

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)*

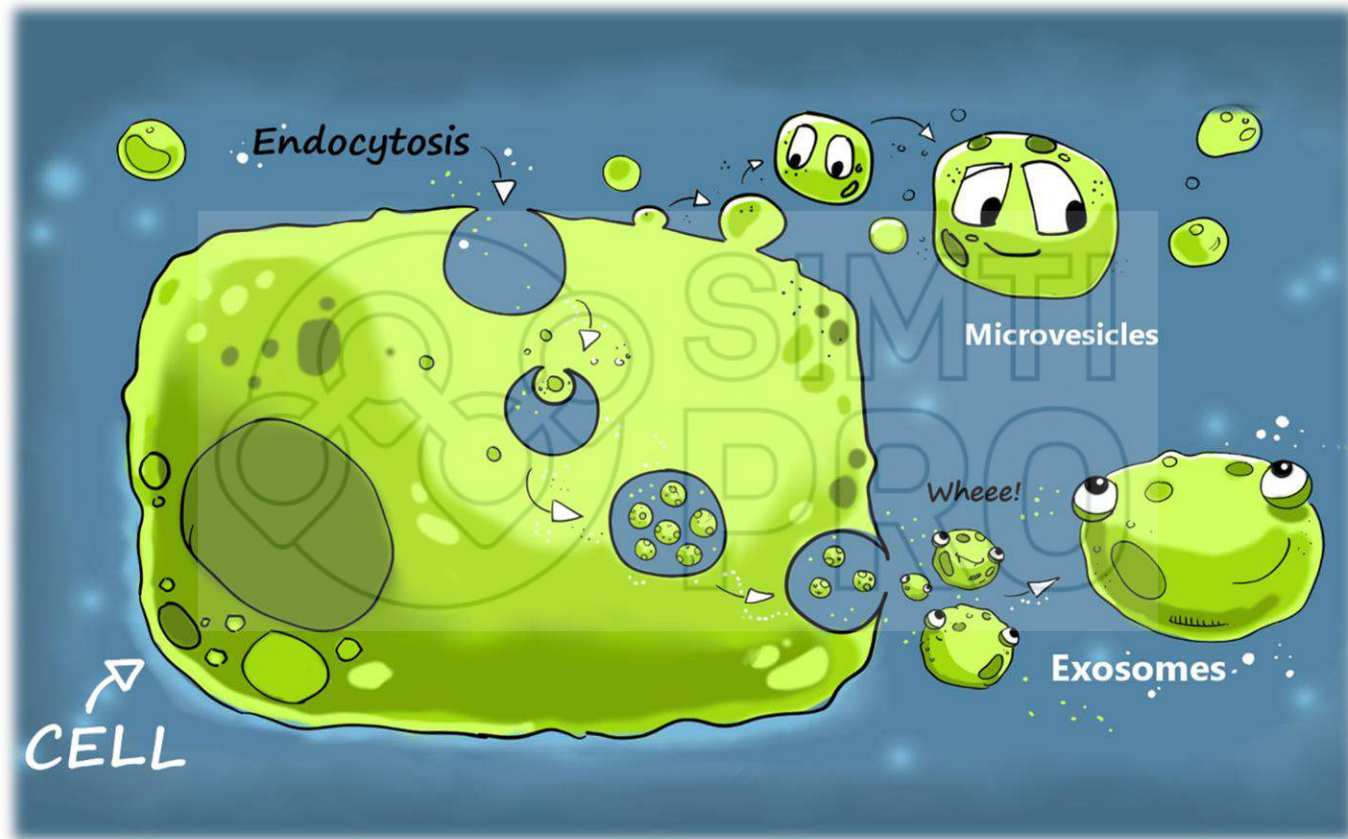
*S.C. BANCA DEL SANGUE E IMMUNOEMATOLOGIA (1); S.C. CHIRURGIA VASCOLARE U. (2);  
DIPARTIMENTO DI SCIENZE MEDICHE (3)*

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

Il sottoscritto, in qualità di Relatore  
dichiara che

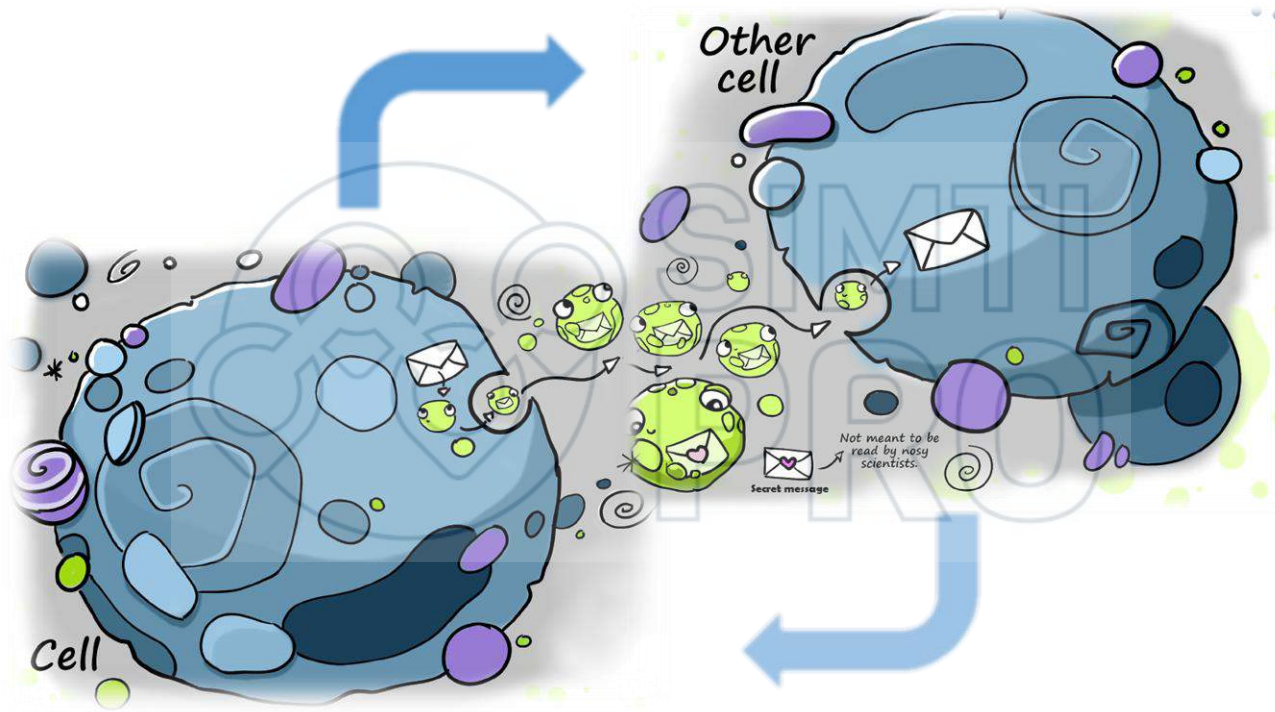
*nell'esercizio della Sua funzione e per l'evento in oggetto, NON È in alcun modo portatore di interessi commerciali propri o di terzi; e che gli eventuali rapporti avuti negli ultimi due anni con soggetti portatori di interessi commerciali non sono tali da permettere a tali soggetti di influenzare le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

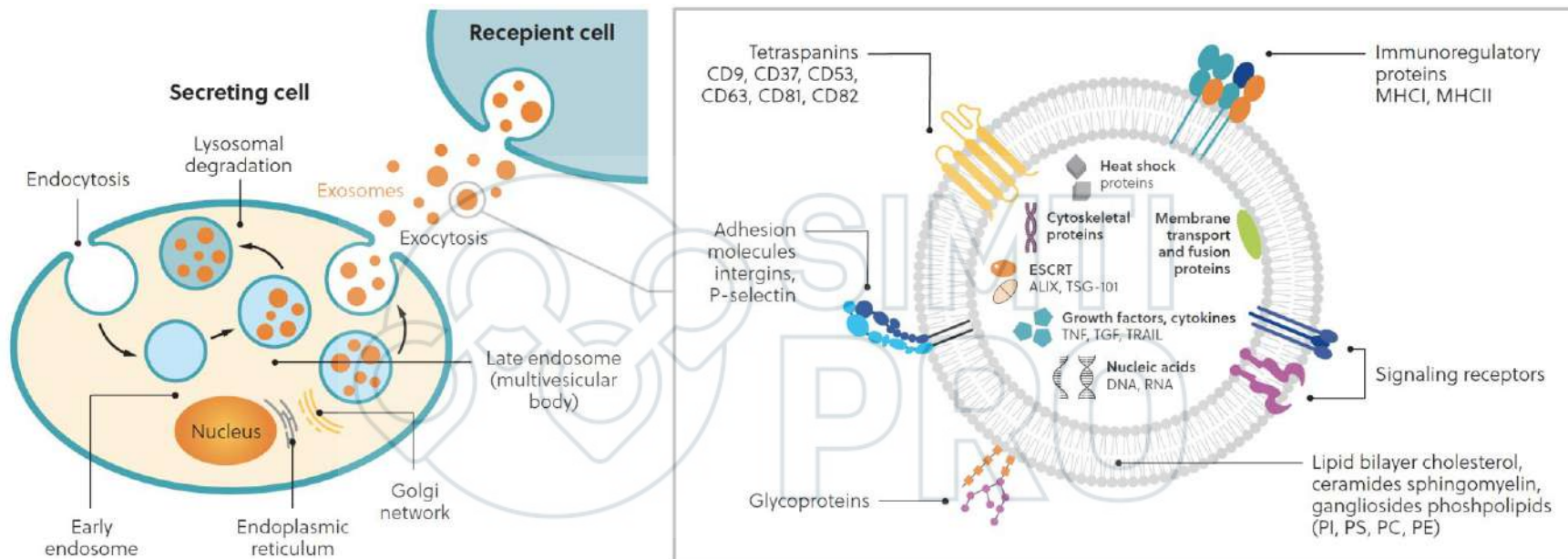
## EXTRACELLULAR VESICLES (EVs)

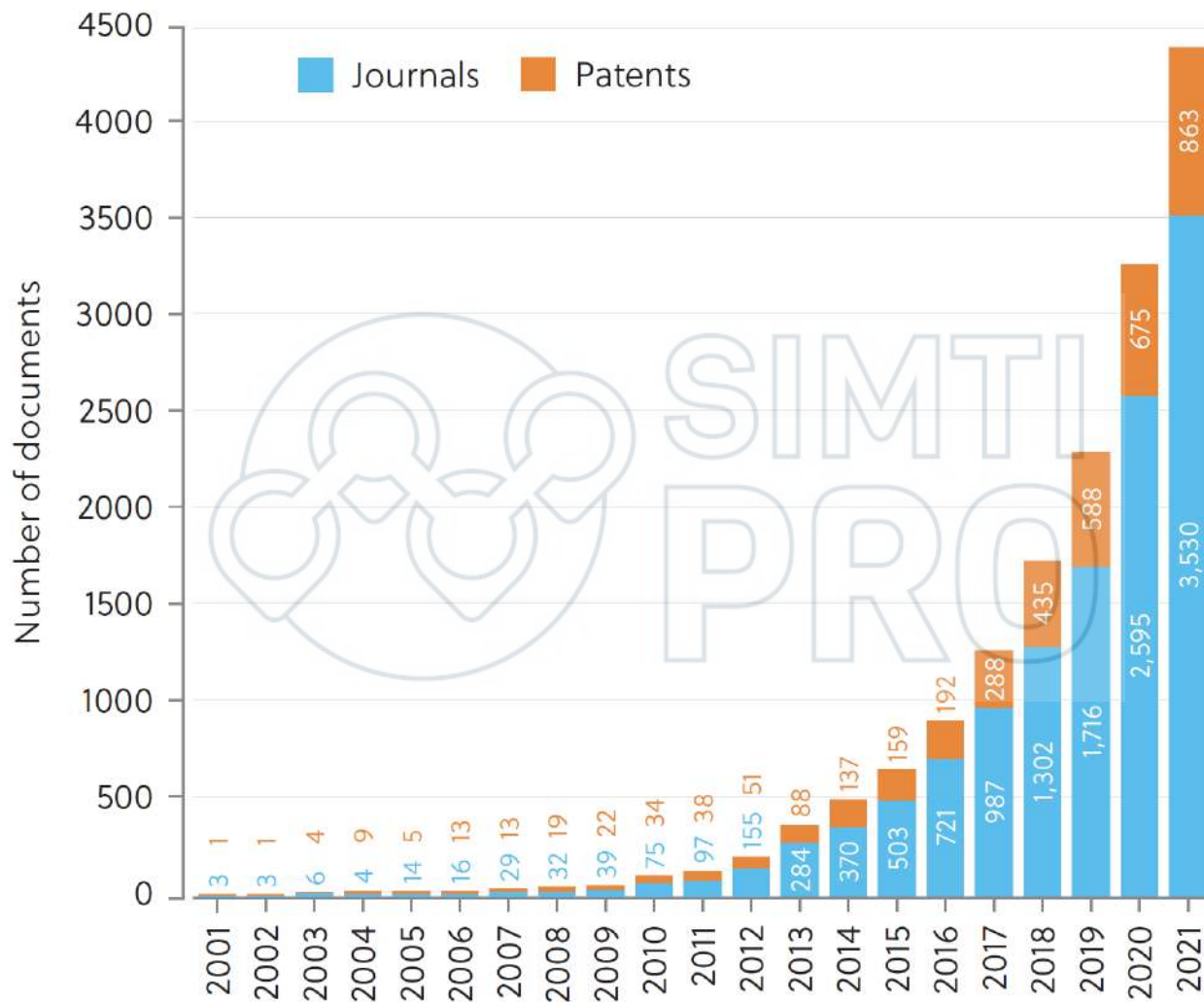


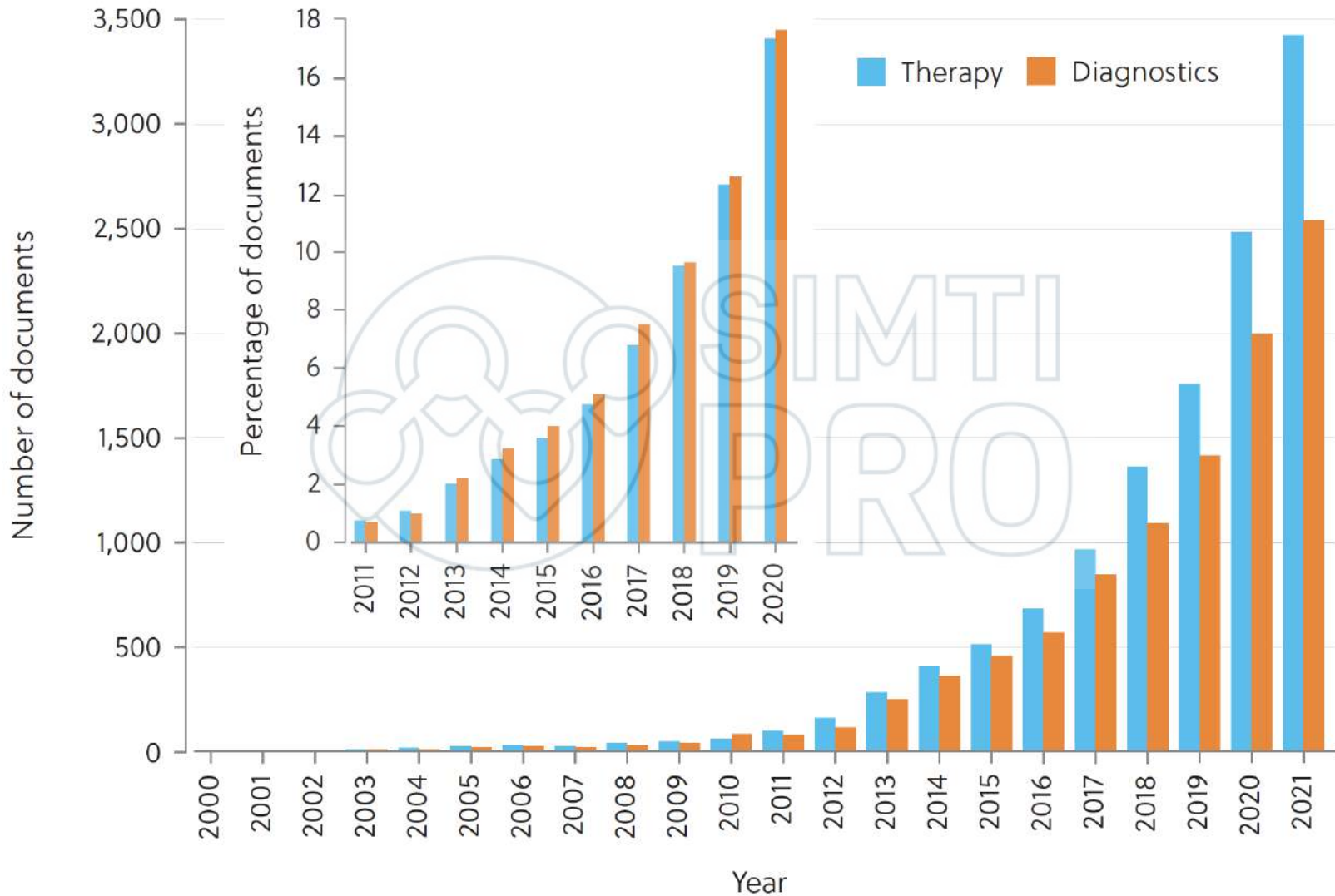
*"Catch the exosomes" by Yuliya Shakalisava and Annett von Katz*

# COMUNICAZIONE INTERCELLULARE









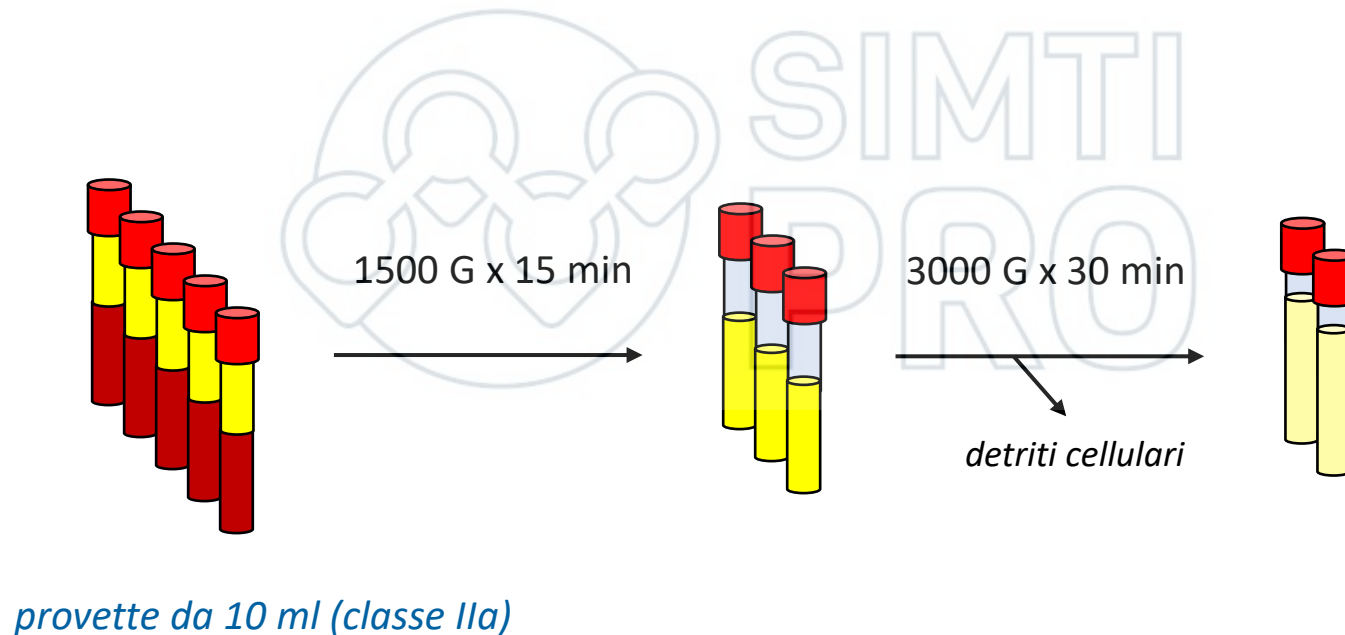
Method	Principle	Advantages	Disadvantages
Ultrafiltration	Utilizing filter membrane with defined size-exclusion limit or molecular weight cut-off	<ul style="list-style-type: none"> <li>- Low cost</li> <li>- Time efficient</li> <li>- Simple</li> </ul>	<ul style="list-style-type: none"> <li>- Potential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



# Charge-based precipitation of extracellular vesicles

MARIA CHIARA DEREGIBUS<sup>1</sup>, FEDERICO FIGLIOLINI<sup>2</sup>, SERGIO D'ANTICO<sup>3</sup>, PAOLA MARIA MANZINI<sup>3</sup>,  
CHIARA PASQUINO<sup>2</sup>, MICHELA DE LENA<sup>2</sup>, CIRO TETTA<sup>4</sup>, MARIA FELICE BRIZZI<sup>1</sup> and GIOVANNI CAMUSSI<sup>1</sup>

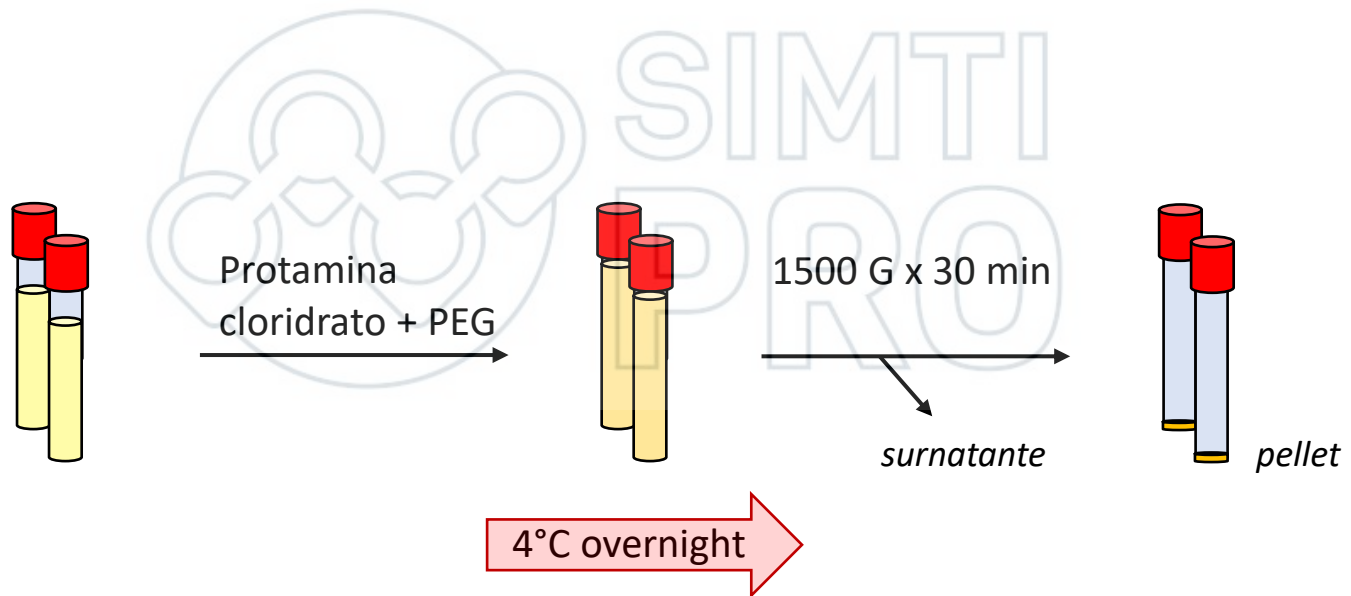
<sup>1</sup>Department of Medical Sciences, University of Turin, <sup>2</sup>i3T - Scarl. - Molecular Biotechnology Center (MBC),  
University of Turin; <sup>3</sup>Blood Bank, A.O.U. Città della Salute e della Scienza,  
I-10126 Turin, Italy; <sup>4</sup>Unicyte AG, 6370 Oberdorf, Switzerland



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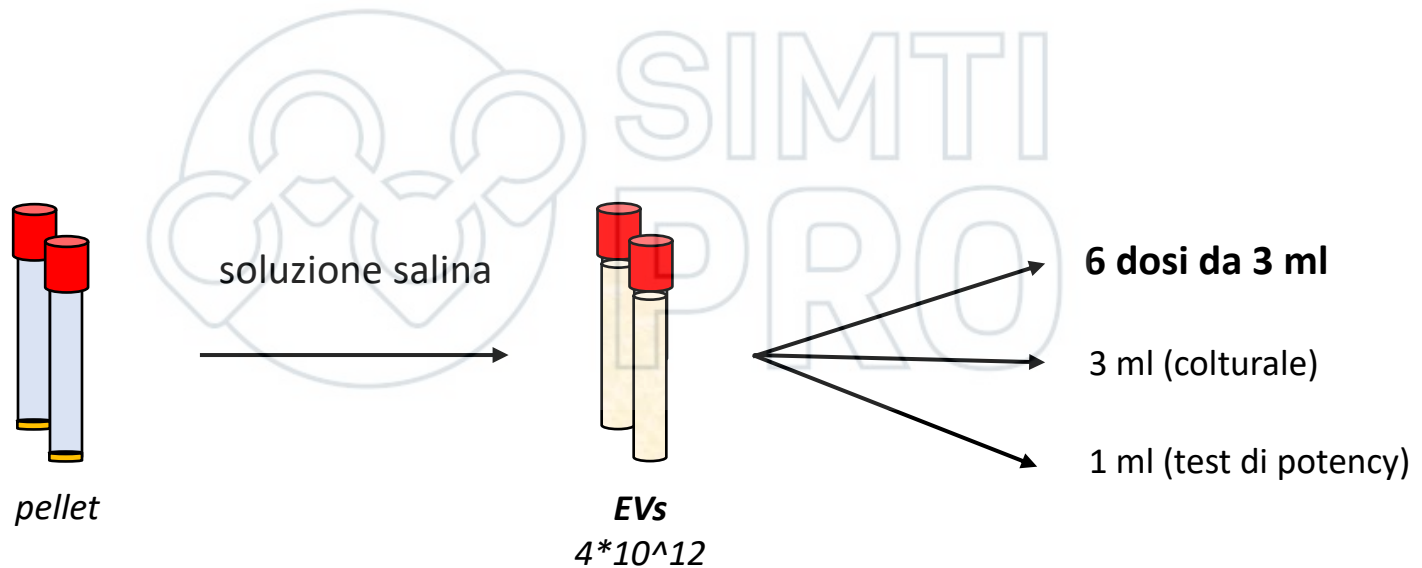
<sup>1</sup>Department of Medical Sciences, University of Turin, <sup>2</sup>i3T - Scarl. - Molecular Biotechnology Center (MBC),  
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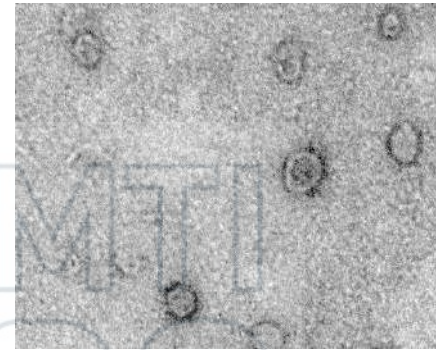
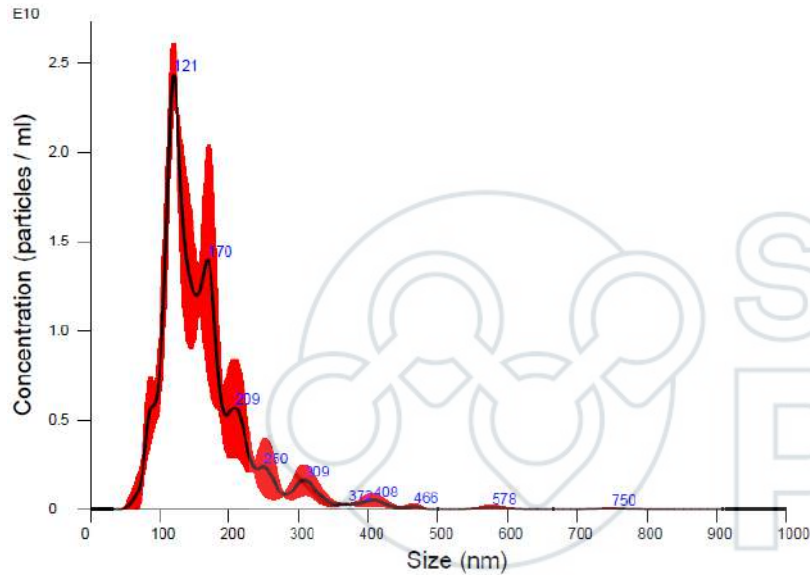
## LAVORAZIONE IN AMBIENTE DI CLASSE A



## 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**

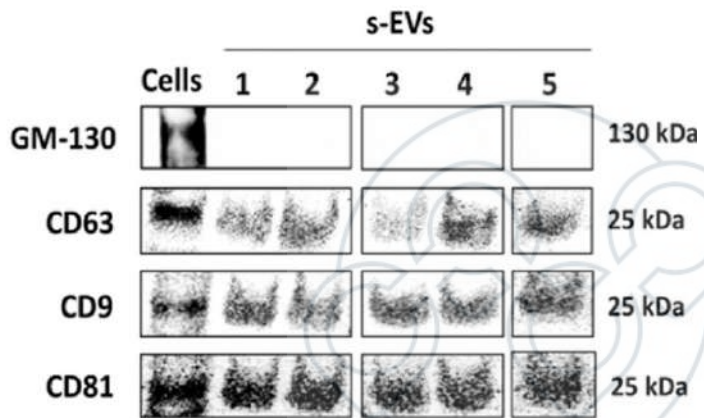
# CARATTERIZZAZIONE



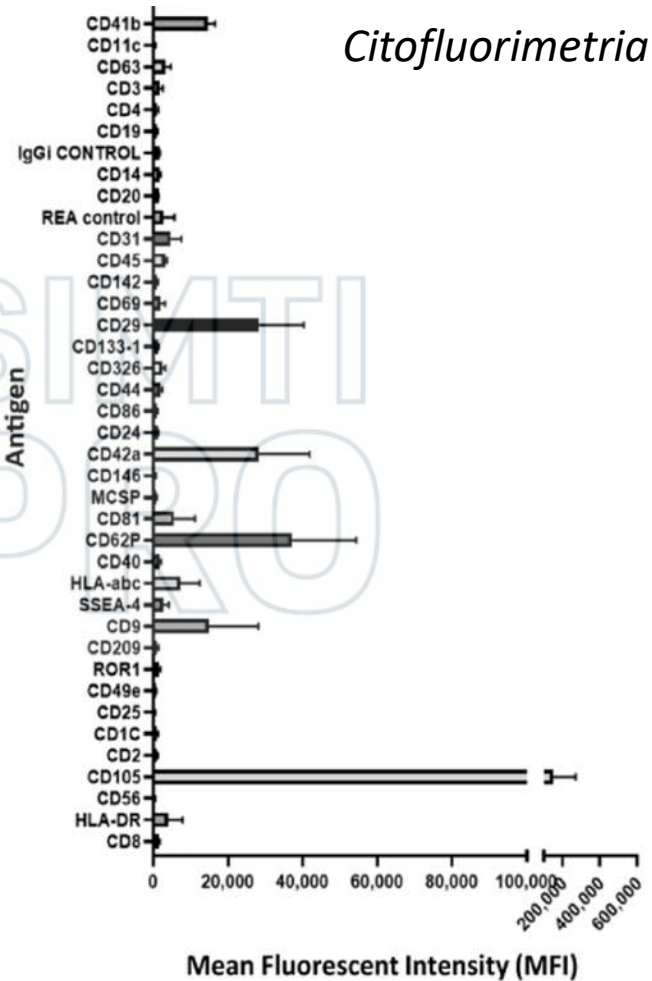
*Microscopia elettronica*

*NTA (nanosight) analysis*

# CARATTERIZZAZIONE



Western blot analysis



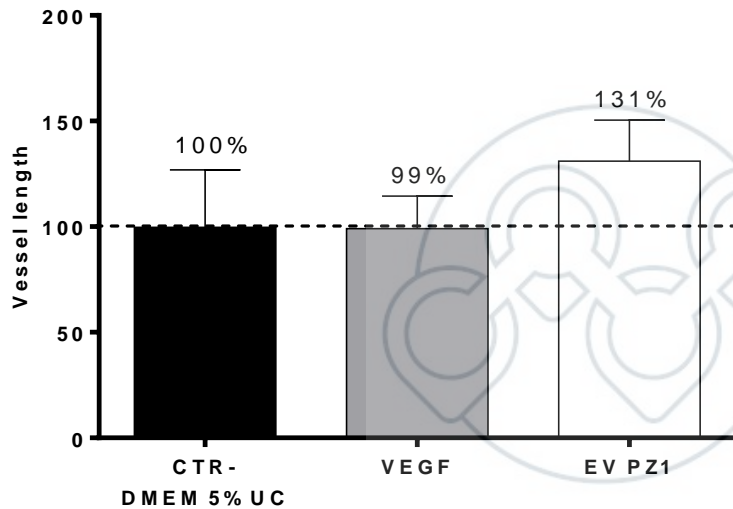
## ANALISI FUNZIONALE (test di potency)



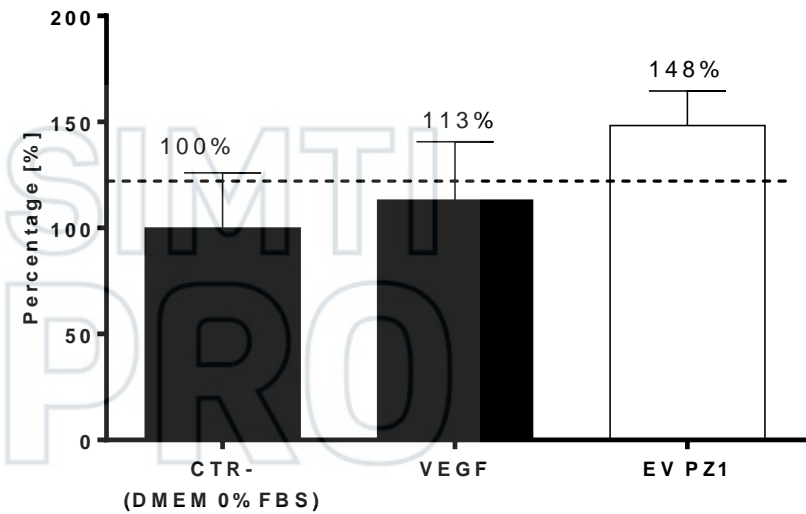
*Tube-assay test: effetto pro-angiogenico su cellule endoteliali*



## ANALISI FUNZIONALE (test di potency)

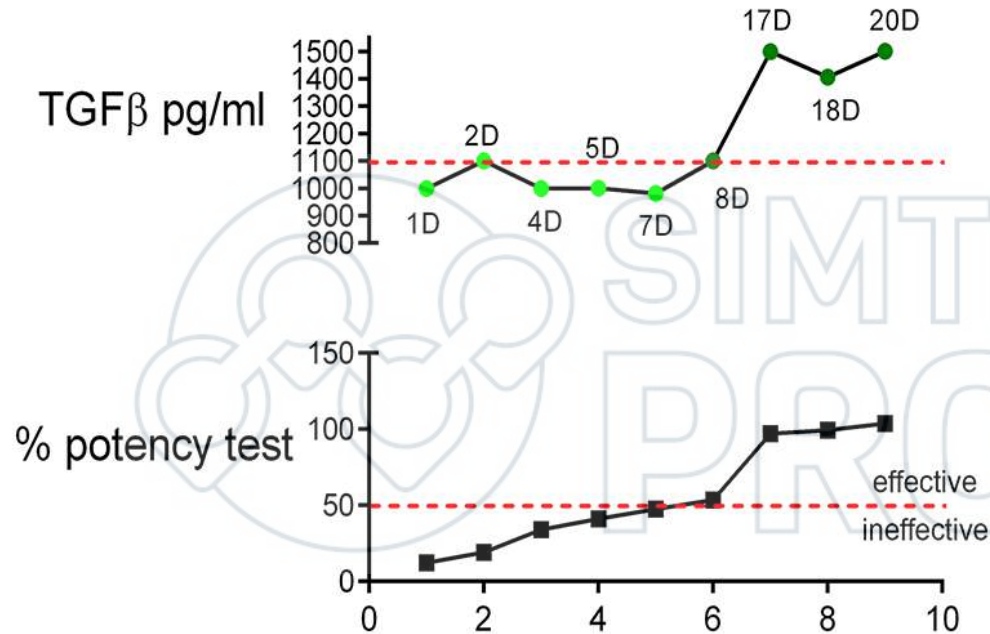


*Tube-assay test (lunghezza dei vasi)*



*% proliferazione*

## CORRELAZIONE TGF $\beta$ E % DI POTENCY

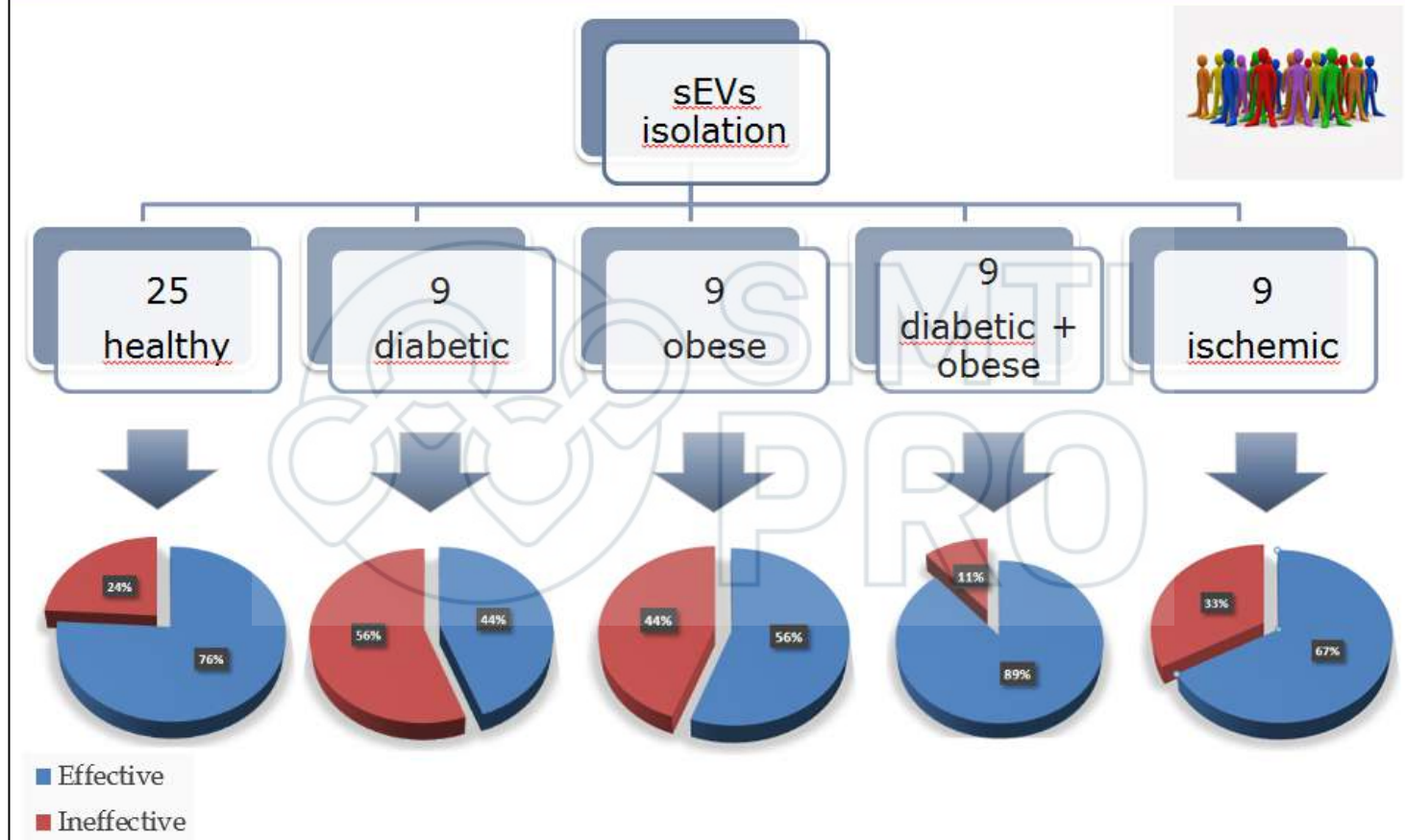


***MiR-130a and Tgf  $\beta$  content in extracellular vesicles derived from the serum of subjects at high cardiovascular risk predicts their in vivo angiogenic potential.***

*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

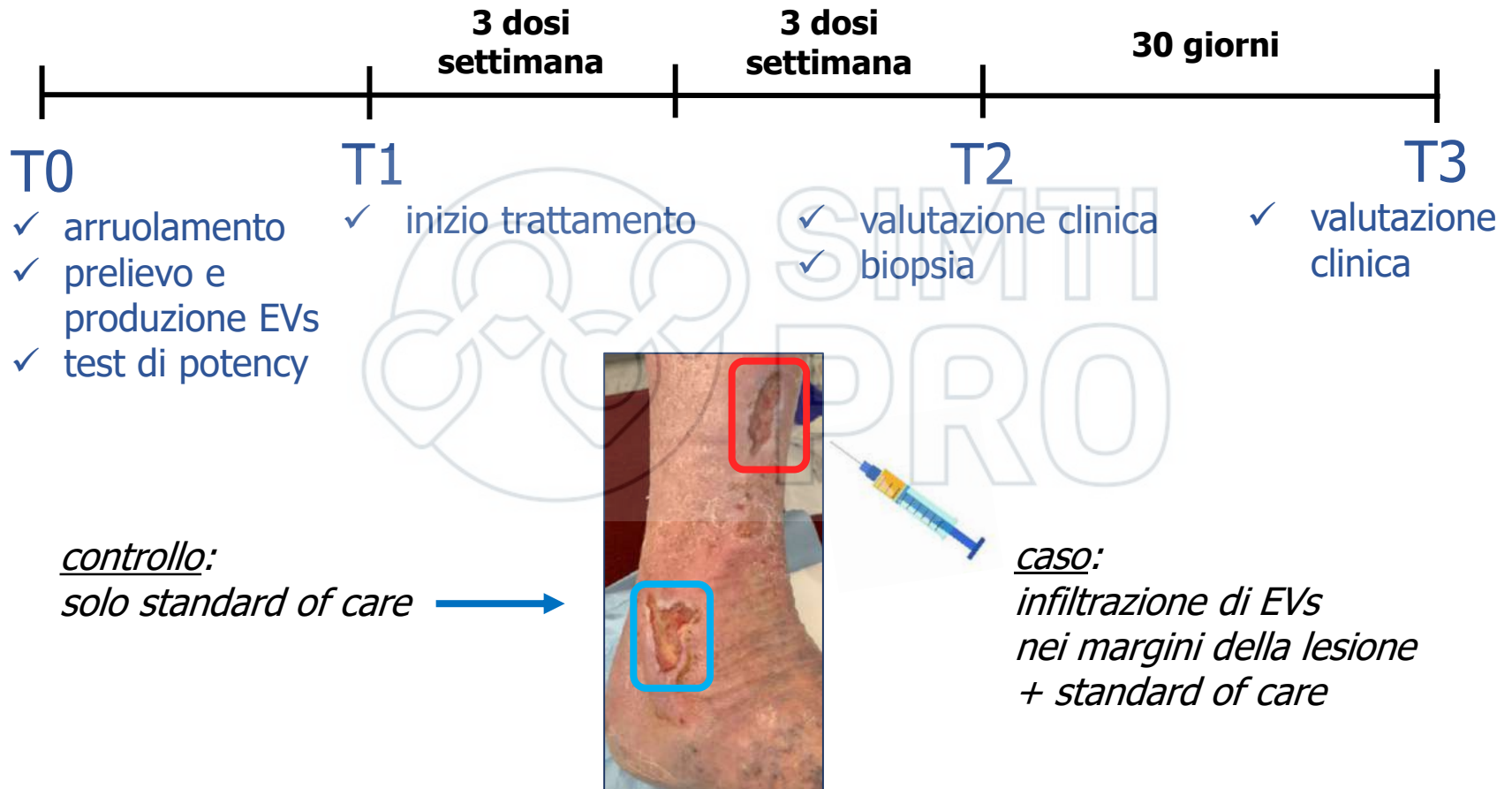
# PROTOCOLLO CLINICO

Inclusion and exclusion criteria.

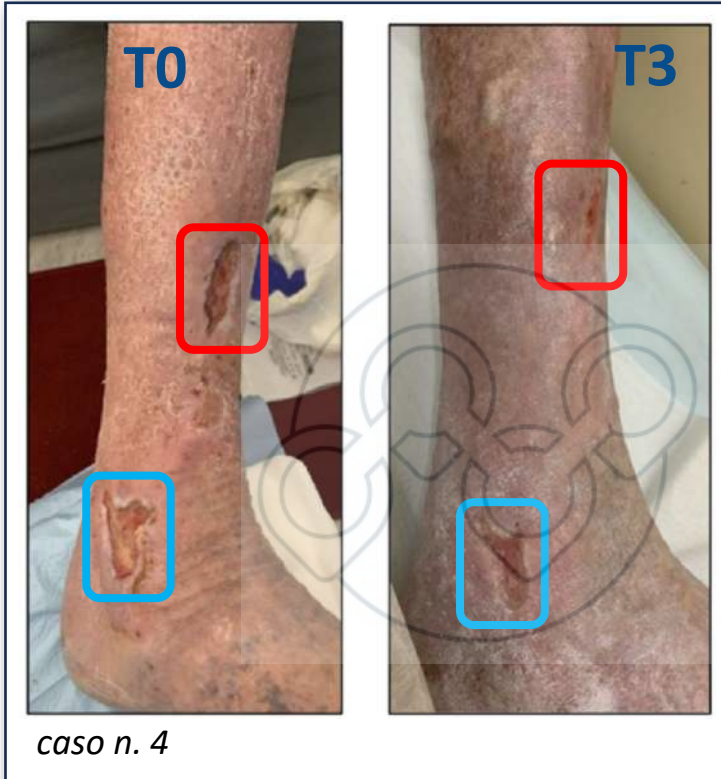
	INCLUSION CRITERIA	EXCLUSION CRITERIA
<b>Demographics &amp; risk factors</b>	<ul style="list-style-type: none"><li>• Age &gt; 18 and &lt; 85</li><li>• Absence of peripheral arterial disease*</li></ul>	<ul style="list-style-type: none"><li>• Cancer</li><li>• Diabetes</li></ul>
<b>Wound condition</b>	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

***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

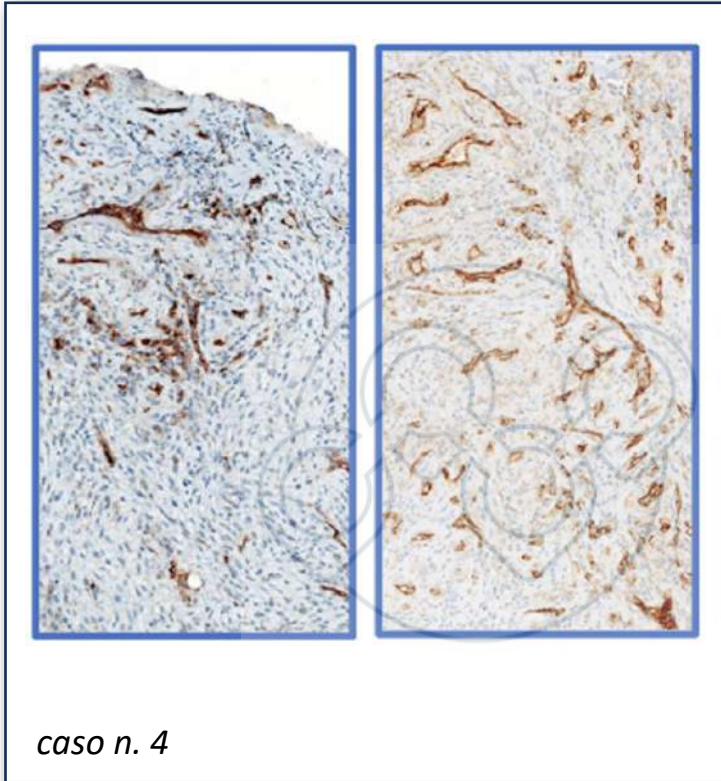


### T3 (30 gg):

- maggior tessuto di granulazione (75-100% in 4/5 lesioni)
- riduzione mediana della superficie di 385 mm<sup>2</sup> vs 106 mm<sup>2</sup> ( $p=0,004$ )
- 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  
 hydrophilic  
 hydrophobic  
 Stuff  
 Proteins  
 More stuff

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!**