenerative effect of allogenic turbulence EVs (1.5×10^{12}) to prevent esophageal stricture in pigs

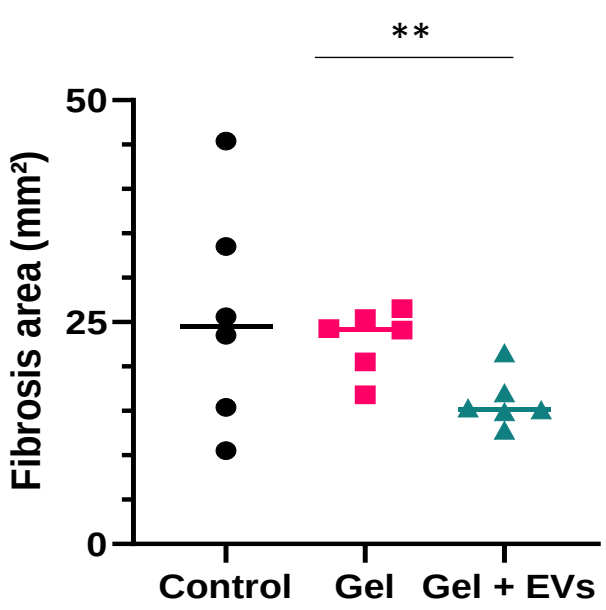
Elise Coffin (MD)
Master student

Animal model: Spontaneous esophageal stricture post submucosal dissection

Reduction of stricture and anti-fibrotic action







Efficient in gastro-cutaneous fistula in pigs

So what would you do next ?

- What is the objective ?
- Creating a company ? Staying academic ? Being both ?
- Business Model ?
- Intellectual property ?
- Team ?
- Regulatory environement ?
- Shares ?
- Location ?
- Relations with academia ?
- Technology transfer ?
- Funding : Grant ? Private money ? Business angels ? Fund ?
- Public relations and networking ?

How to choose an indication ?

Need ?

Science ?

IP ?

Market ?

Clinical trial design and outcome ?

Competition ?

Reimbursement ?

Partnership opportunity ?

Therafast bio

A therapeutic start-up that aims at putting in the clinic Caloric restriction mimetics

Therafast Bio

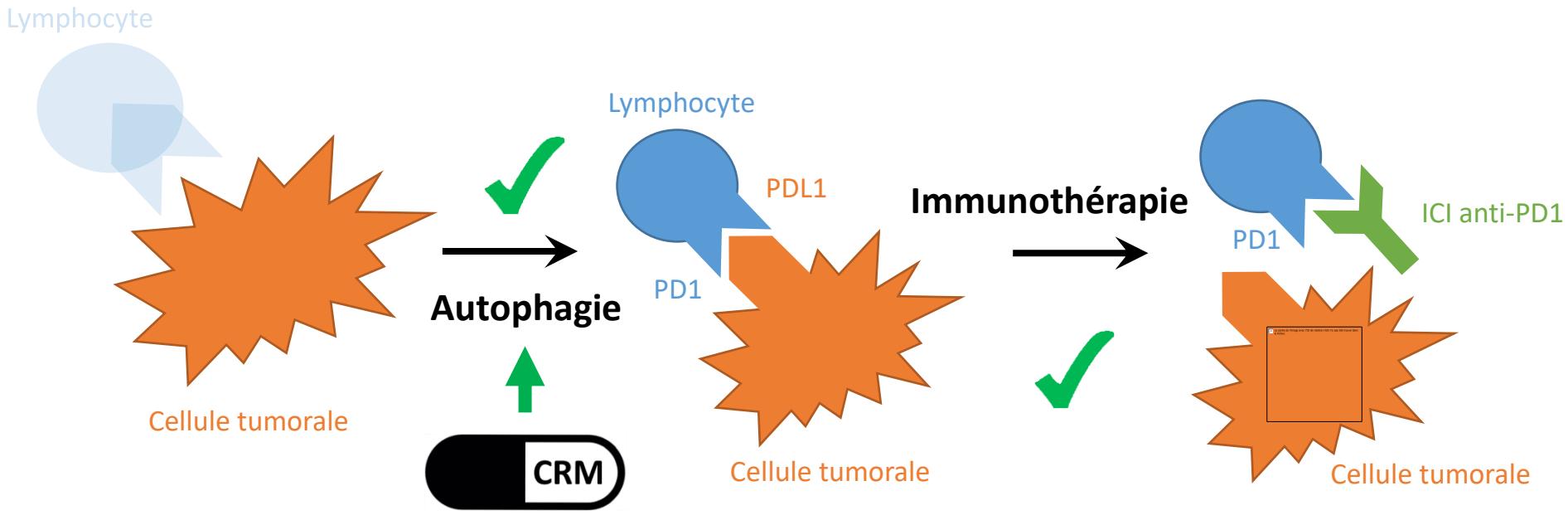
Caloric Restriction Mimetics

Indication	Response rate (%)
Non-small cell lung cancer (NSCLC), squamous and non-squamous	15-20%
Small cell lung cancer (SCLC)	15%
Renal cell Carcinoma (RCC)	15-20%
Bladder cancer	25%
Head & neck squamous cell carcinoma (HNSCC)	15-25%
Gastric cancer	20%
Hepatocellular carcinoma (HCC)	20%
Hodgkin's Lymphoma (HL)	65-85%
Ovarian cancer	15%
Triple negative breast cancer (TNBC)	20%

Source: Curie Institute; Bryan, Garnier & Co.ests.

→Objectif : Augmenter le taux de réponse aux immunothérapies

Concept scientifique



Nobel Prize 2016

Caloric Restriction Mimetics

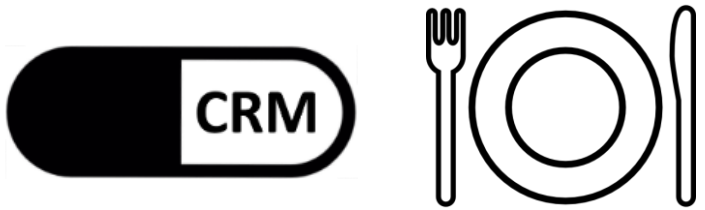
Restriction Calorique (jeûne)

Effets thérapeutiques connus

Mécanisme biologique bien décrit

Mais **<u>dénutrition sévère</u>**

Mimétiques de restriction calorique



Mime l'action de la restriction calorique au niveau cellulaire

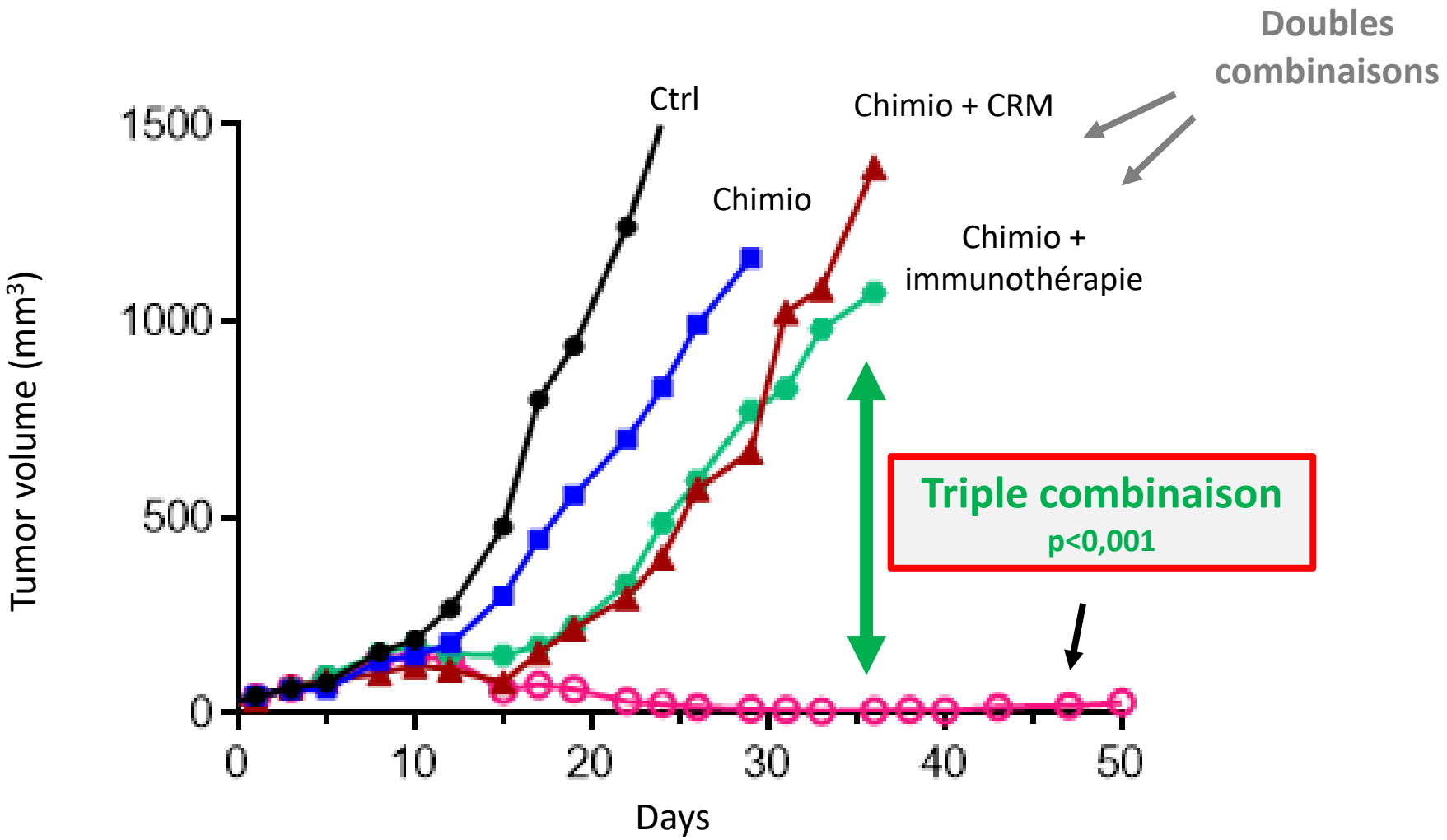
↓

reproduit les effets thérapeutiques du jeûne

Alimentation ad libitum

<u>sans dénutrition</u>

Chimiothérapie, Immunothérapie et CRM



Modèle syngénique immunocompétent , souche MCA205 résistante aux immunothérapies, Chimiothérapie n>8/groupe

Nature reviews Drug Dis 2014
Nature reviews Drug Dis 2017
Nature reviews Clin Onc 2016
Molecular Cell 2014
Autophagy 2014
Autophagy 2016
Cancer cell 2016
Oncoimmunology 2019
Kroemer *et al*, patent 2018

Données précliniques et cliniques

Notre Equipe a démontré :

⇒ Effet des CRM sur système immunitaire bien identifié

⇒ En combinaison avec chimiothérapie et immunothérapie : synergie

⇒ Validé sur 3 modèles

Une équipe indépendante a démontré :

⇒ Valide l'intérêt des CRM

⇒ Validé sur 3 modèles

Données cliniques

⇒ La toxicité est très limitée chez l'humain

⇒ Dose maximale tolérée connue

⇒ Signal préliminaire d'efficacité sur >50 patients

⇒ Effet intrinsèque de la combinaison en l'absence de chimiothérapie et immunothérapie

So what would you do next ?

- What is the objective ?
- Creating a company ? Staying academic ? Being both ?
- Business Model ?
- Intellectual property ?
- Team ?
- Regulatory environement ?
- Shares ?
- Location ?
- Relations with academia ?
- Technology transfer ?
- Funding : Grant ? Private money ? Business angels ? Fund ?
- Public relations and networking ?

How to choose an indication ?

Need ?

Science ?

IP ?

Market ?

Clinical trial design and outcome ?

Competition ?

Reimbursement ?

Partnership opportunity ?

Thank you !

Contact : Max.piffoux@cri-paris.org

Laboratoire MSC (Paris) / CHU Lyon Sud / Centre Léon Bérard (Lyon)