UNIVERSITÀ DI PAVIA
Department of Drug Sciences

Nano Innovation 2020
Rome, 15-18 September
Conference & Exhibition

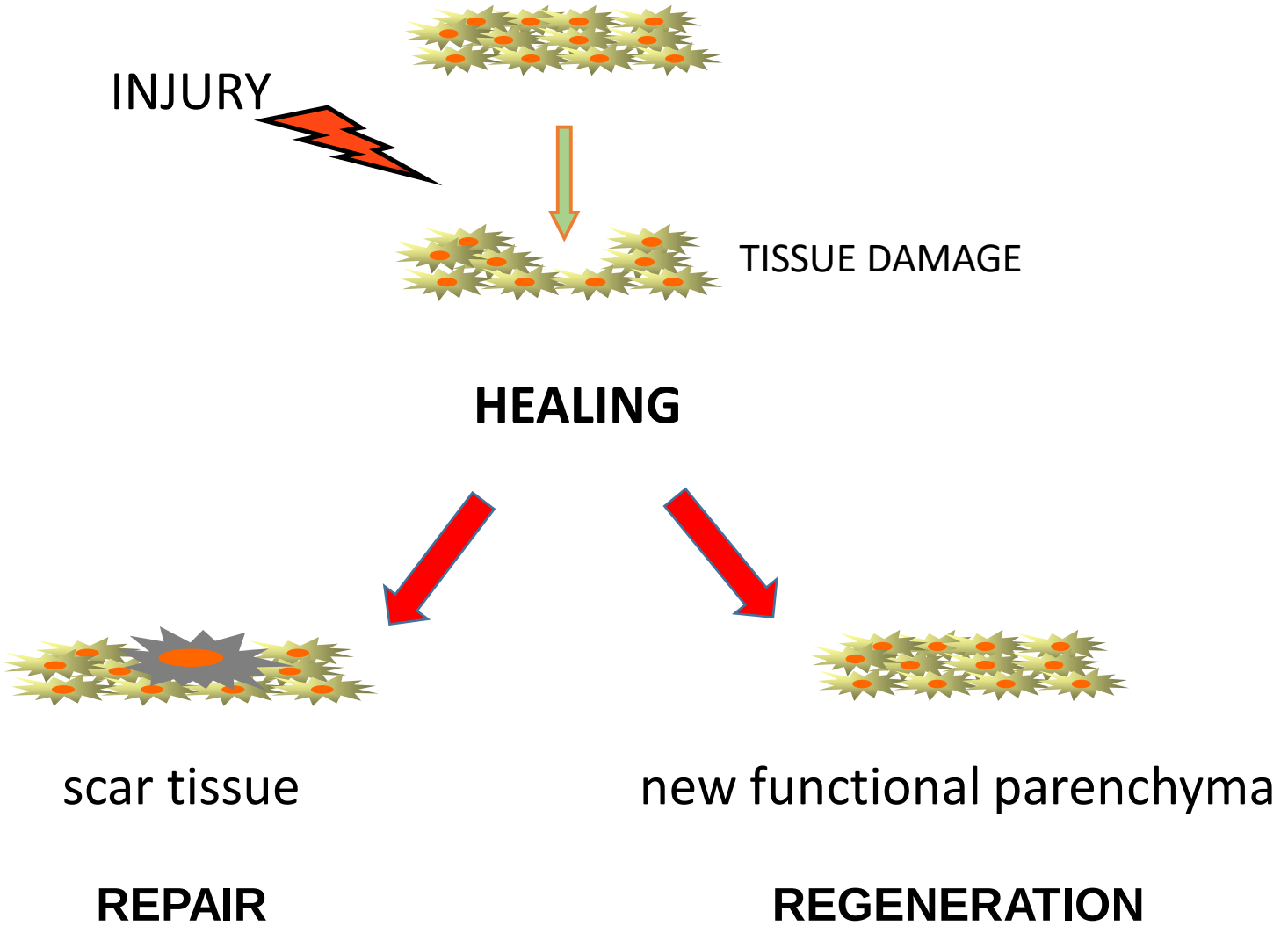
Mesenchymal stem/stromal cell secretome for regenerative medicine and drug delivery

Pharmaceutical challenges for clinical use

Maria Luisa Torre, PhD

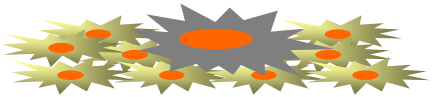
September, 17th, 2020

Tissue Regeneration and Repair

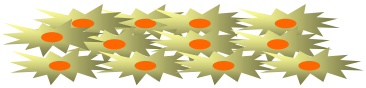


A brief definition of Regenerative Medicine

REPAIR



REGENERATION



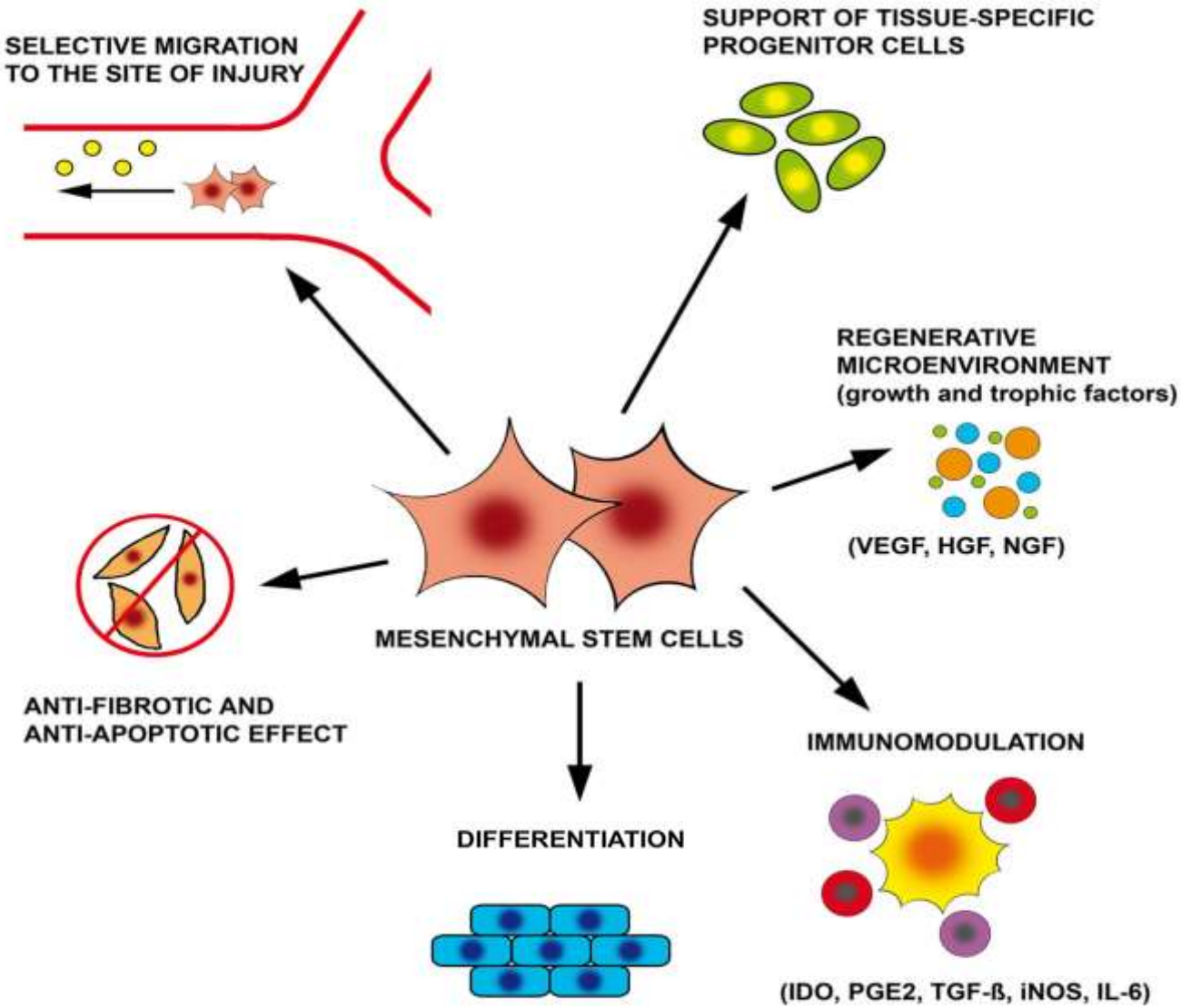
type of tissue
size and the depth of damage
individual's health status
age

"Regenerative medicine replaces or regenerates human cells, tissue or organs, to restore or establish normal function"

"These approaches may include, but are not limited to, the use of soluble molecules, gene therapy, stem cell therapy, tissue engineering and the reprogramming of cell and tissue types"

Mason and Dunnill, Future Medicine 2008

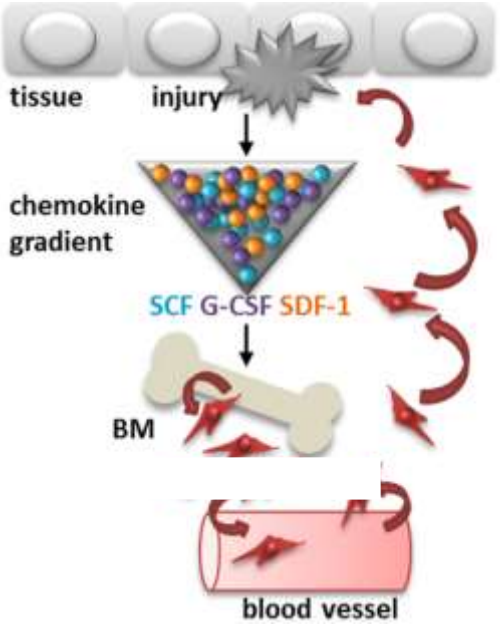
Tissue regeneration and Mesenchymal stem cells (MSC)



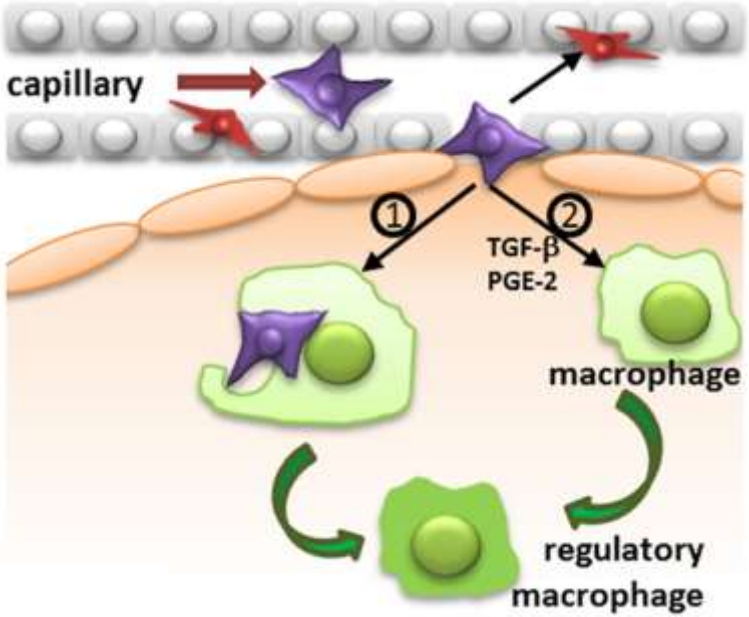
Sahar, Stem Cells Regen Med. 2017

MSC homing and recruitment by injured tissue

Physiological Migration



Cell Therapy



Eggenhofer et al Front. Immunol 2014

MSCs as drugs for regenerative therapy

Current Pharmaceutical Design, 2013, *19*, 2459-2473

Mesenchymal Stem/Stromal Cells: A New "Cells as Drugs" Paradigm. *Efficacy and Critical Aspects in Cell Therapy*

Laura de Girolamo[1], Enrico Lucarelli[2], Giulio Alessandri[3], Maria Antonietta Avanzini[4], Maria Ester Bernardo[5], Ettore Biagi[6], Anna Teresa Brini[7], Giovanna D'Amico[8], Franca Fagioli[9], Ivana Ferrero[10], Franco Locatelli[5,11], Rita Maccario[4], Mario Marazzi[12], Ornella Parolini[13], Augusto Pessina[14,*] and Maria Luisa Torre[15]; Italian Mesenchymal Stem Cell Group (GISM).

STEM CELLS AND DEVELOPMENT
Volume 24, Number 6, 2015

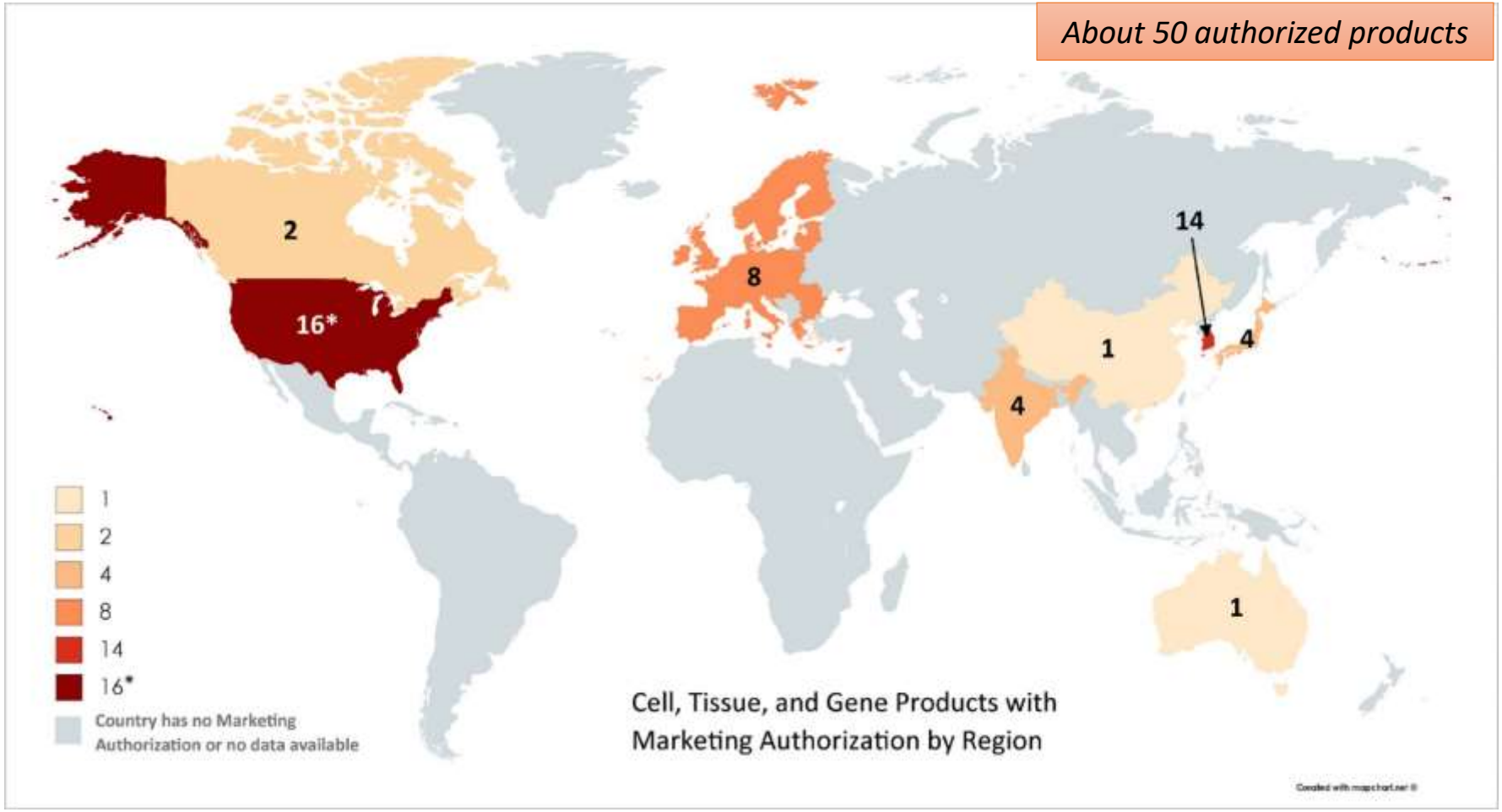
DOI: 10.1089/scd.2014.0299

COMPREHENSIVE REVIEW

Ex Vivo Expanded Mesenchymal Stromal Cell Minimal Quality Requirements for Clinical Application

Maria Luisa Torre,[1,*] Enrico Lucarelli,[2,*] Simona Guidi,[3] Maura Ferrari,[4] Giulio Alessandri,[5] Laura De Girolamo,[6] Augusto Pessina,[7] Ivana Ferrero,[8] on behalf of the Gruppo Italiano Staminali Mesenchimali (GISM)

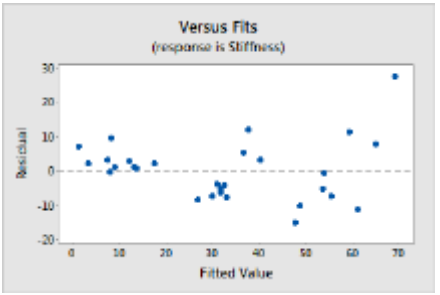
ATMP marketing authorization (2018)



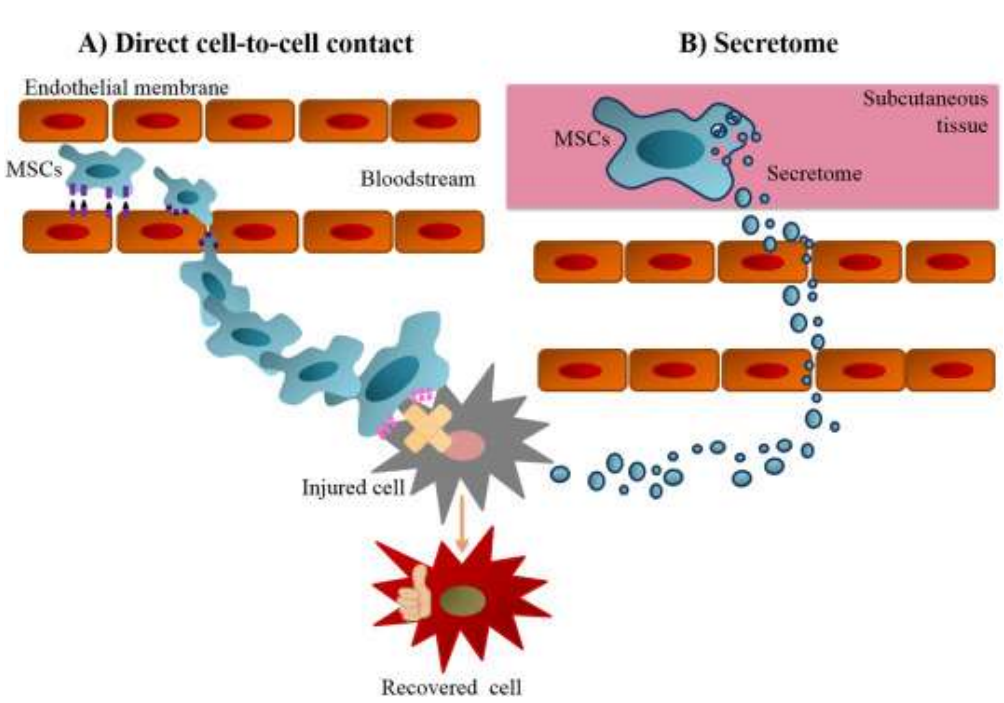
Cuende et al. Cytotherapy 2018

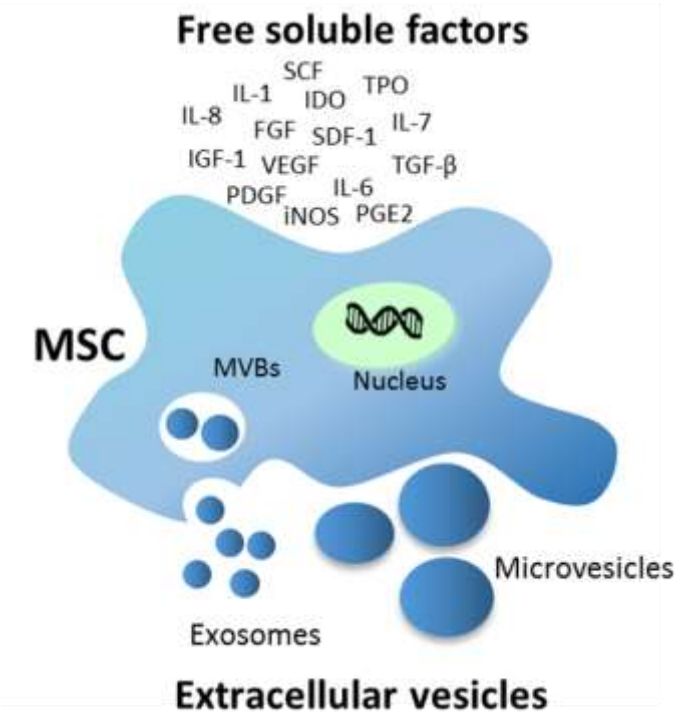
Some of the current issues facing MSCs therapies

1. High **cost** of products
2. Production/distribution bottlenecks (cryopreservation/**logistic**)
3. Size of MSC dramatically increases in culture and the expression of adhesion molecules is strongly up regulated. This affects the biodistribution of MSC after e.v. administration (lung accumulation)
4. **Variable efficacy** (duration/persistence)



MSC secretome and tissue regeneration





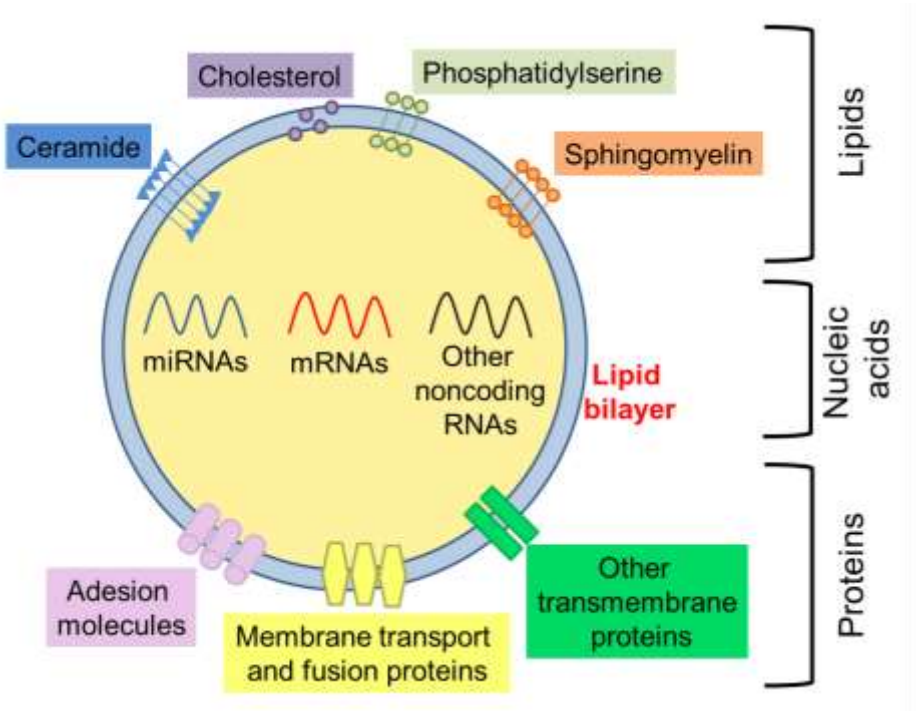
*"The **multipotency** of MSCs **is not the key** aspect for their current therapeutic use"*

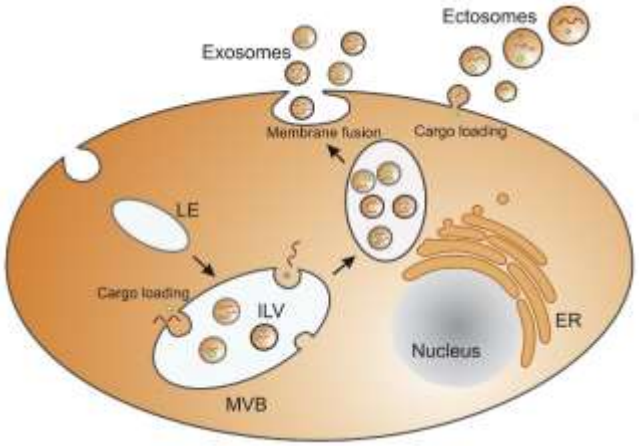
*"MSCs are powerful **site-regulated DRUG STORES** that may serve as modulatory or curative agents for a variety of human maladies"*

Caplan Tissue Eng. Part A, 2010

Crivelli et al. J Control Release, 2017

MSC secretome contains extracellular vesicles (EVs)

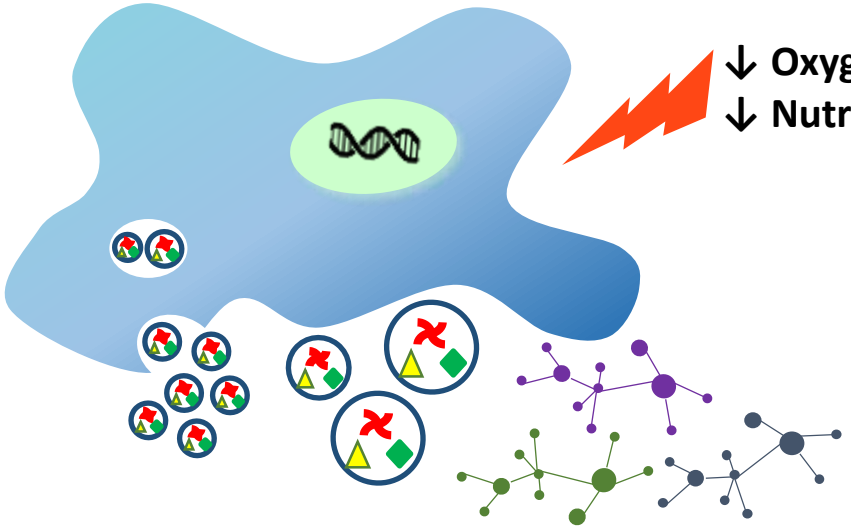




	EXOSOMES	MICROVESICLES (OR ECTOSOMES)
Size (nm)	40 – 150	150 – 600
Biogenesis	Multivesicular bodies (MVBs) fusion with cell membrane	Outward budding of cell membrane

Stremersch et al, J Control Release 2016
Crivelli et al. J. Control. Release 2017

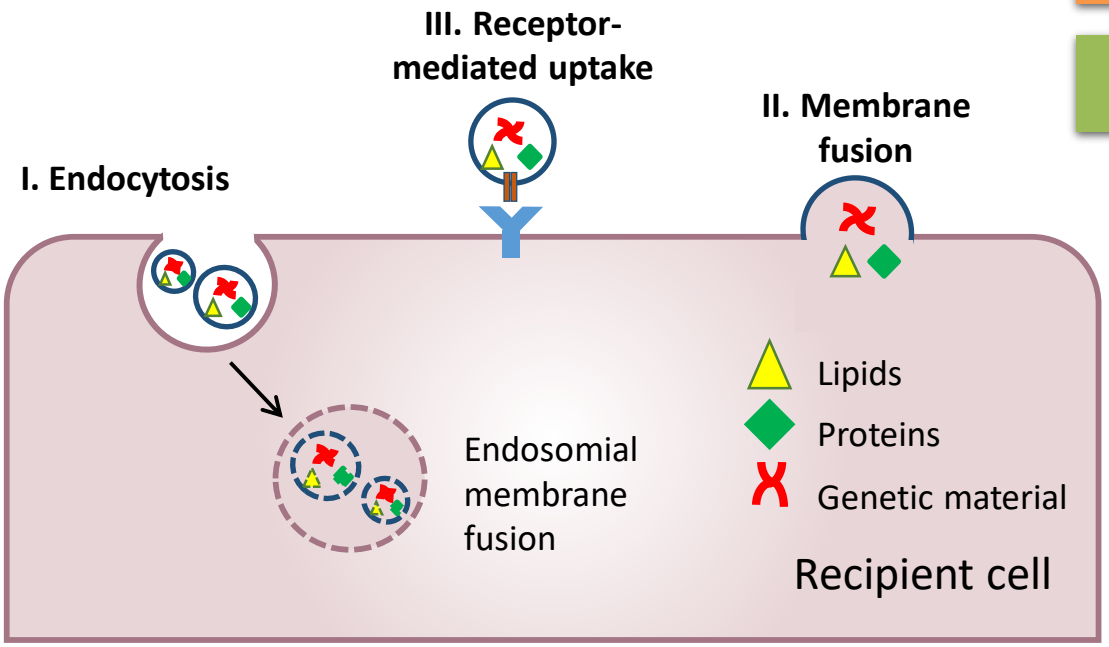
MSC-secretome is a physiological therapeutic agent



Transfer of bioactive compounds

Regulation of receptors

Phenotype changes



Bari et al. J. Control. Release 2019

Secretome can replace MSC regenerative therapy

MSCs

Secretome

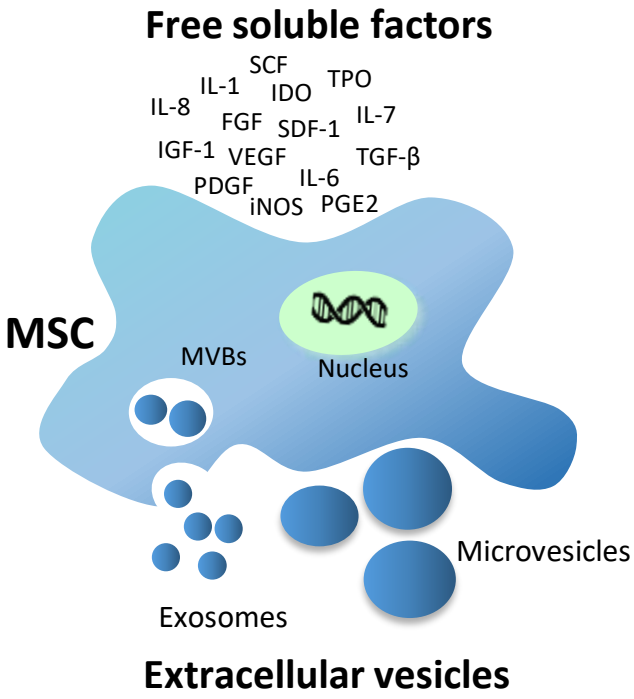
Ability to adapt and respond to the microenvironment

Safer (non-living, lower immunogenicity, no vascular clotting)
Nanoscale (enhanced permeability and retention effect)
Physiological mediators of tissue regeneration
Effectively **cross biological membrane**
Scalable production process

MSC-secretome shows fewer limitations than its parental cells

Crivelli et al. J. Control. Release 2017

Our challenge: secretome pharmaceuticalization



Quality

Safety

Efficacy

GOOD MANUFACTURING PRACTICE GMP

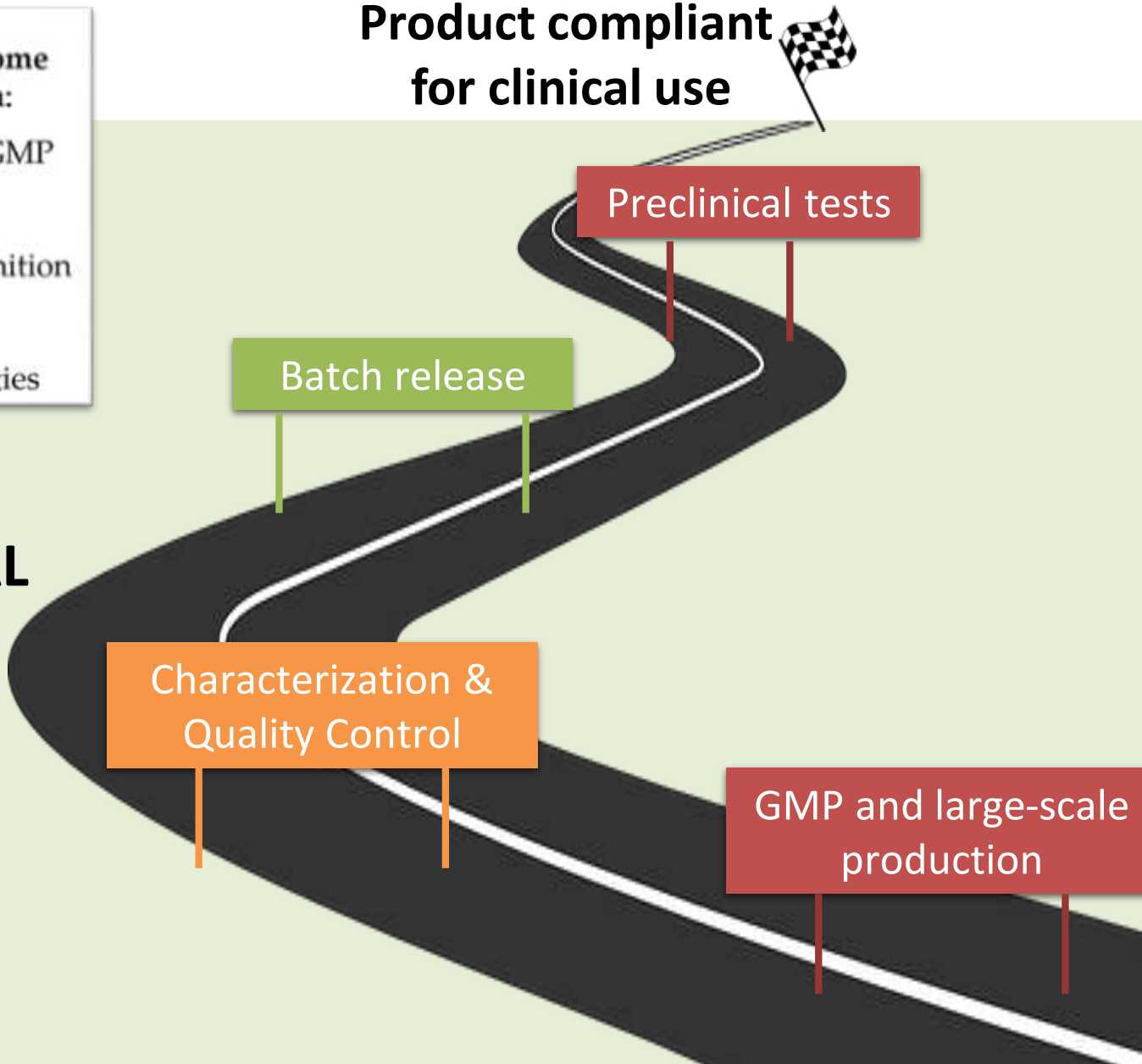
Industrial scale-up

From bench to clinical use

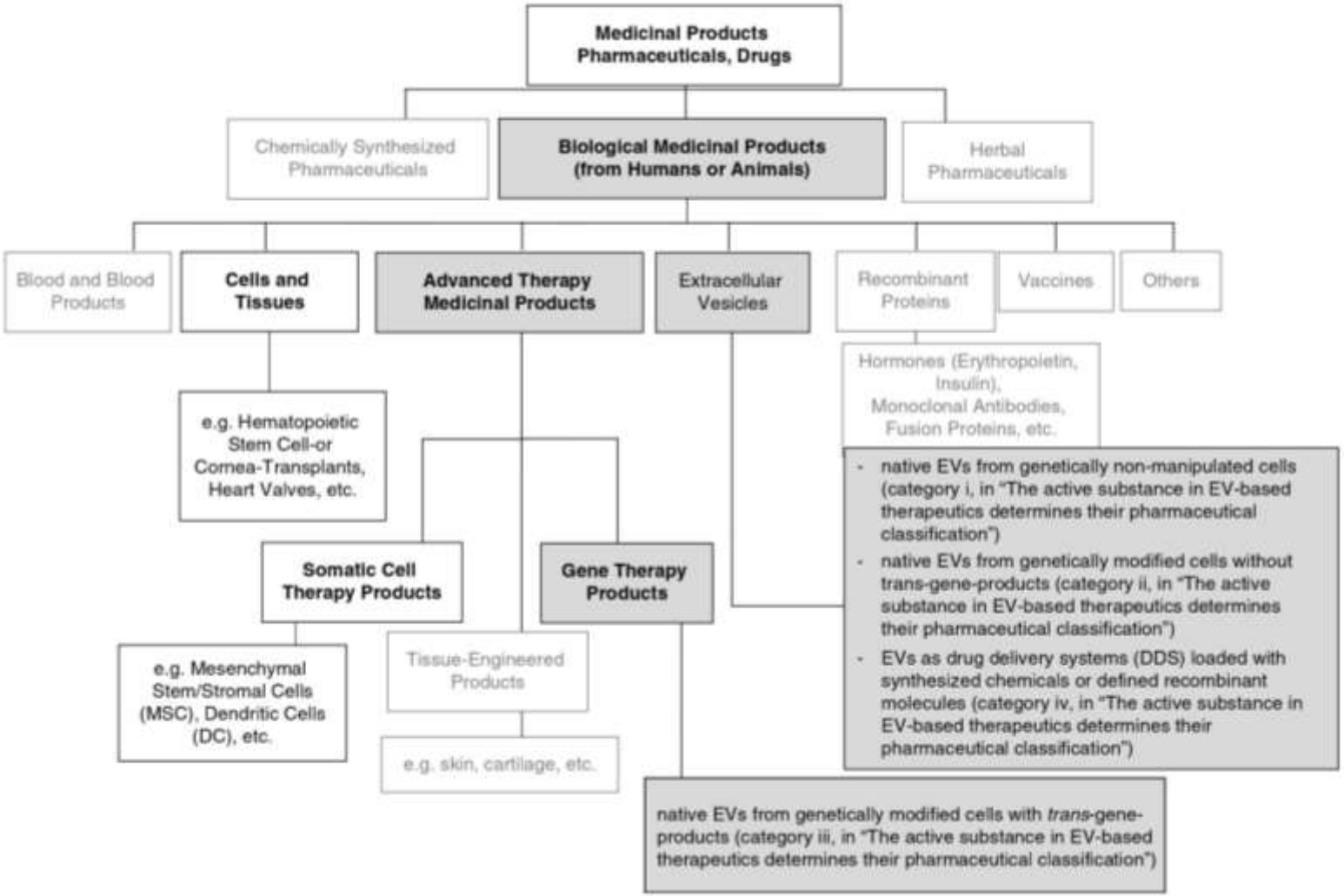
Missing steps for secretome pharmaceuticalization:

- Scalable, reproducible and GMP purification protocols
- In-depth characterization
- Mechanism(s) of action definition
- Dosage and frequency of administration studies
- Effective formulation strategies

PHARMACEUTICAL REGULATORY FRAMEWORK



Pharmaceutical regulatory framework



Special guidelines targeting EV-based therapeutics may be needed

Lener et al. J Extracell Vesicles 2015

Scale-up for GMP Lyosecretome production

cells

MDPI

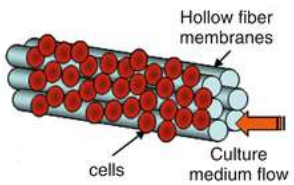
Article

Pilot Production of Mesenchymal Stem/Stromal Freeze-Dried Secretome for Cell-Free Regenerative Nanomedicine: A Validated GMP-Compliant Process

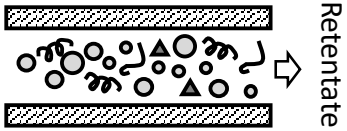
Elia Bari [1], Sara Perteghella [1,2,*], Dario Di Silvestre [3], Marzio Sorlini [2,4], Laura Catenacci [1], Milena Sorrenti [1], Giorgio Marrubini [1], Rossana Rossi [3], Giuseppe Tripodo [1], Pierluigi Mauri [3], Mario Marazzi [5] and Maria Luisa Torre [1,2]

GMP

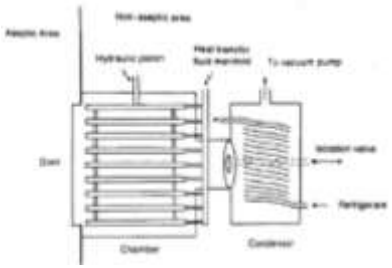
Industrial scale-up



Supernatant recovery



Formulation



Serum starvation

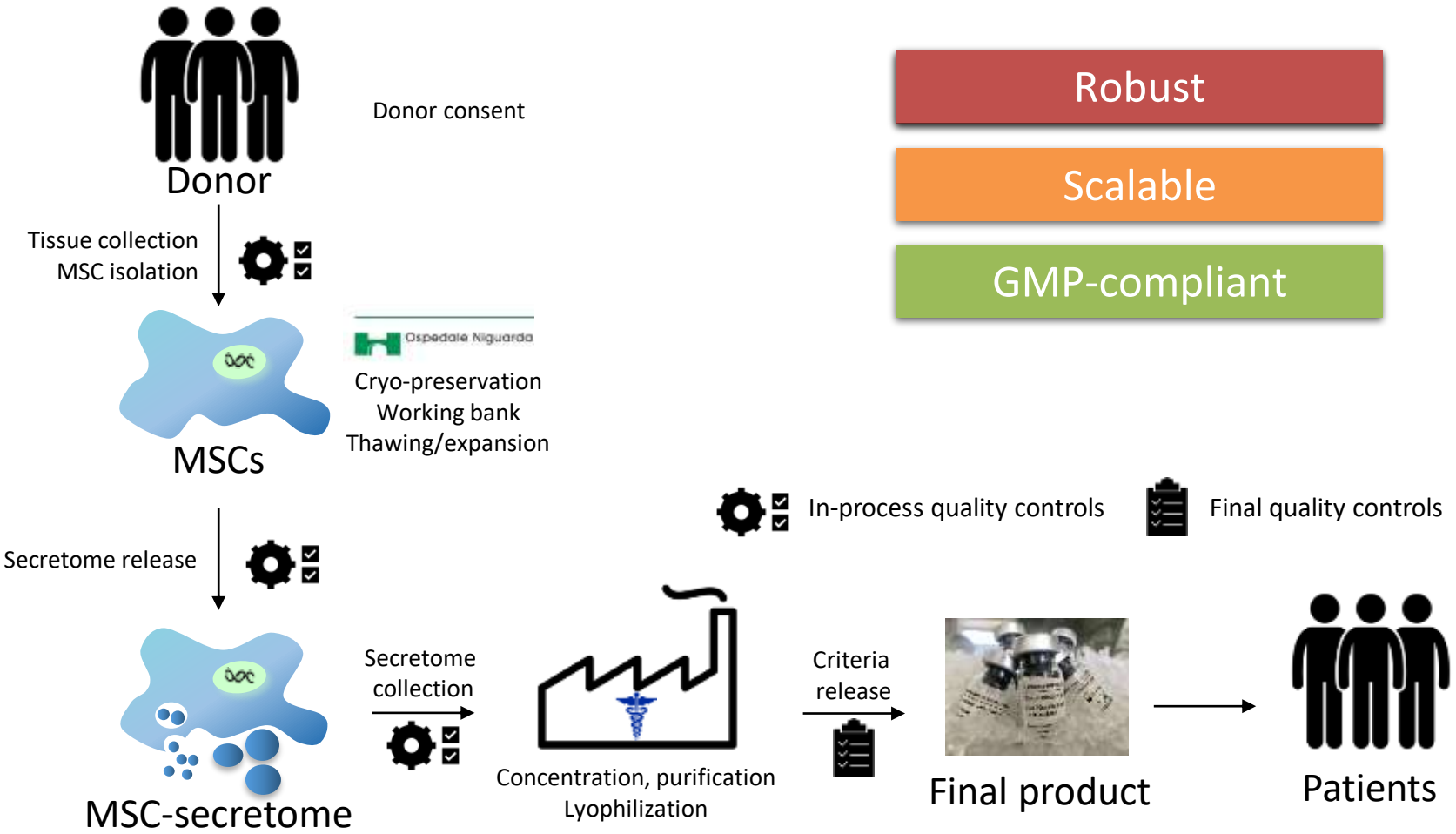
Ultrafiltration

Freeze-drying

Perteghella, Bari et al. WO2018078524 (A1)

Bari et al. Cells 2018

Lyosecretome pharmaceutical production process



Robust

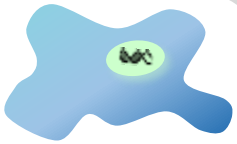
Scalable

GMP-compliant

"The process is the product"

Perteghella, Bari et al. WO2018078524 (A1)
Bari et al. Cells 2018

Quality controls and minimal criteria for clinical use



MSCs

Cell viability

Above 98%

Identity

Concordance with ISCT criteria

Sterility

No microbial, endotoxin, viral or mycoplasma contamination

MSC-EV

Molecular fingerprinting

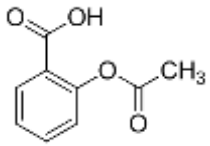
Components and composition

Pharmacopoeia test

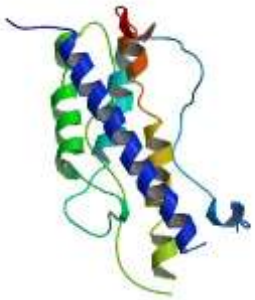
For the specific dosage form

Sterility

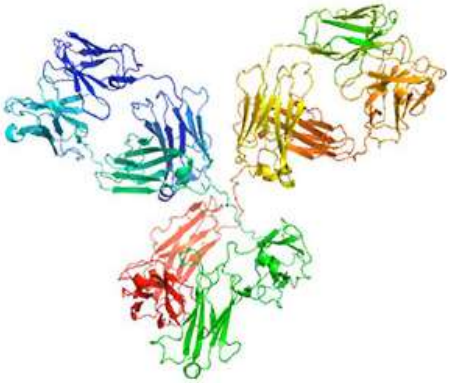
No microbial, endotoxin, viral or mycoplasma contamination



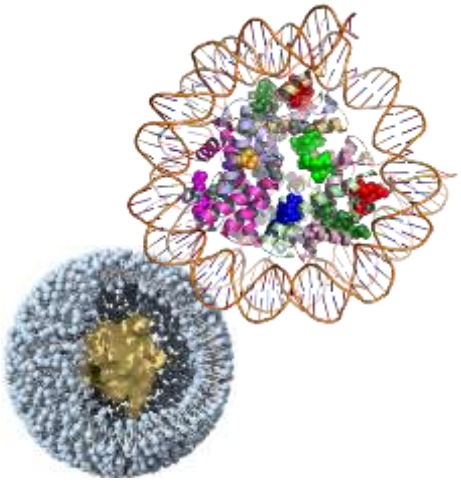
Small molecule drugs



Large molecule drugs



Biologic



Secretome

COMPLEXITY

Bari et al. Cells 2018

Extracellular Vesicle Characterization methods

Flow Cytometry	•EV phenotype, count and size •Fast technique •Unable to detect exosomes •Combined with imaging
Resistive Pulse Sensing	•EV count and size •Low sample volumes (40µl) •"Clogging" of the instrument
Electron Microscopy	•Transmission electron microscopy (TEM) and CryoEM •EV image, phenotype and count •Phenotype limited to few surface markers •Number of images achieved for statistical significance
Atomic Force Microscopy	•Cellular source determined by using antibody-coated substrates •Antibody aggregation •Number of images achieved for statistical significance
Nanoparticle Tracking Analysis	•Size from 30 to 1000 nm •EV concentration •Low precision with heterogeneous samples
Dynamic Light Scattering	•Size from 5-10 nm to 6 µm •Zeta potential •Limitations for polydispersed samples
Colorimetric assays	•Bradford and BCA assays for total protein content determination •Sulfophosphovanilin (SPV) assay for total lipid determination
Western blot	•Identification of specific proteins
RT-PCR	•miRNA

Erdbrügger and Lannigan. Cytometry 2016

“Good and Bad” extracellular vesicles

Physiological role

- Immune surveillance
- Blood coagulation
- Synaptic plasticity
- Stem cell maintenance

Pathological role

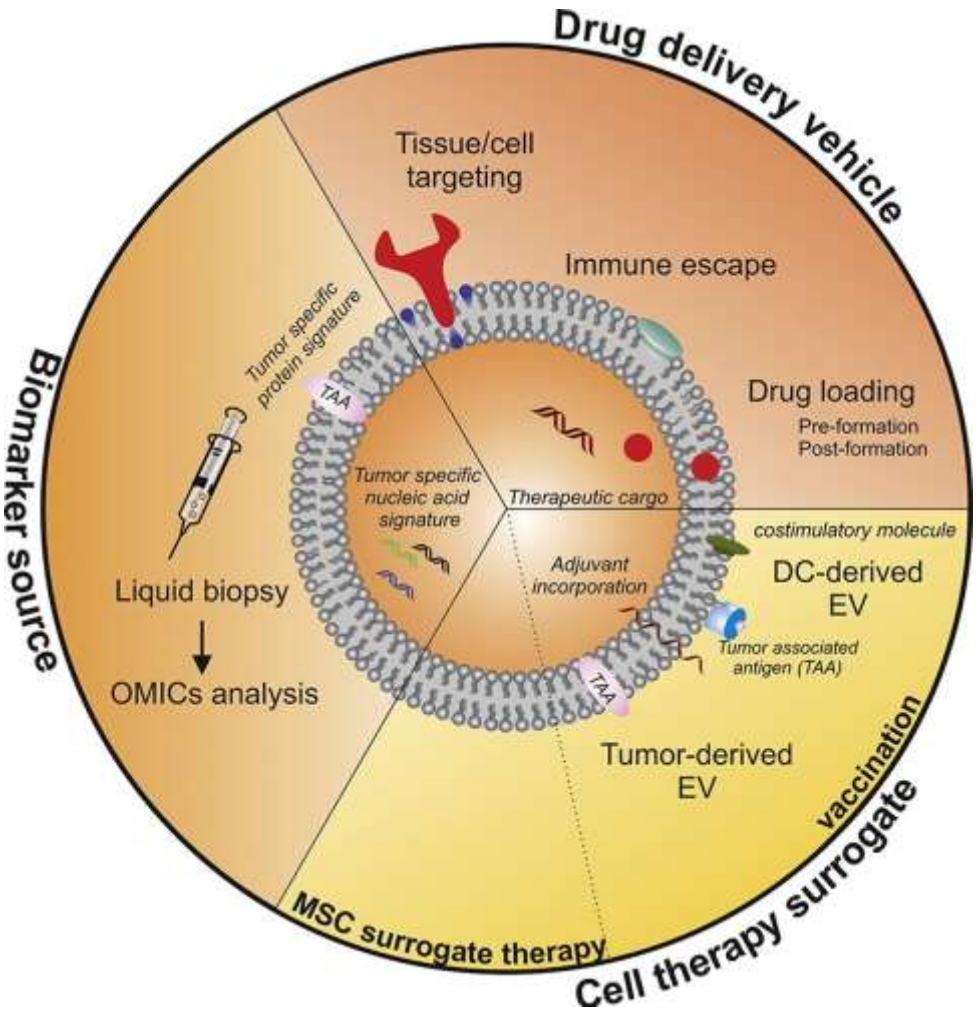
- Metastasis
- Prion diseases
- HIV-infection

All roles played by the transport of molecules

EVs act as natural carriers

Andalussi et al Nat Rev Drug Discov. 2013

Diagnostic and therapeutic applications of EVs



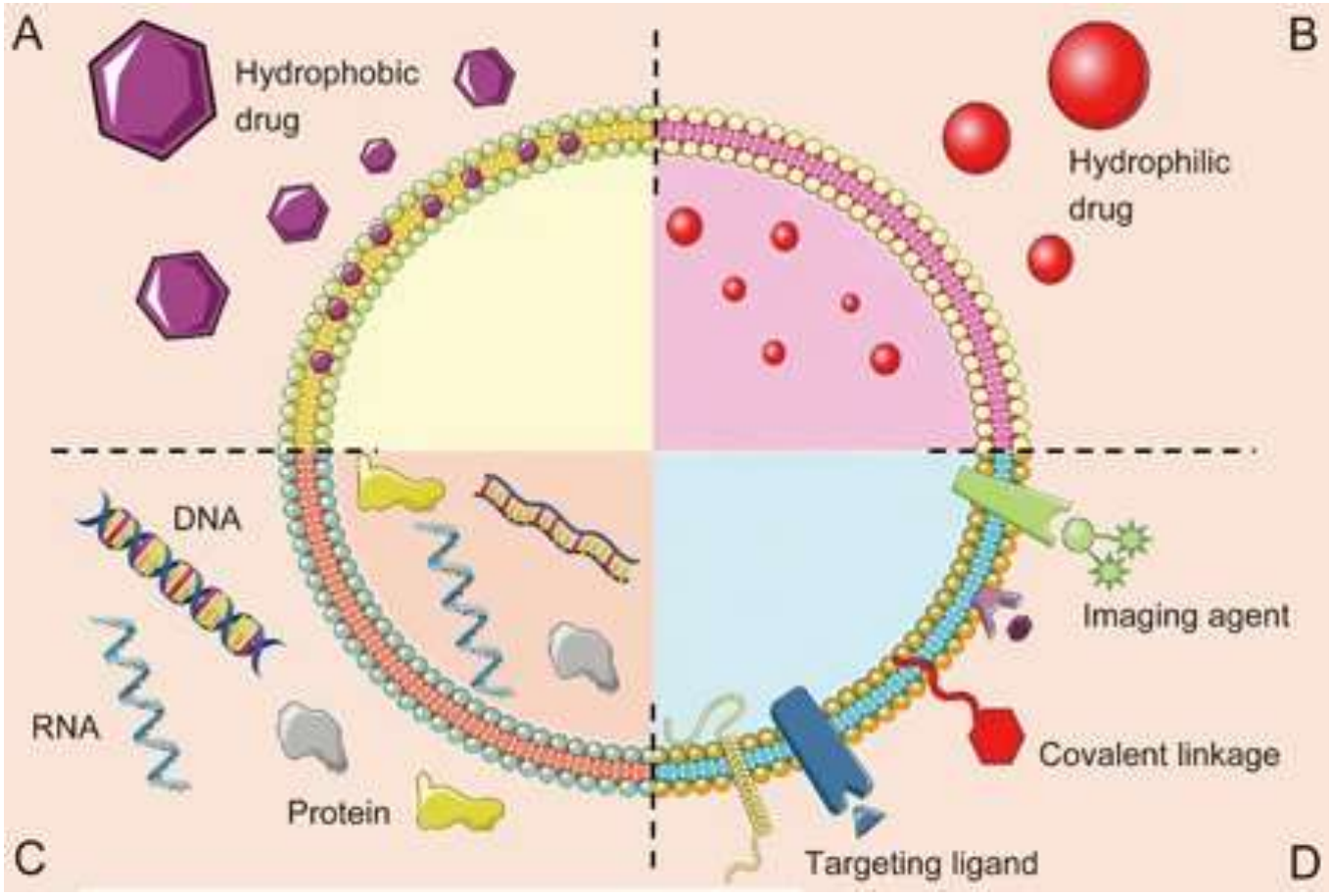
Diagnostic tool

Active Pharmaceutical Ingredient

Drug Delivery System

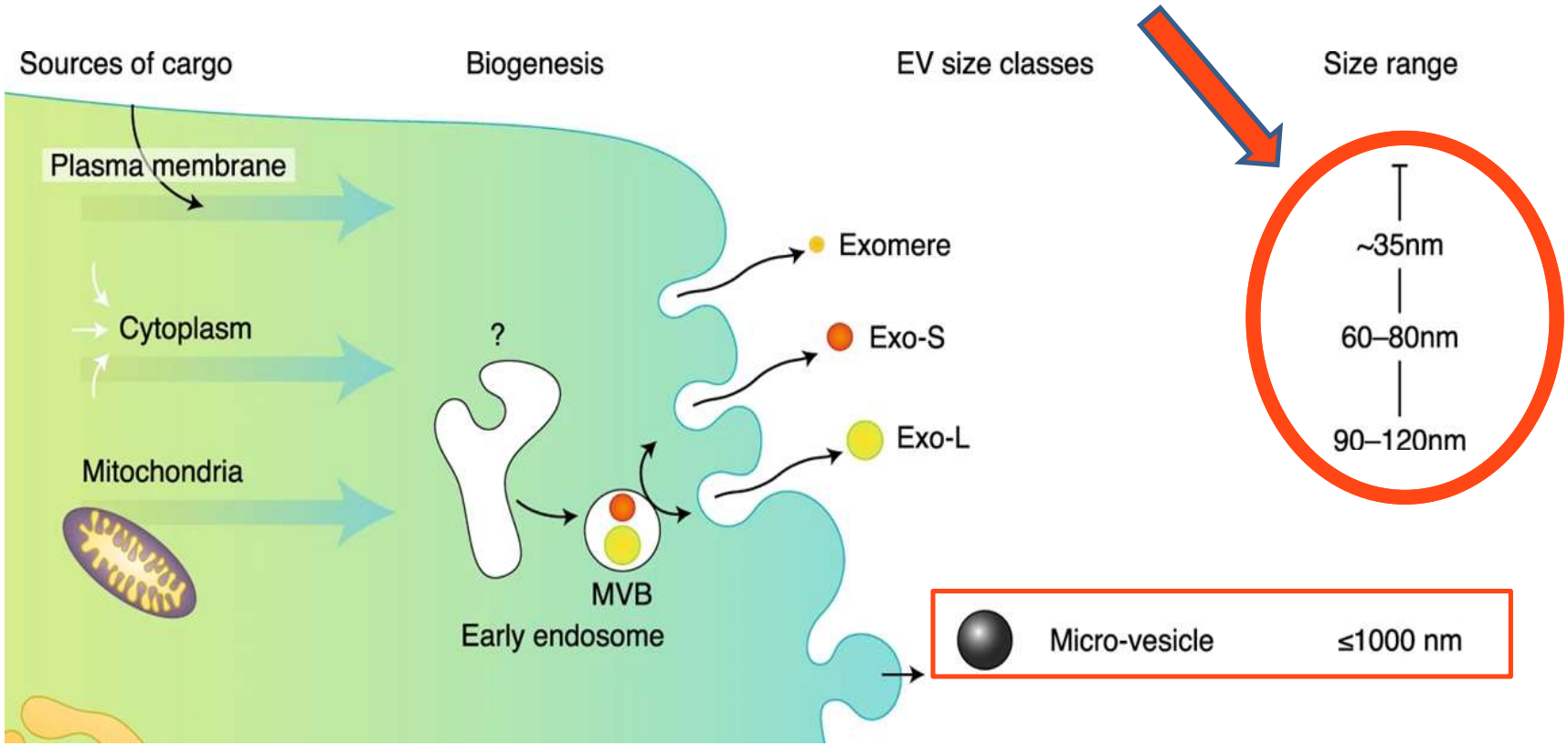
Stremersch et al. J Control Release 2016

MSC-EV Drug Delivery System: a liposomal structure



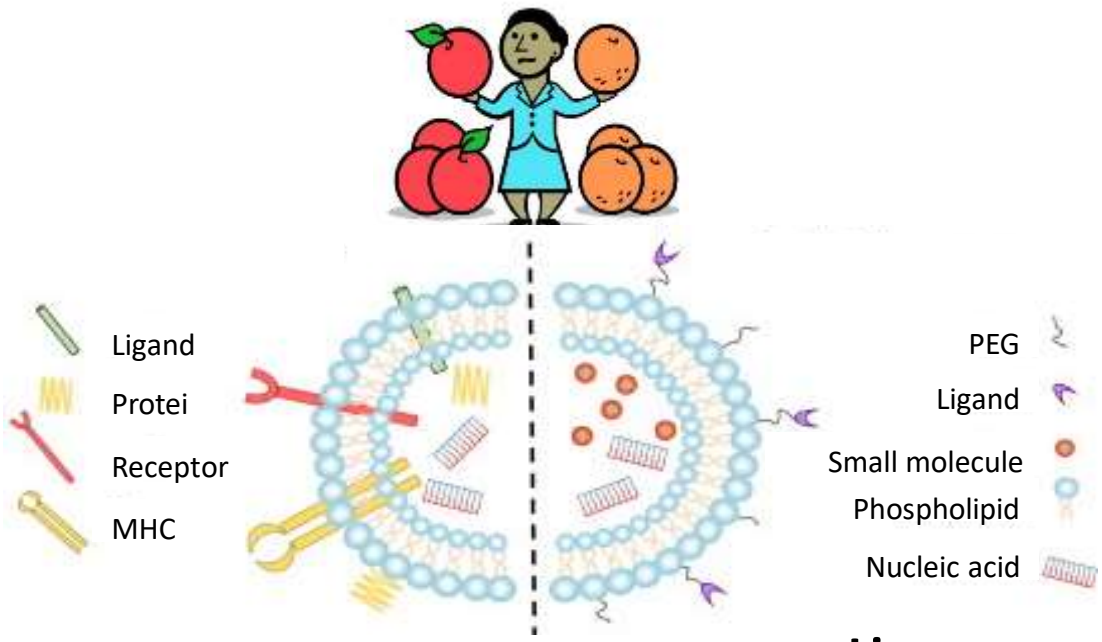
Luan et al. Acta Pharmacologica Sinica 2017

MSC-EV Drug Delivery System: a nanocarrier



Zijlstra and Di Vizio, Nature Cell Biology 2018

MSCs-EVs and liposomes: similarities and differences



Extracellular Vesicles

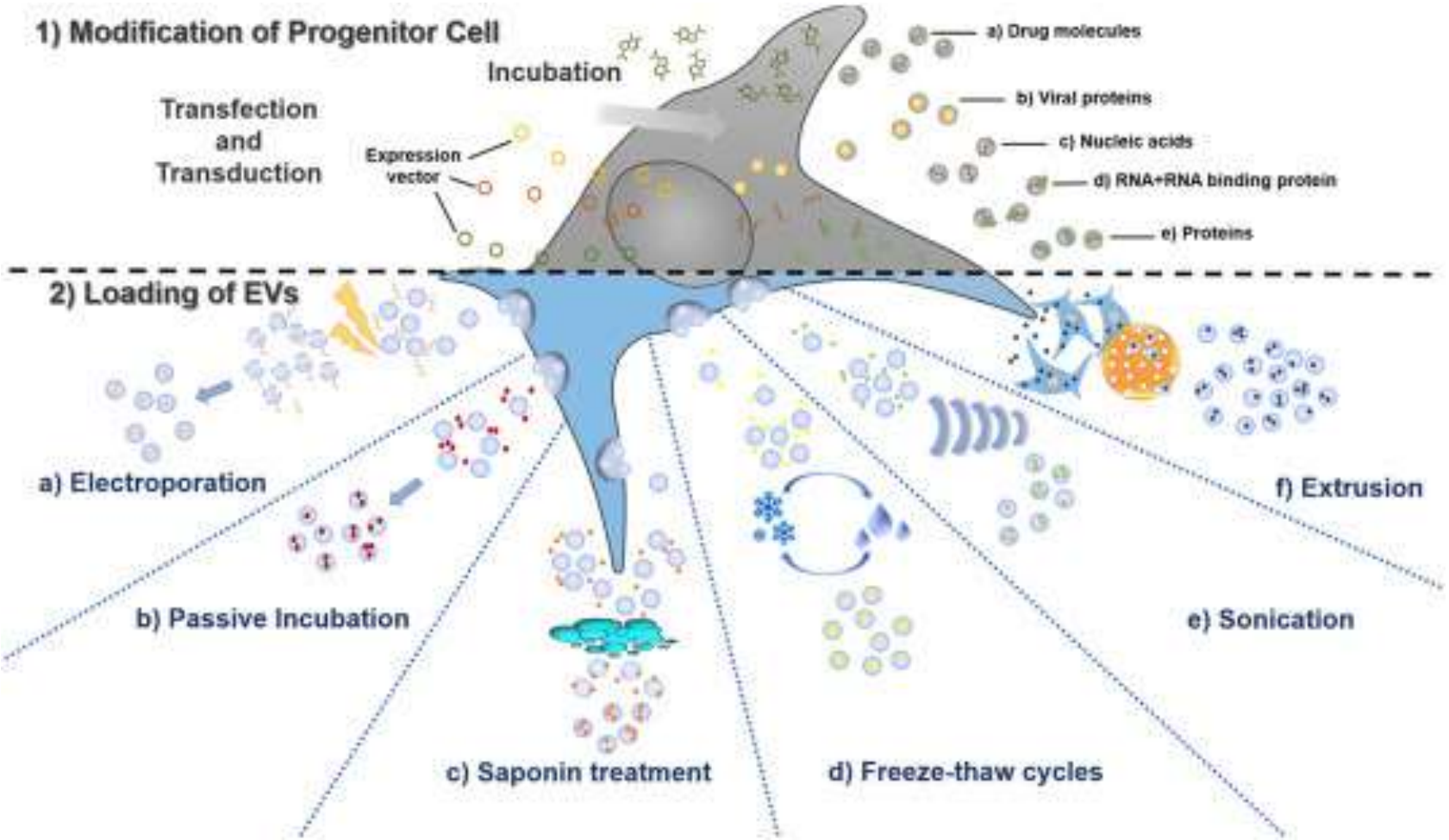
Liposomes

Long circulating
Intrinsic ability to target tissues
Able to cross BBB
Not immunogenic, no toxicity
Biocompatible

Modulation of properties (size, composition, z-potential)

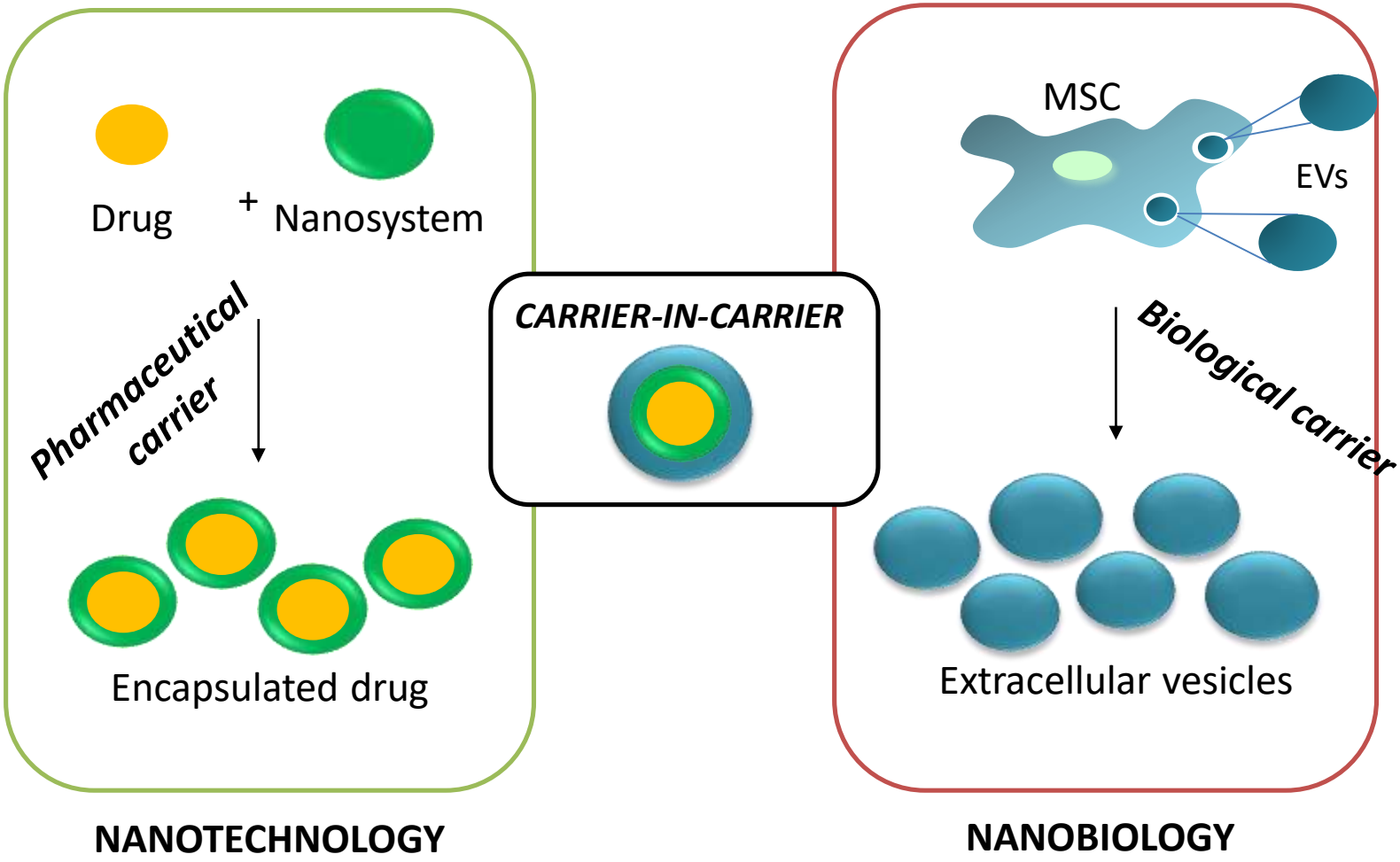
Raimondo et al. Int J Mol Sci 2019
van der Meel et al. J Control Release 2014

MSCs-EVs drug loading strategies



Rufino-Ramos et al. J Control Release 2017

MSCs-EVs and the “carrier-in-carrier”



Tripodo et al. Colloids Surf B Biointerfaces 2015
Crivelli et al. Eur J Pharm Biopharm 2019

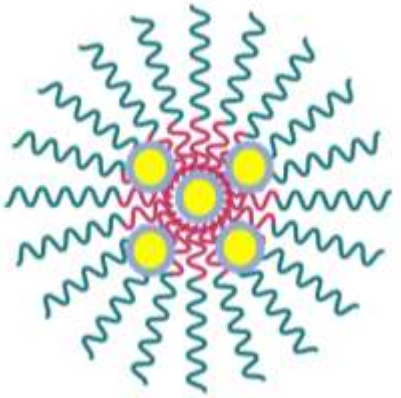
MSCs-EVs and the “carrier-in-carrier”

Colloids and Surfaces B: Biointerfaces 125 (2015) 300–308

Contents lists available at ScienceDirect

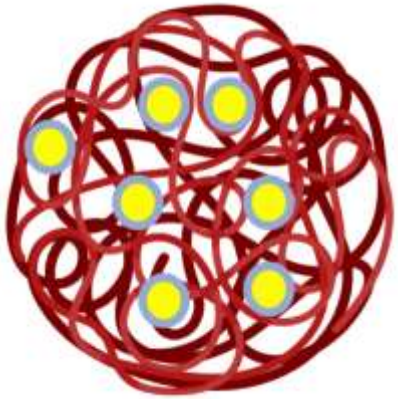
Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb



Mesenchymal stromal cells loading curcumin-INVITE-micelles: A drug delivery system for neurodegenerative diseases

Giuseppe Tripodo[a,1], Theodora Chlapanidas[a,*,1], Sara Perteghella[a], Barbara Vigani[a], Delia Mandracchia[b], Adriana Trapani[b], Marta Galuzzi[a,c], Marta Cecilia Tosca[c], Barbara Antonioli[c], Paolo Gaetani[d], Mario Marazzi[c], Maria Luisa Torre[a]



European Journal of Pharmaceutics and Biopharmaceutics 137 (2019) 37–45

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Silk fibroin nanoparticles for celecoxib and curcumin delivery: ROS-scavenging and anti-inflammatory activities in an *in vitro* model of osteoarthritis

Barbara Crivelli[a,1], Elia Bari[a,1], Sara Perteghella[a,b,*], Laura Catenacci[a], Milena Sorrenti[a], Michela Mocchi[a,c], Silvio Faragò[d], Giuseppe Tripodo[a], Adriele Prina-Mello[c], Maria Luisa Torre[a,b]

Conclusions

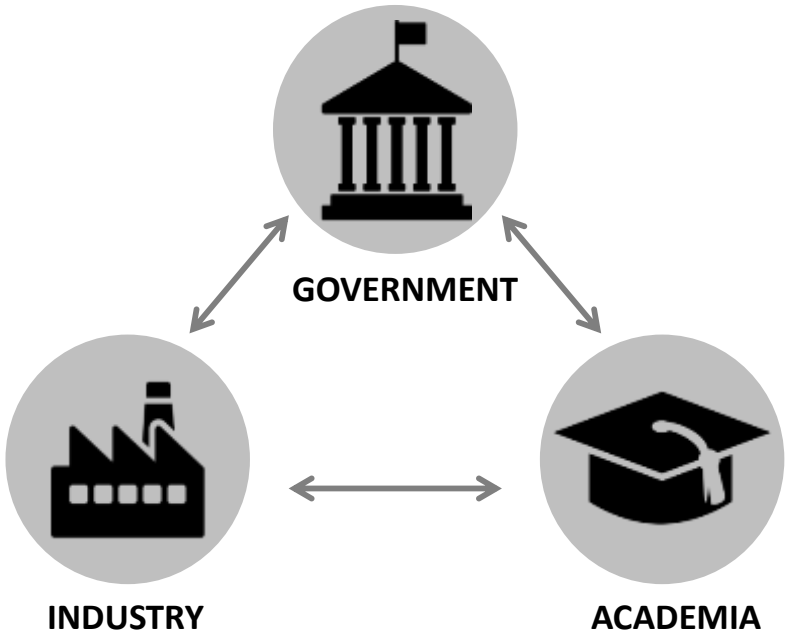
Lyosecretome GMP production process at affordable costs is defined ✓

A new API for regenerative medicine is available in large amount ✓

A variety of new Drug Delivery Systems can be designed ✓

Characterization, Composition
Mechanisms of action
Potency/Efficacy

The missing piece
for clinical translation



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Cell Factory and Regenerative Medicine research center

G Sesana
M.C. Tosca

Sistema Socio Sanitario
Ospedale Niguarda
Regione Lombardia

and Mario Marazzi

Thank you for your attention