

45°

Convegno Nazionale
di Studi di Medicina Trasfusionale

Rimini | 29-31 maggio 2024



**PRIMA ESPERIENZA DI PRODUZIONE E IMPIEGO DI
VESCICOLE EXTRACELLULARI (EVs) AUTOLOGHE NEL
TRATTAMENTO DI ULCERE VENOSE CRONICHE (UVC)
NON RESPONSIVE A TERAPIA CONVENZIONALE**

*Sergio D'Antico (1) - Marika Salafia (1) - Lorenzo Gibello (2) - Maria Felice Brizzi (3)
- Margherita Alba Carlotta Pomatto (3) - Marco Lorenzi (1)*

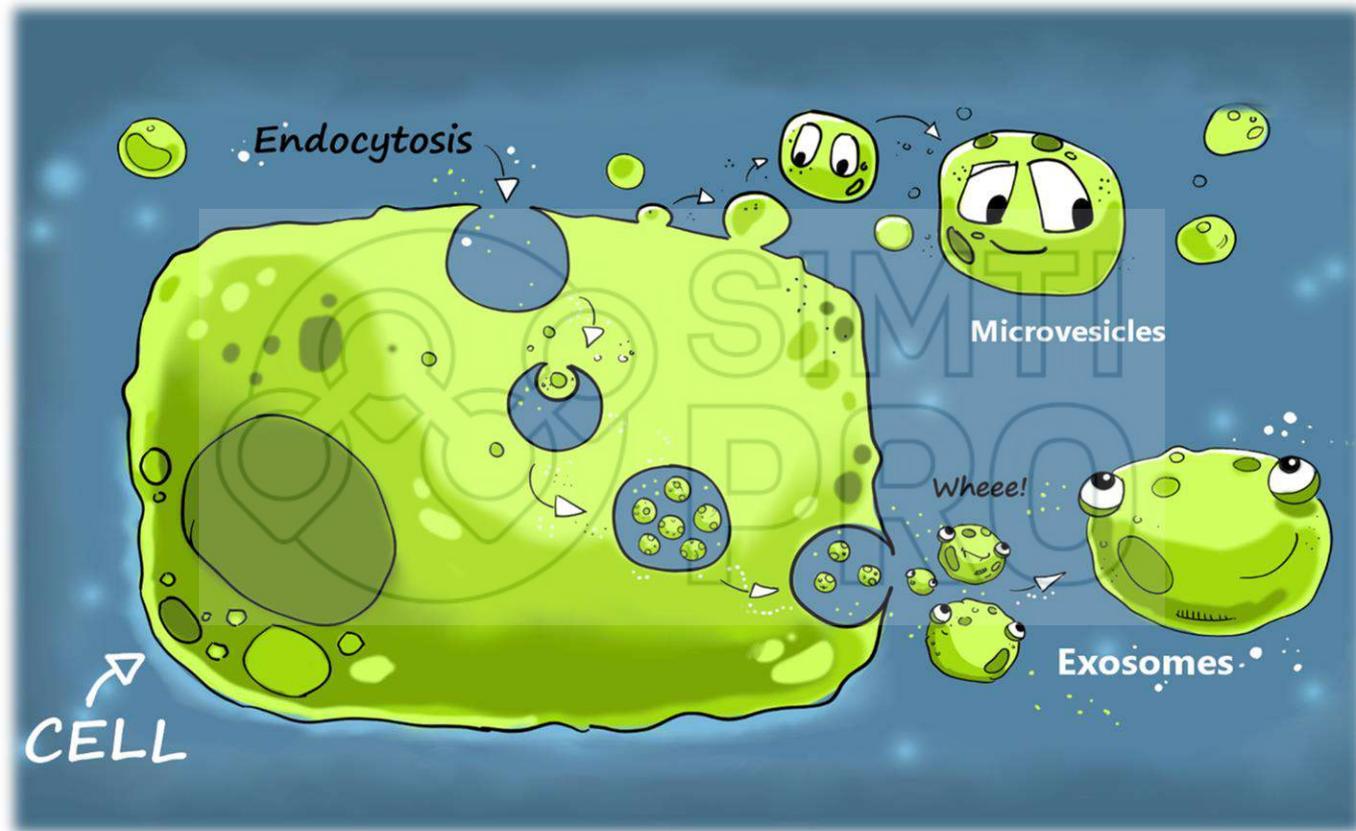
*S.C. BANCA DEL SANGUE E IMMUNOEMATOLOGIA (1); S.C. CHIRURGIA VASCOLARE U. (2);
DIPARTIMENTO DI SCIENZE MEDICHE (3)*

A.O.U. CITTA' DELLA SALUTE E DELLA SCIENZA DI TORINO

Il sottoscritto, in qualità di Relatore
dichiara che

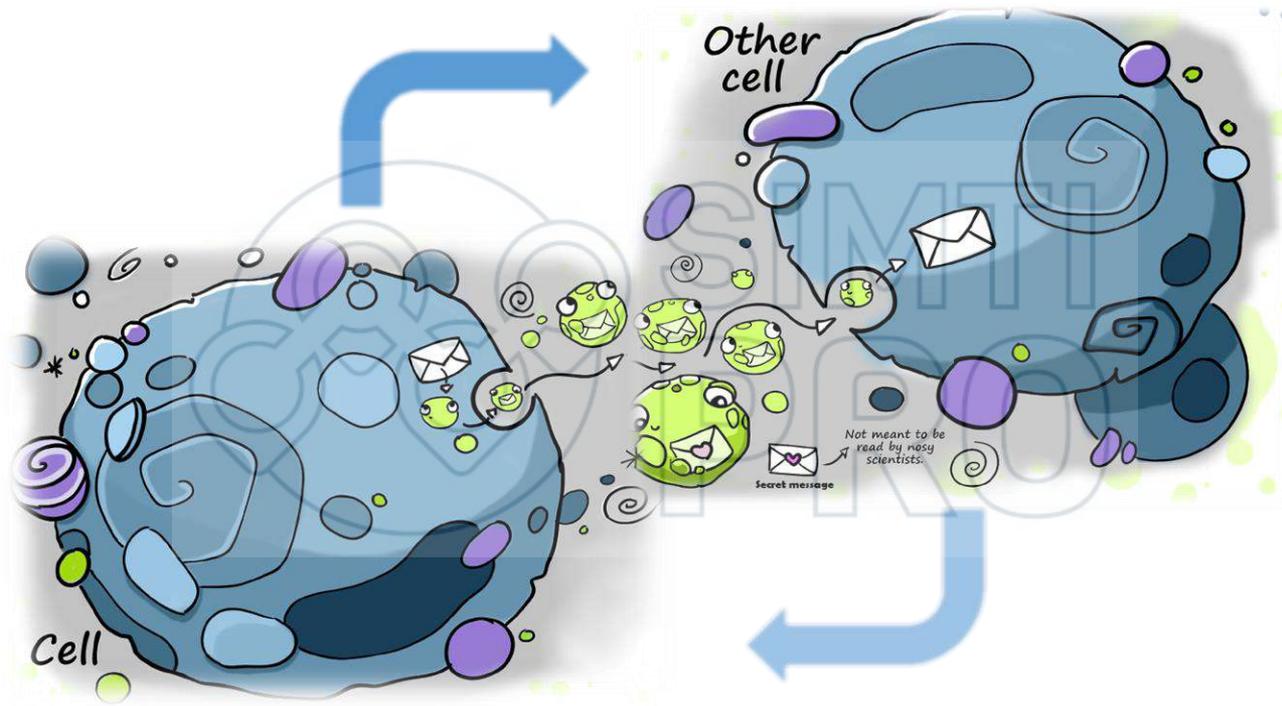
nell'esercizio della Sua funzione e per l'evento in oggetto, NON È in alcun modo portatore di interessi commerciali propri o di terzi; e che gli eventuali rapporti avuti negli ultimi due anni con soggetti portatori di interessi commerciali non sono tali da permettere a tali soggetti di influenzare le sue funzioni al fine di trarne vantaggio.

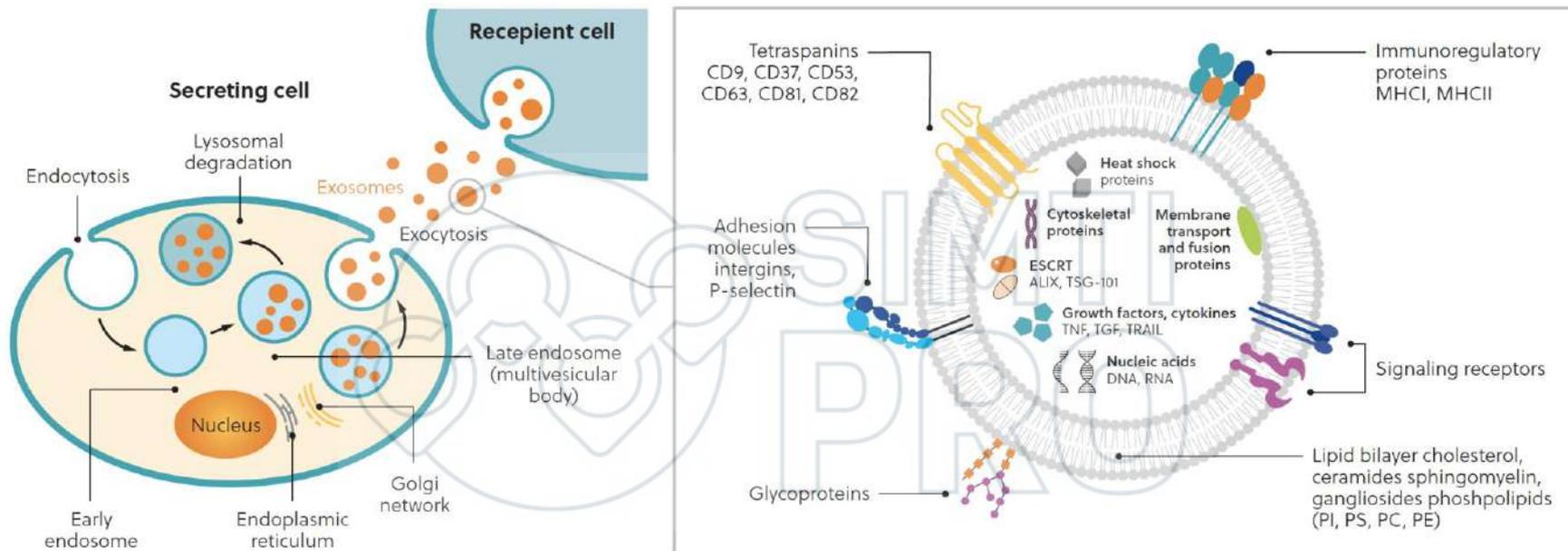
EXTRACELLULAR VESICLES (EVs)

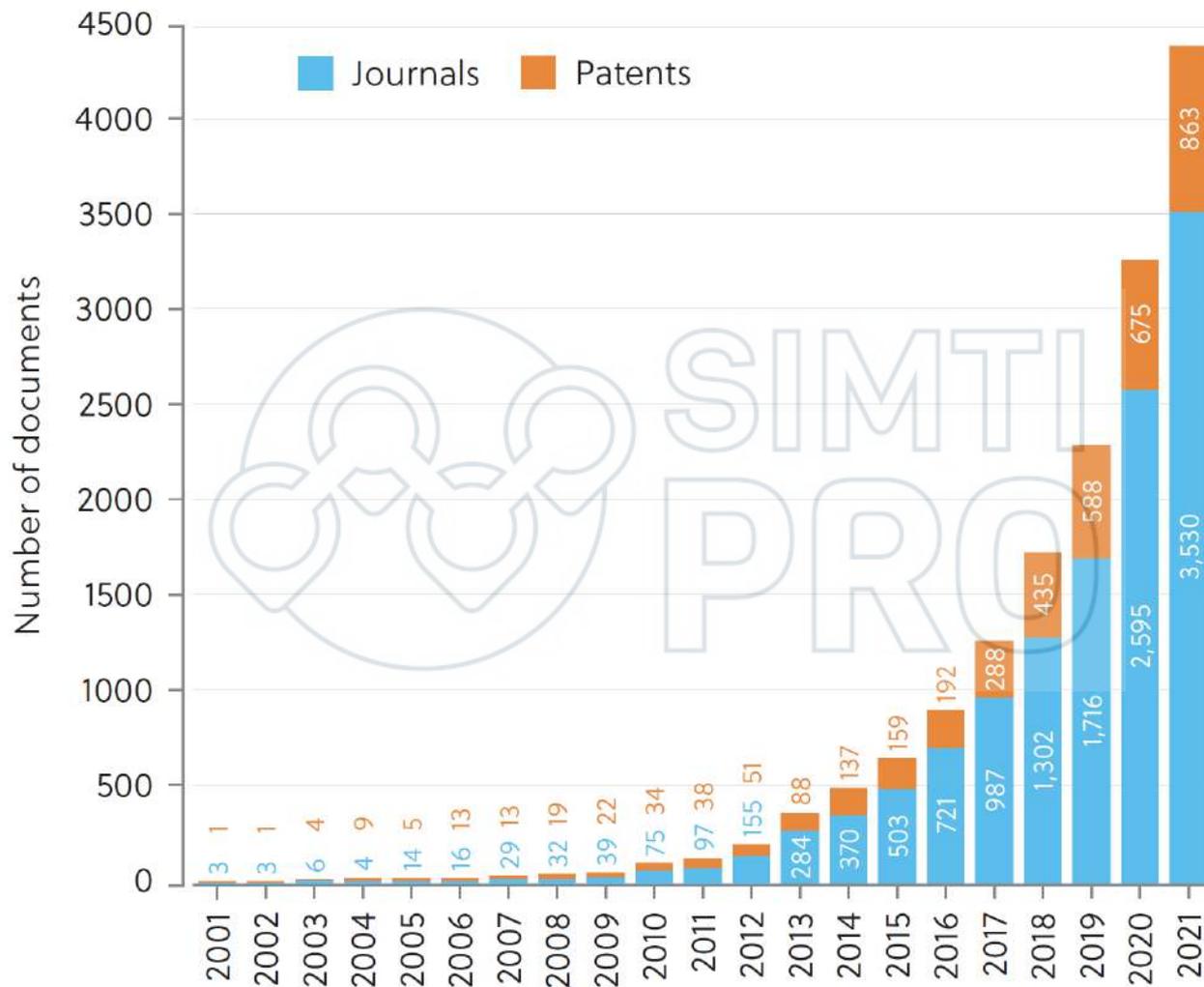


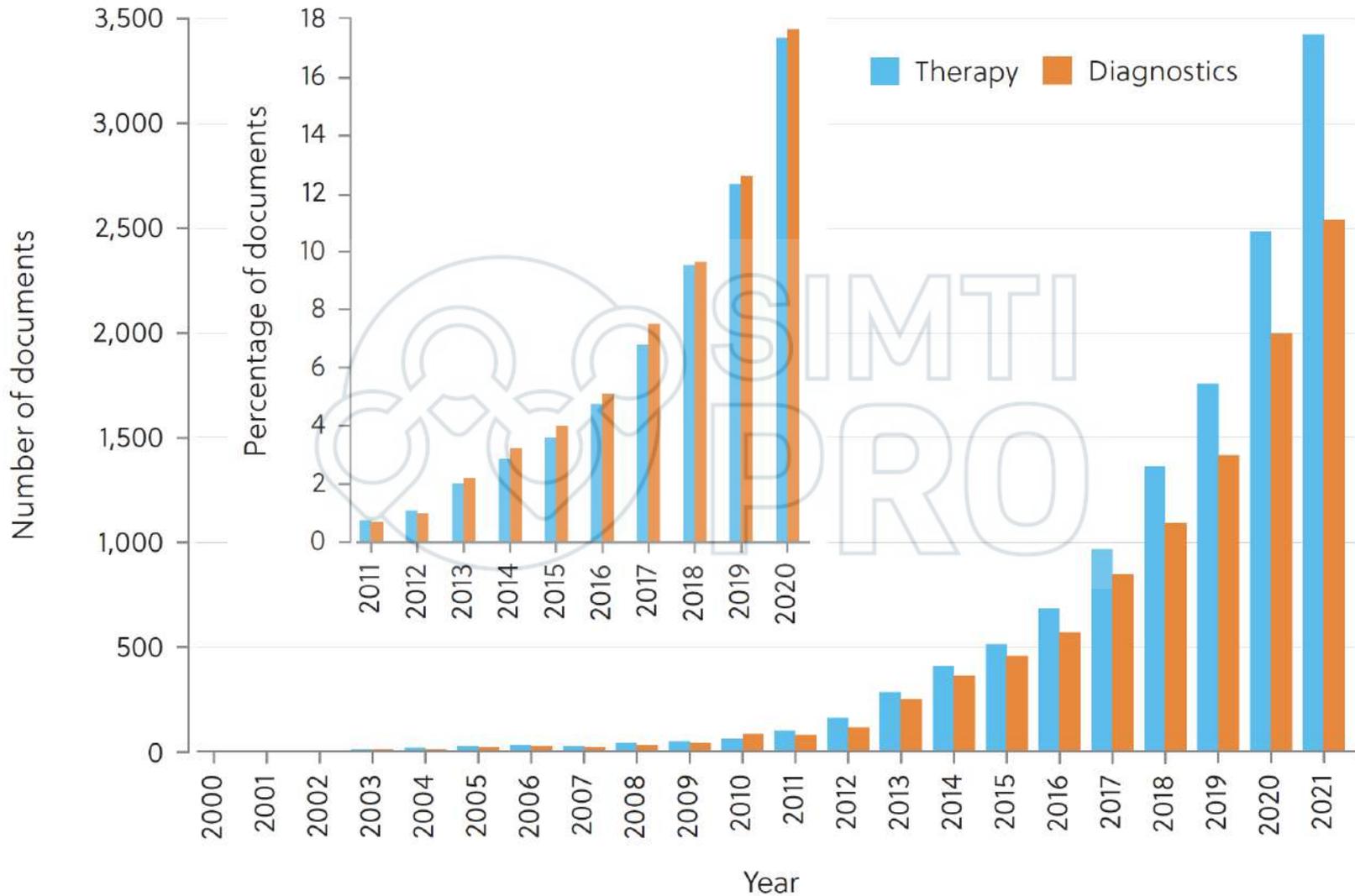
"Catch the exosomes" by Yuliya Shakalisava and Annett von Katz

COMUNICAZIONE INTERCELLULARE







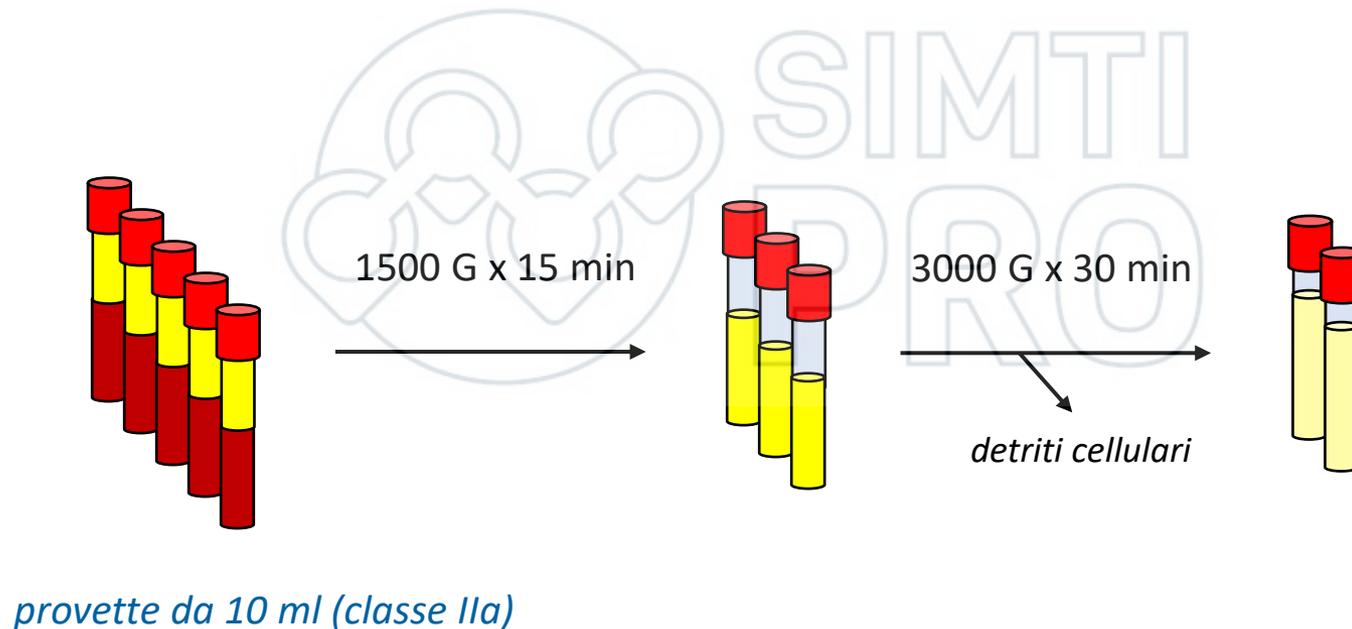


Method	Principle	Advantages	Disadvantages
Ultrafiltration	Utilizing filter membrane with defined size-exclusion limit or molecular weight cut-off	<ul style="list-style-type: none"> - Low cost - Time efficient - Simple 	<ul style="list-style-type: none"> - Potential damage of exosomes - Membrane clogging and blockage
Ultracentrifugation	Density and size-based sequential separations	<ul style="list-style-type: none"> - Suitable for large-volume samples - No other markers introduced - Low cost 	<ul style="list-style-type: none"> - High equipment cost - Labor-intensive - Potential damage of exosomes - Low yield
Immunoaffinity	Exosome capture based on antigen-antibody specific recognition and binding	<ul style="list-style-type: none"> - High specificity - Simple - Scalability 	<ul style="list-style-type: none"> - Potential damage of exosome integrity - Expensive reagents - Nonspecific binding
Polymer precipitation	Hydrophilic water-excluding polymer adhering and precipitating exosomes	<ul style="list-style-type: none"> - Broad applicability - Simple and rapid - No exosome deformation 	<ul style="list-style-type: none"> - Lack of specificity and selectivity - Low purity - Contamination with polymers
Size-exclusion chromatography	Exosome separation based on hydrodynamic radii	<ul style="list-style-type: none"> - Preserve biological activity - No preprocessing 	<ul style="list-style-type: none"> - Potential contamination - High equipment cost
Microfluidics	Immunoaffinity, size, density	<ul style="list-style-type: none"> - High efficiency - Fast sample processing - High portability - Easy automation and integration 	<ul style="list-style-type: none"> - Large amounts of starting materials - Low sample capacity

Charge-based precipitation of extracellular vesicles

MARIA CHIARA DEREGIBUS¹, FEDERICO FIGLIOLINI², SERGIO D'ANTICO³, PAOLA MARIA MANZINI³,
CHIARA PASQUINO², MICHELA DE LENA², CIRO TETTA⁴, MARIA FELICE BRIZZI¹ and GIOVANNI CAMUSSI¹

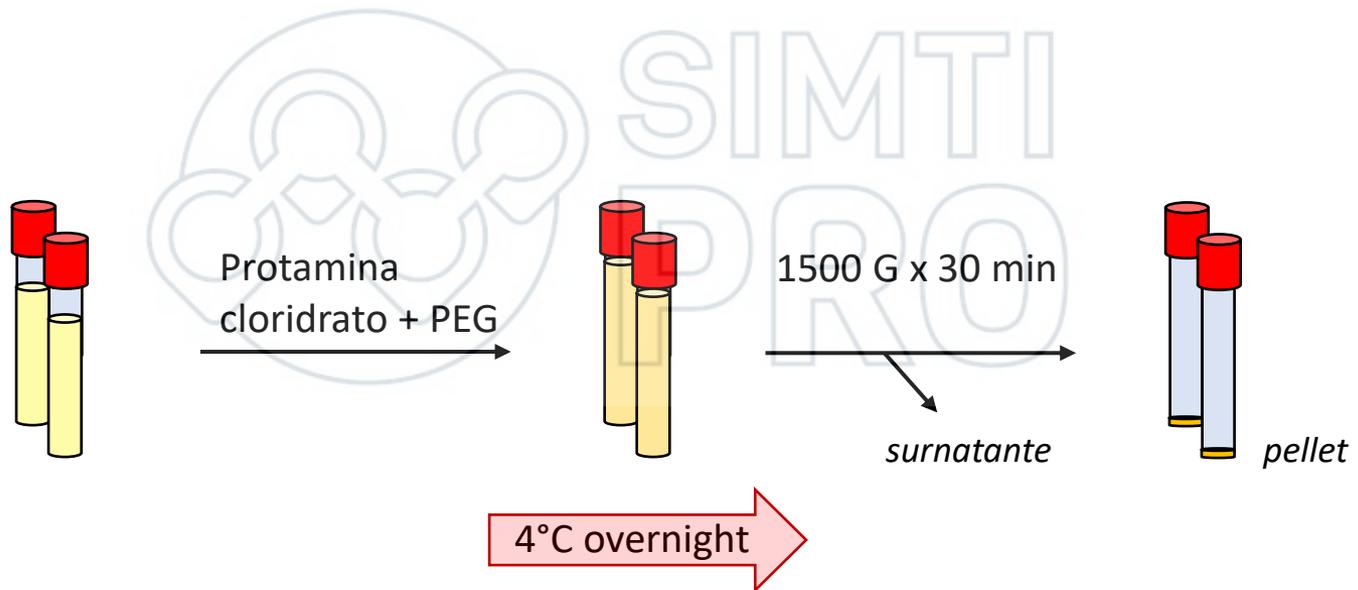
¹Department of Medical Sciences, University of Turin, ²i3T - Scarl. - Molecular Biotechnology Center (MBC),
University of Turin; ³Blood Bank, A.O.U. Città della Salute e della Scienza,
I-10126 Turin, Italy; ⁴Unicyte AG, 6370 Oberdorf, Switzerland



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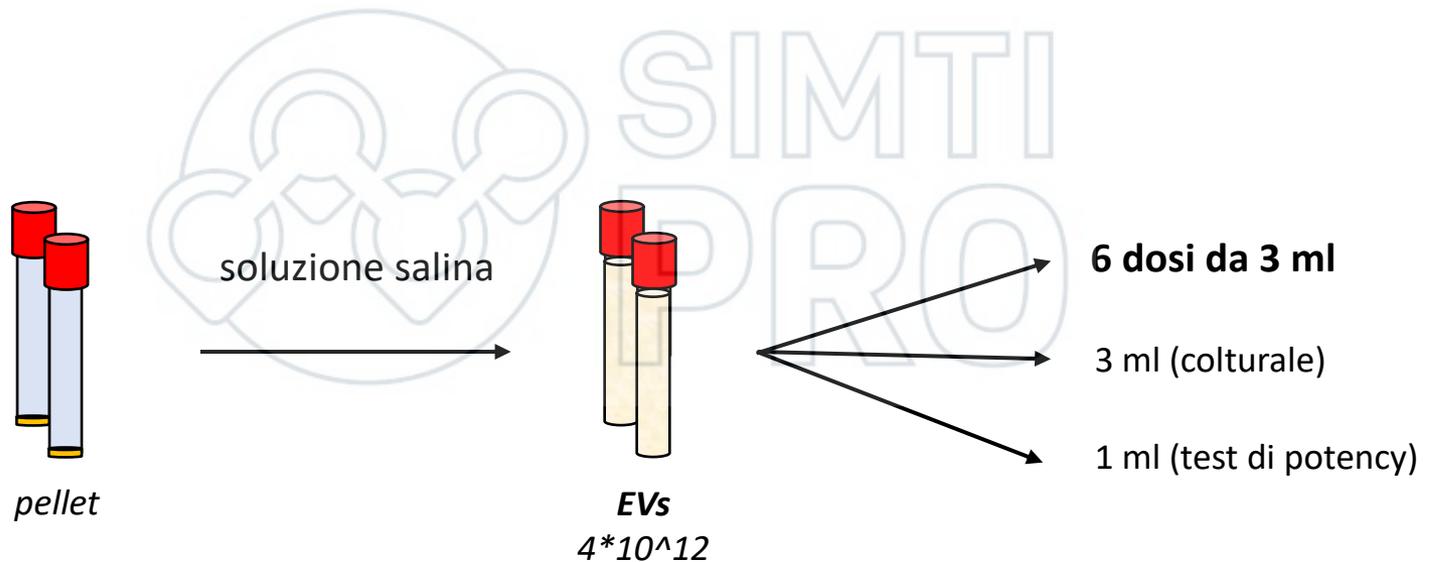
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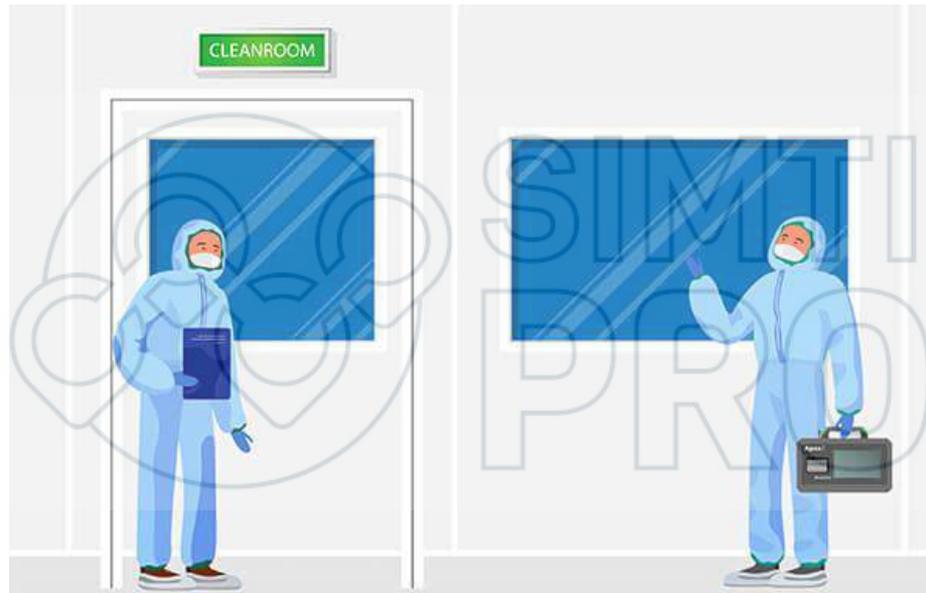
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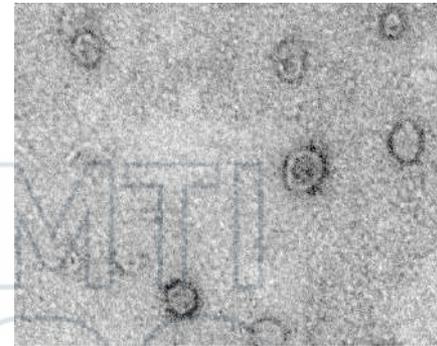
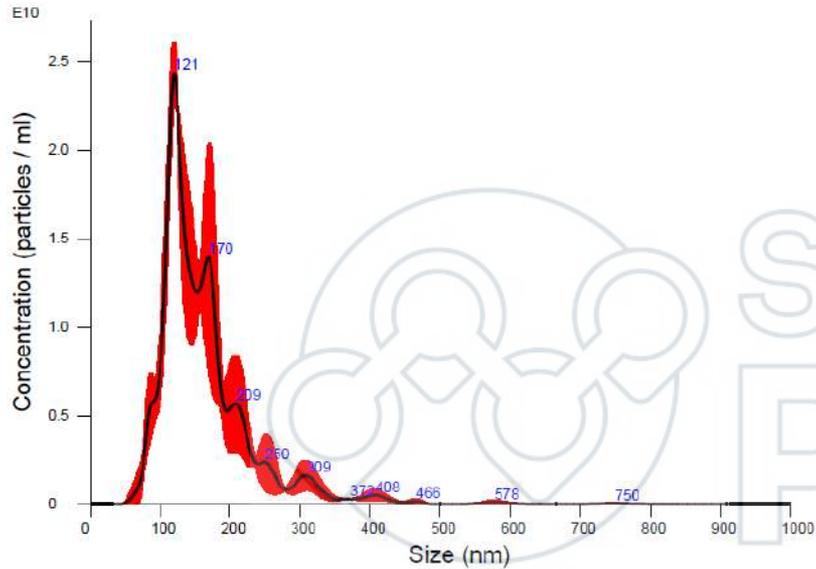
LAVORAZIONE IN AMBIENTE DI CLASSE A



GESTIONE EMOCOMPONENTE

- **AUTODONAZIONE** *Sangue intero (50 ml senza anticoagulante)*
- **SEPARAZIONE** *“Siero EV” (nuova codifica)*
- **DIVISIONE** *“Siero EV” aliquota 1, aliquota 2, ... aliquota 6*
- **VALIDAZIONE** *Esami eseguiti <30 gg precedenti*
- **PRENOTAZIONE**
- **ASSEGNAZIONE**
- **CONSEGNA** *(dopo scongelamento)*
- **AVVENUTO UTILIZZO**

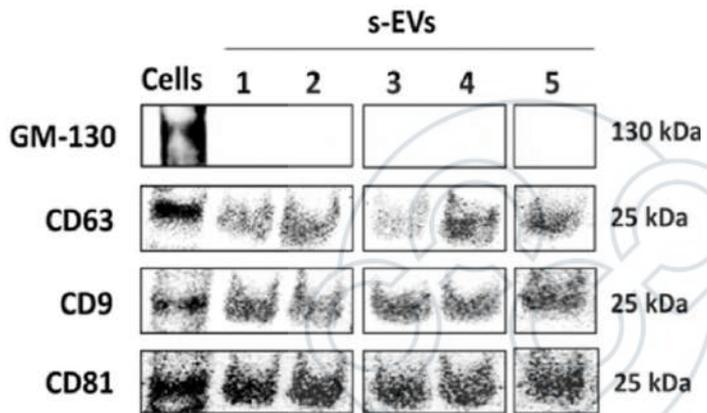
CARATTERIZZAZIONE



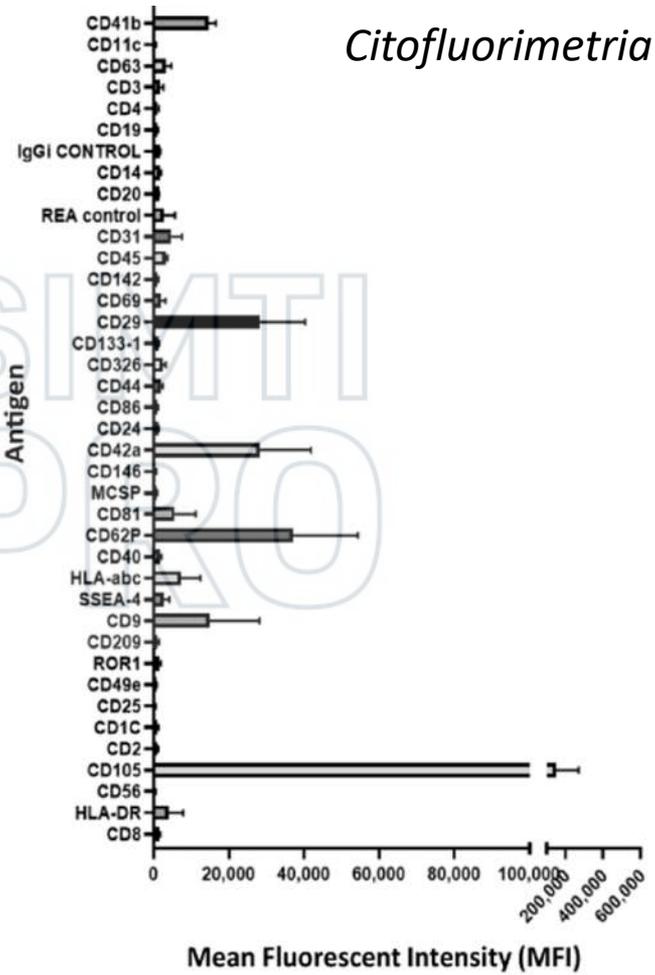
Microscopia elettronica

NTA (nanosight) analysis

CARATTERIZZAZIONE

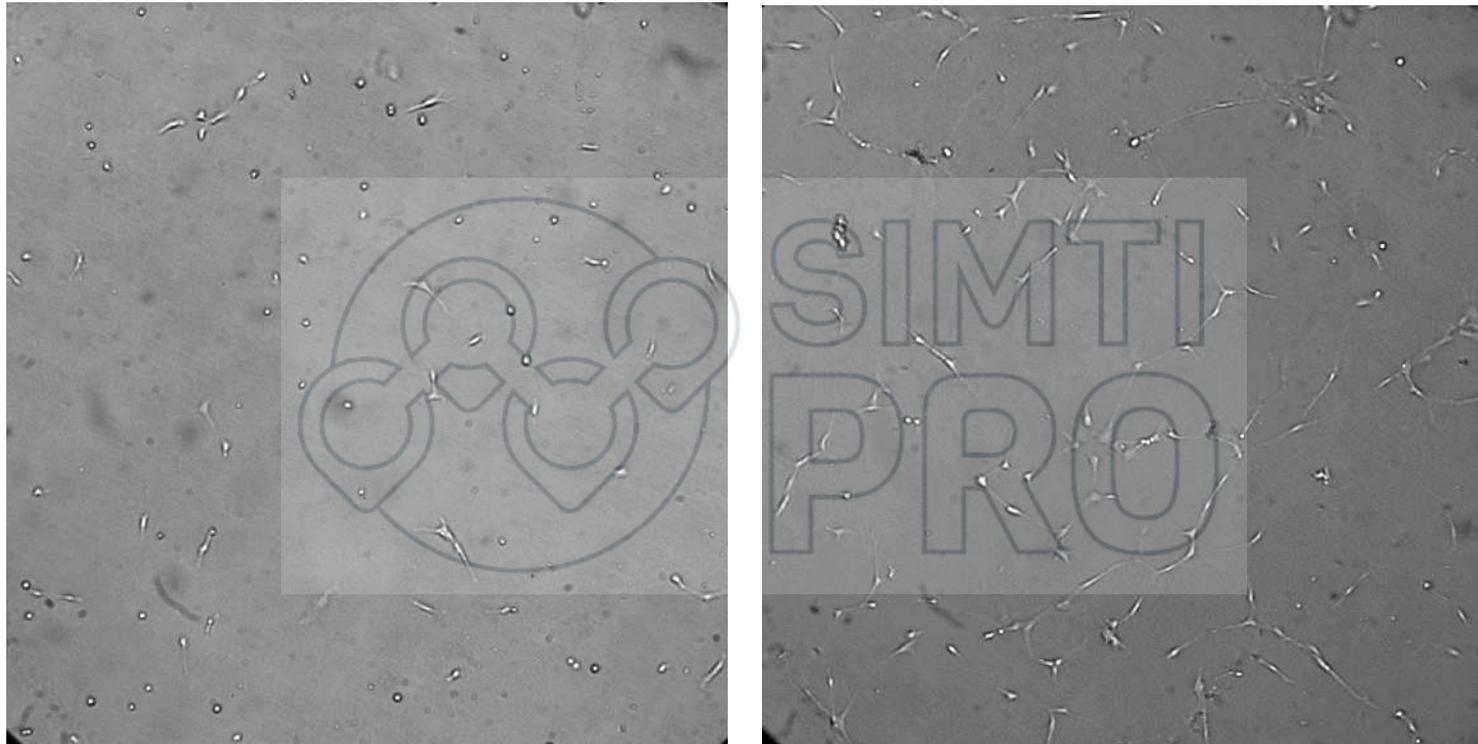


Western blot analysis



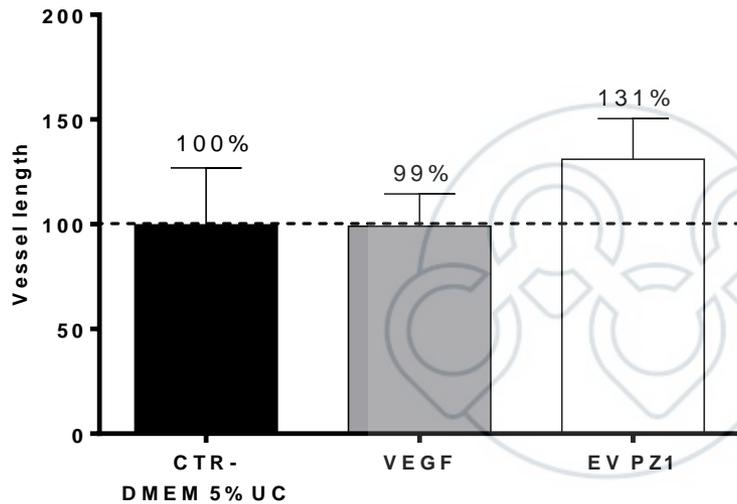
Citofluorimetria

ANALISI FUNZIONALE (test di potency)

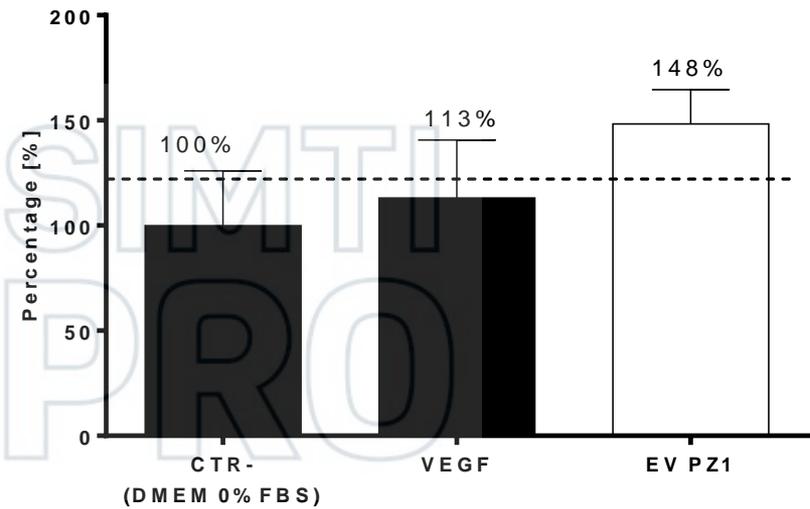


Tube-assay test: effetto pro-angiogenico su cellule endoteliali

ANALISI FUNZIONALE (test di potency)

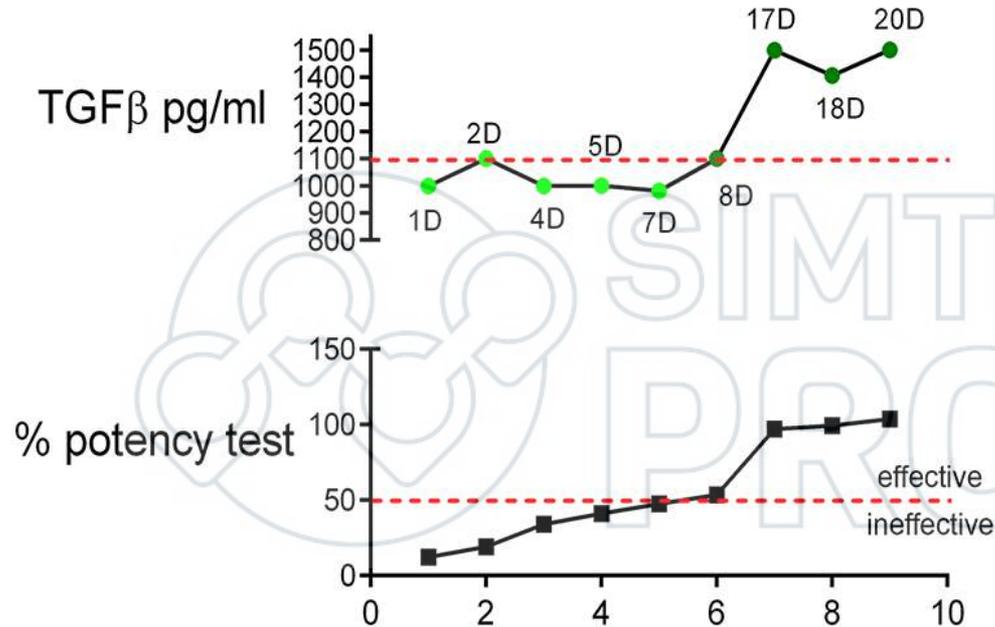


Tube-assay test (lunghezza dei vasi)



% proliferazione

CORRELAZIONE TGF β E % DI POTENCY

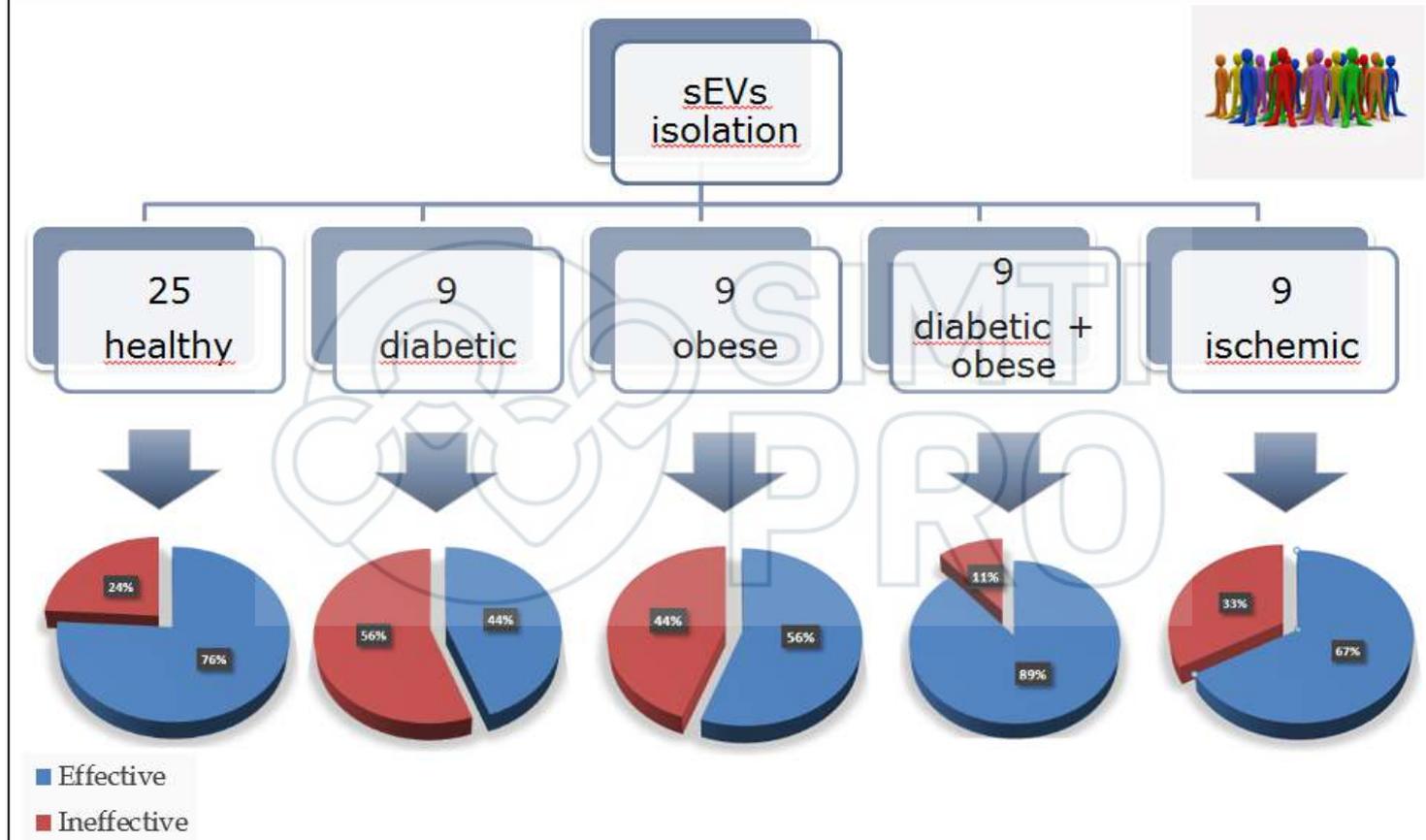


MiR-130a and Tgf β content in extracellular vesicles derived from the serum of subjects at high cardiovascular risk predicts their in vivo angiogenic potential.

C. Cavallari, F. Figliolini, M. Tapparo, M. Cedrino, A. Trevisan, L. Positello, et al.;

Sci. Rep. 10 (1) (2020) 706.

sEV angiogenesis potency assay profiling



M.F. Brizzi

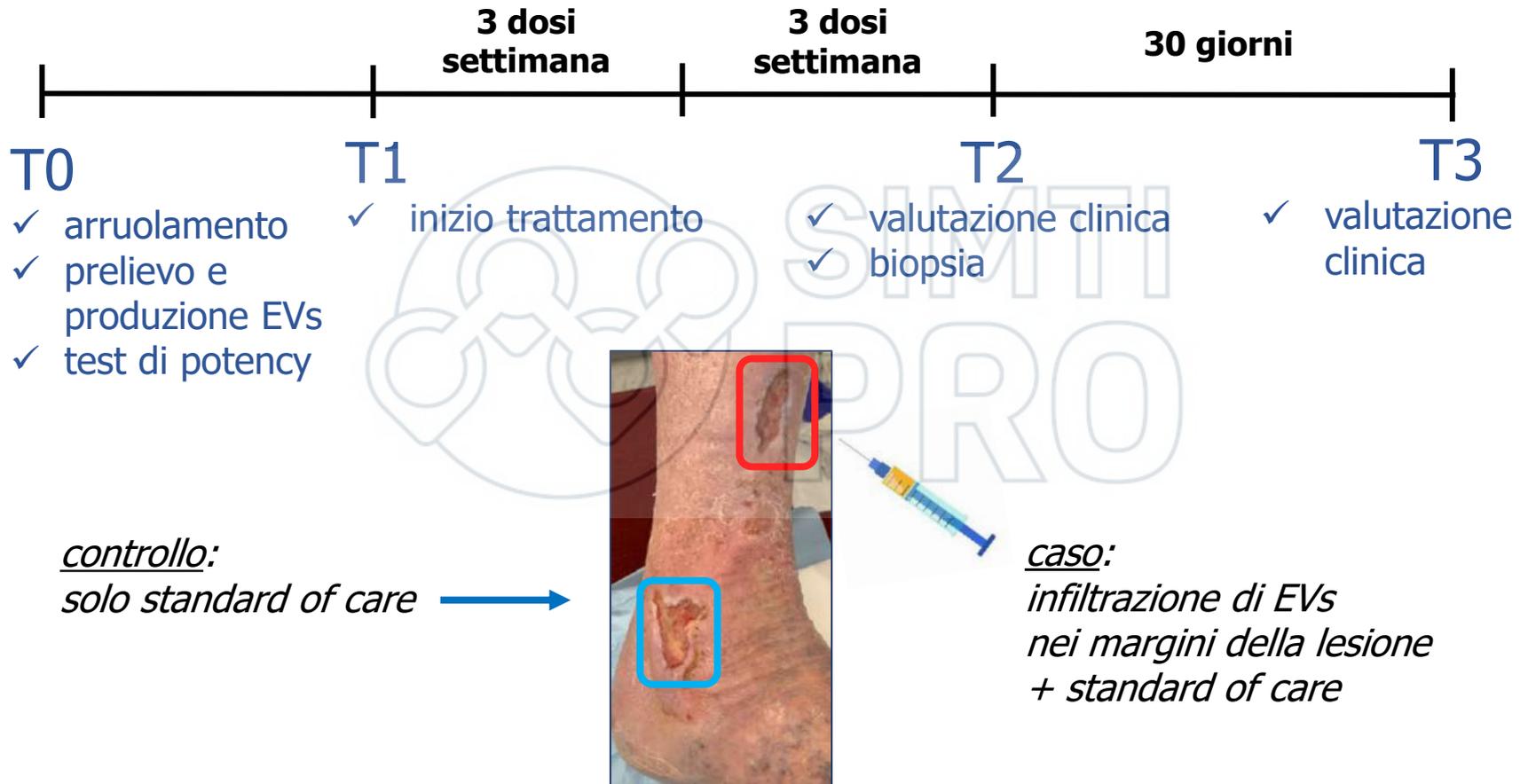
PROTOCOLLO CLINICO

Inclusion and exclusion criteria.

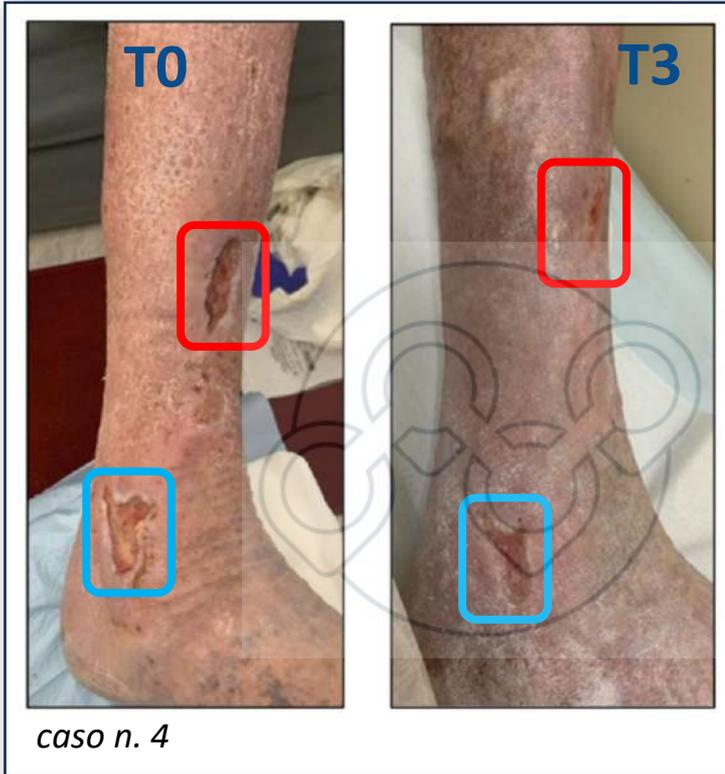
	INCLUSION CRITERIA	EXCLUSION CRITERIA
Demographics & risk factors	<ul style="list-style-type: none">• Age > 18 and < 85• Absence of peripheral arterial disease*	<ul style="list-style-type: none">• Cancer• Diabetes
Wound condition	<ul style="list-style-type: none">• Ulcer in granulation phase§	<ul style="list-style-type: none">• Active wound infection°• Tendon or bone exposure
Laboratory tests	<ul style="list-style-type: none">• Hgb > 10 g/dl• Plts > $100 \times 10^9/L$• Positivity to the potency test	<ul style="list-style-type: none">• HBsAg +• Anti-HCV+• Anti-HIV +
Study consent	<ul style="list-style-type: none">• Written agreement to participate to study protocol	

First pilot case-control interventional study using autologous extracellular vesicles to treat chronic venous ulcers unresponsive to conventional treatments. L Gibello, S D'Antico, M Salafia, R Senetta, MAC Pomatto, G Orlando, A Sarcinella, T Lopatina, P Quaglino, M Lorenzi, F Verzini, G Camussi, MF Brizzi; Pharmacol. Research 190 (2023)

PROTOCOLLO CLINICO



RISULTATI

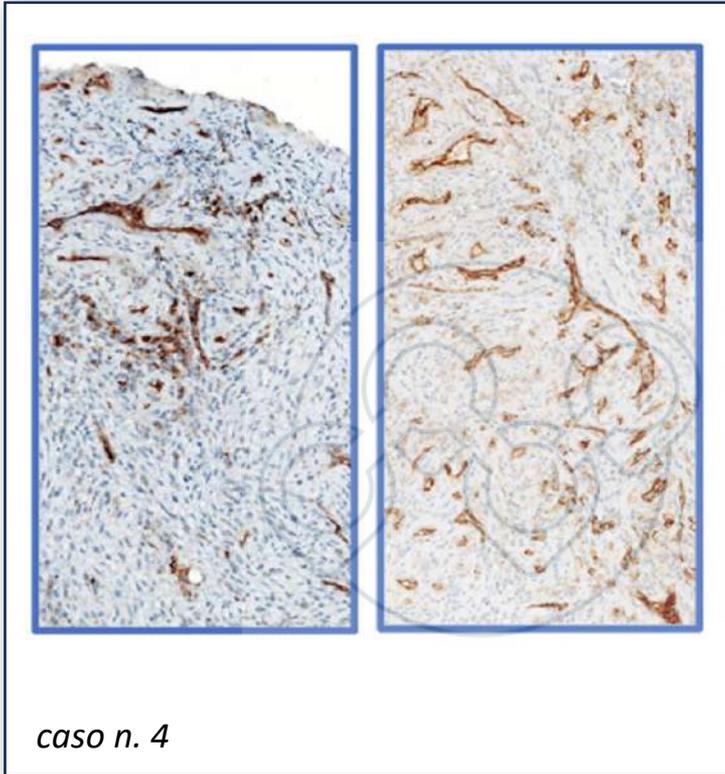


T3 (30 gg):

- maggior tessuto di granulazione (75-100% in 4/5 lesioni)
- riduzione mediana della superficie di 385 mm² vs 106 mm² ($p=0,004$)
- non reazioni avverse o infezione

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Pharmacol. Research 190 (2023)

RISULTATI



Istologia:

- maggiore proliferazione microvascolare
- minore necrosi fibrinoide

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CONCLUSIONI

- Le EVs da siero possono essere considerate un emocomponente (manipolazione minima)
- Le EVs per uso terapeutico in vivo dovrebbero essere preparate nel laboratorio di un ST in ambienti di classe A, secondo gli standard qualitativi di produzione (GPGs, EDQM)
- E' necessaria la collaborazione con laboratori specializzati in biotecnologie (metodica, CQ, test di potency)
- L'applicazione è sperimentale e necessita di un protocollo clinico approvato da un Comitato Etico.

Size: 40-150nm
Or just really, really small like tiny-small.

STRUCTURE:

miRNA
 Secret message (don't tell anyone)
 Lipid bilayer
 Stuff
 Proteins
 More stuff

hydrophilic
 hydrophobic

They don't actually have eyes!
 Artist insisted that without eyes
 it wouldn't look cute.

Potential new
 superhero!!!

Can be found in blood,
 urine, saliva and even
 tears.

Might help us detect and
 cure diseases in the future.

Other random fun fact.

LIKES:

- 1) Playing Hide-and-Seek.
- 2) Confusing scientists.
- 3) Bringing stuff from one cell to another.
- 4) Being all over the place.
- 5) Communicating

ID Card Exosomes

GRAZIE PER L'ATTENZIONE!