



**Università
di Brescia**

Dipartimento di Scienze Cliniche e Sperimentali

Dottorato di Ricerca in
«Intelligenza Artificiale in Medicina e Innovazione
nella Ricerca clinica e metodologica»

Coordinatore: Prof. Domenico Russo

The missing link: **Ex vivo** ad in **Vitro** Analysis for a comprehensive characterization of the “**Secretome**” in the scenario of the CAR-T cell therapy and study of its Impact on CAR-T cell **Efficacy** and **Toxicity** (**EVASIET CAR-T**)

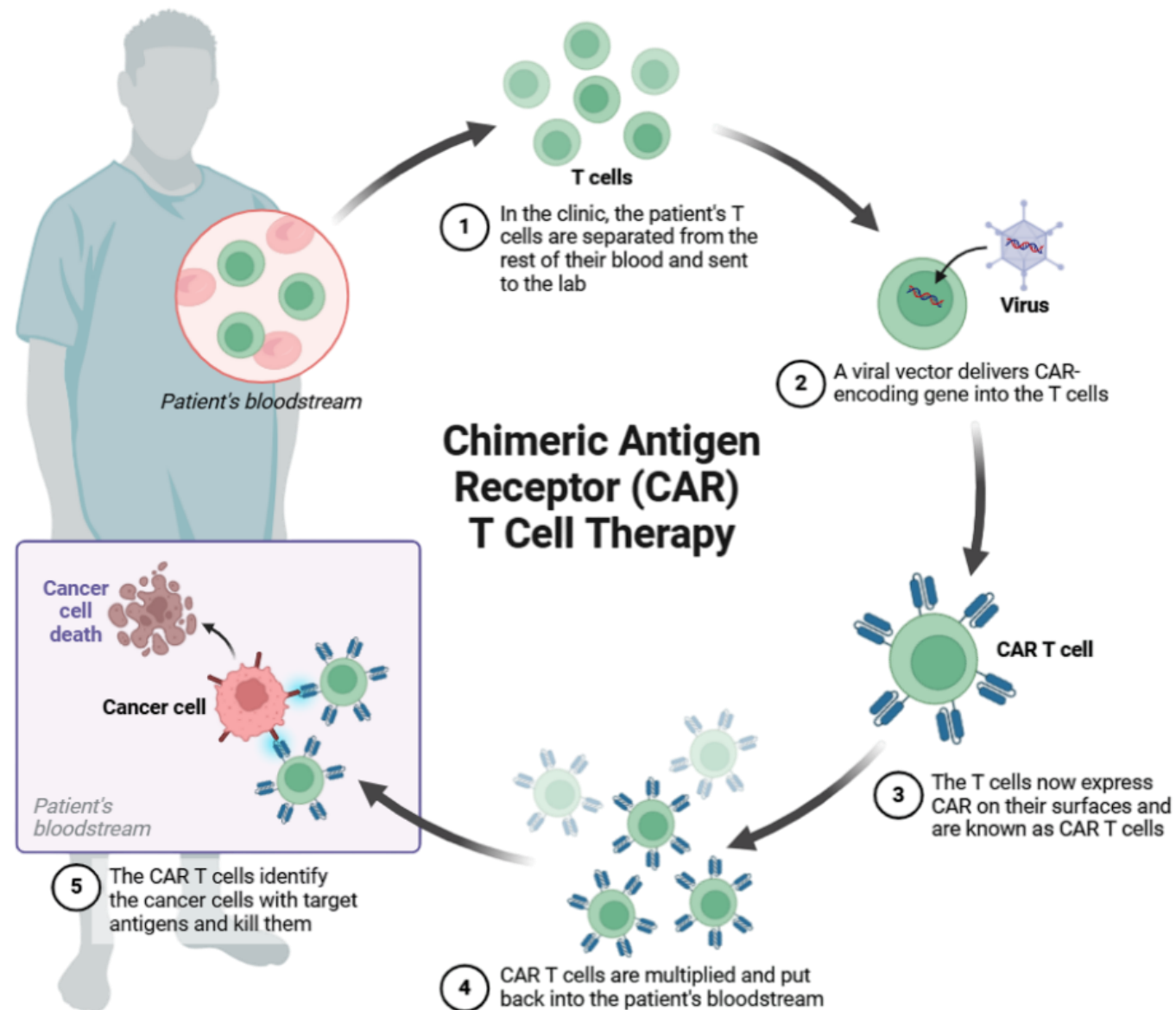
XHAHYSA BESJANA

XL ciclo

Supervisor: Prof.ssa Simona Bernardi

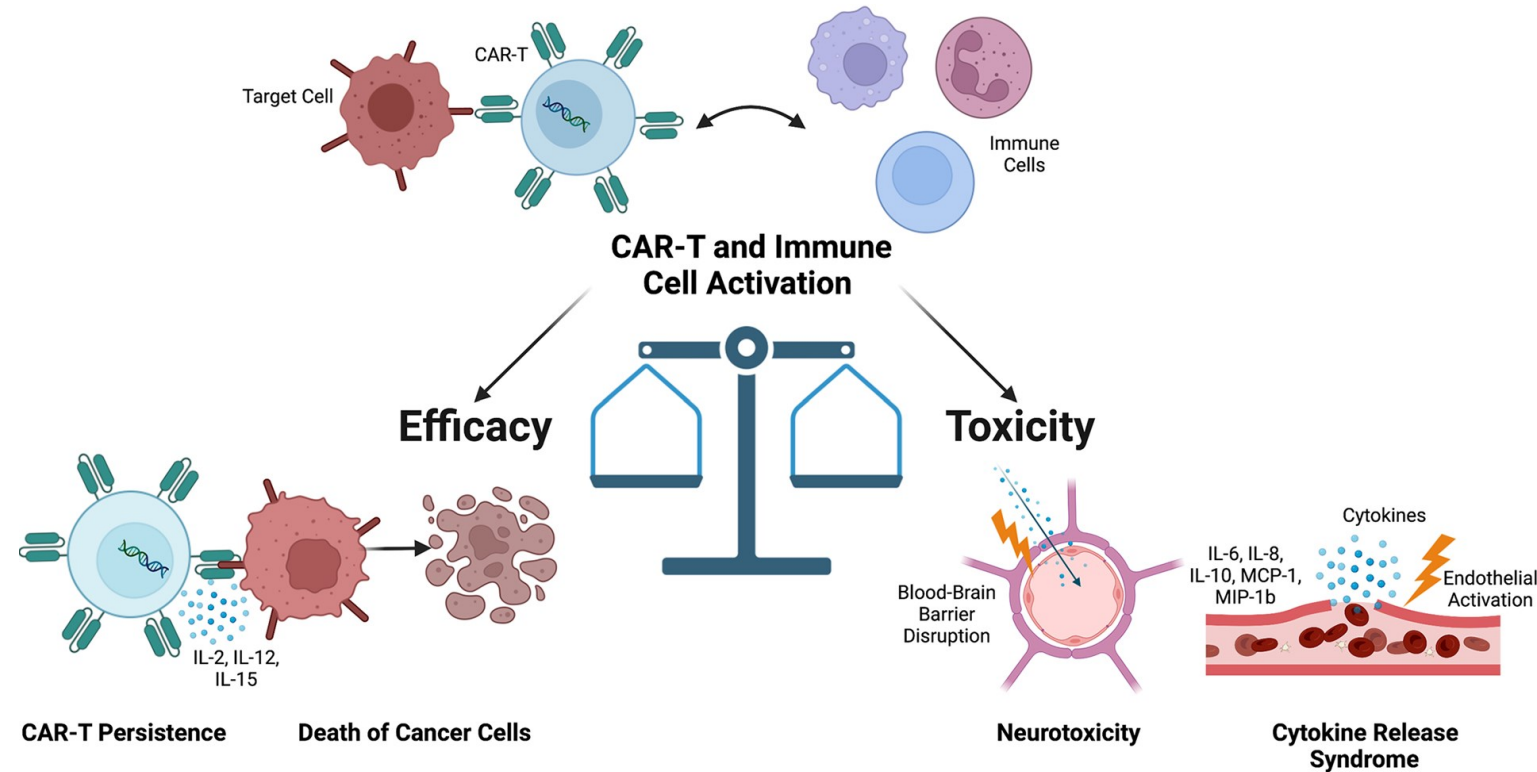
Introduction

- Chimeric Antigen Receptor-T (CAR-T) cell therapy has transformed treatment of hematological malignancies



Introduction

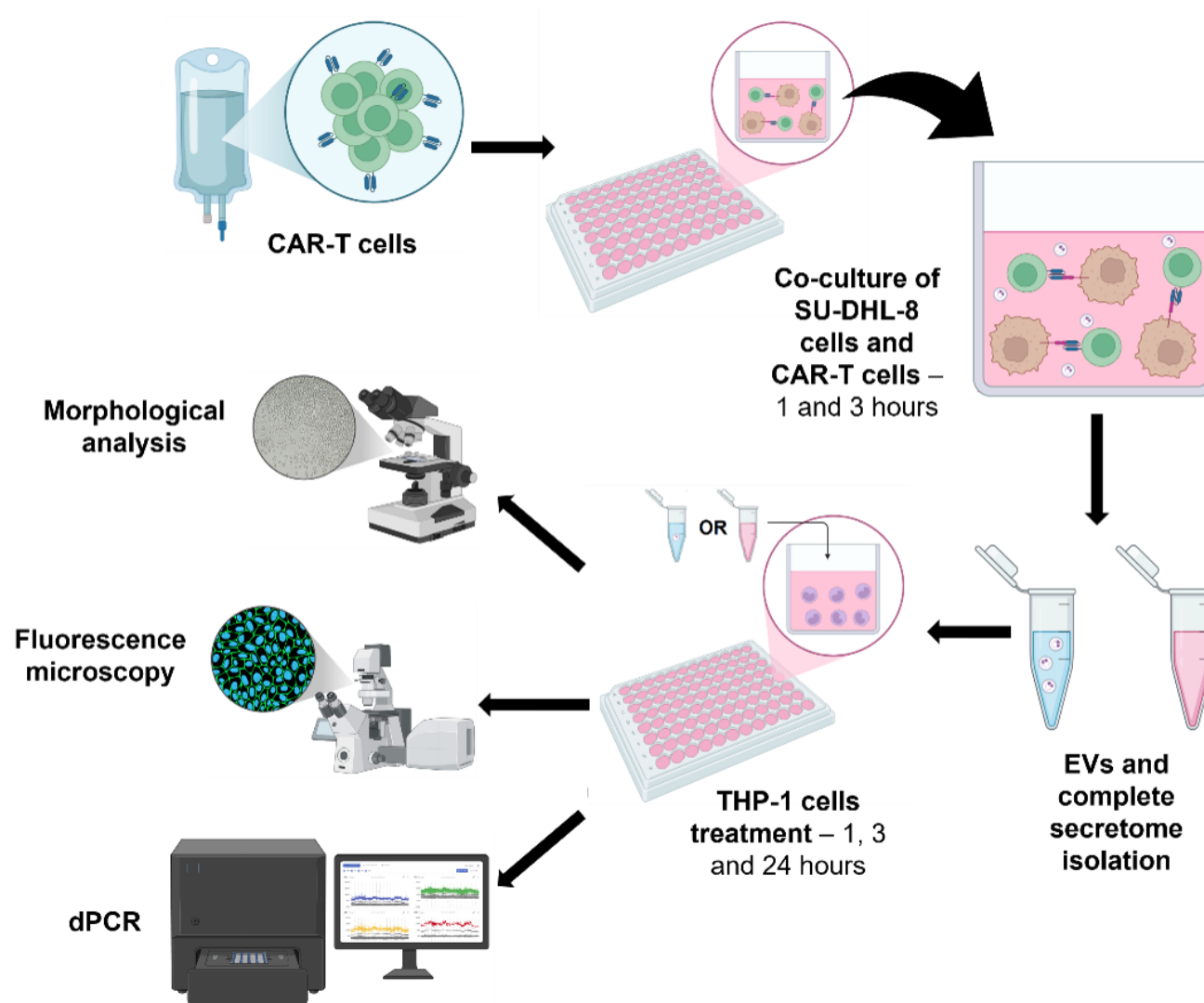
- Chimeric Antigen Receptor-T (CAR-T) cell therapy has transformed treatment of hematological malignancies
- Accompanied by varying types and grades of immune-mediated toxicities
 - Still not fully elucidated
 - The monocyte-macrophage lineage appears to play a significant role
- We investigated how CAR-T cells interact with monocytes and trigger immune activation



Chohan K.L., et al. *Curr Hematol Malig*, 2023

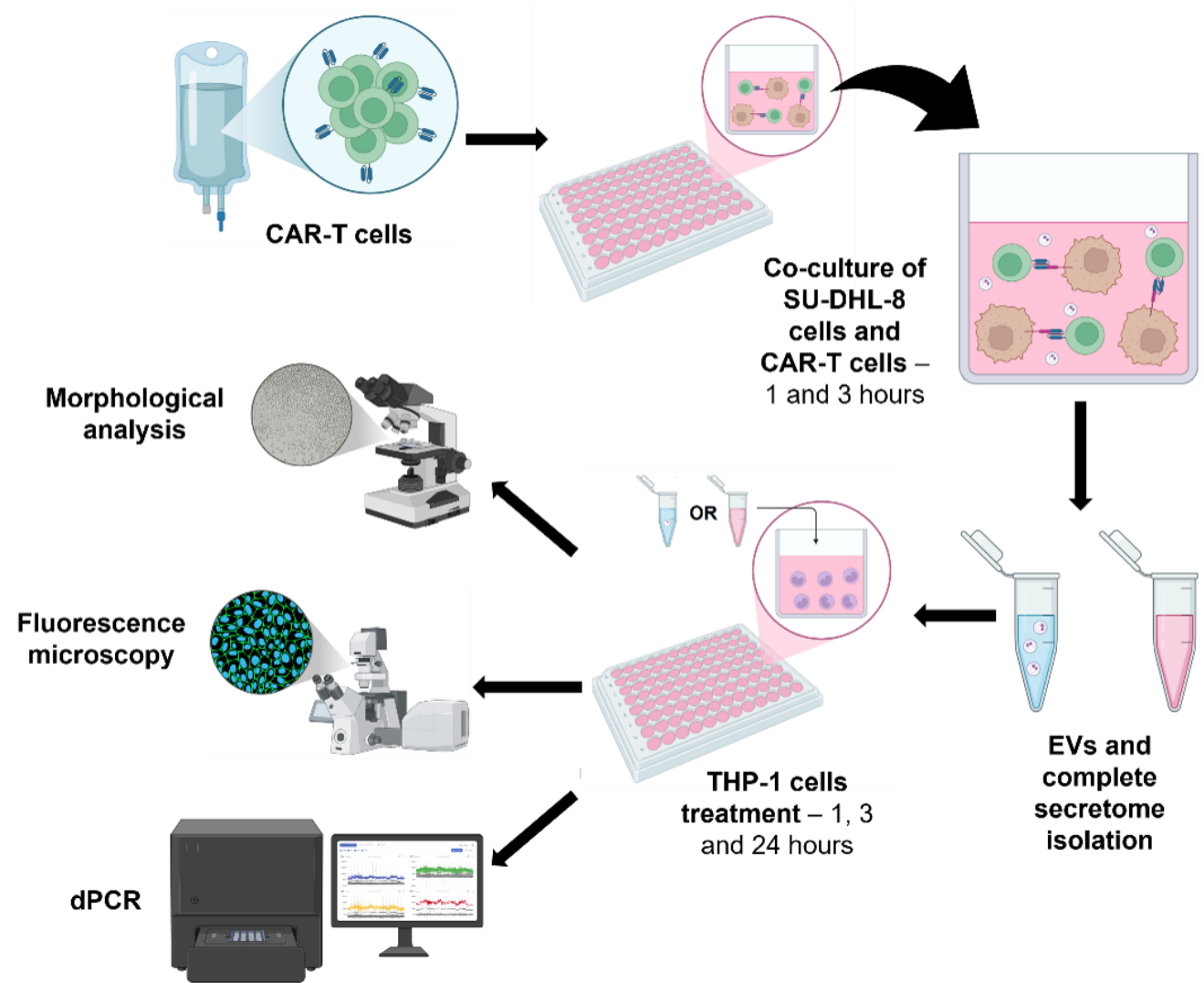
Materials & Methods

- 8 patients
 - 4 with Diffuse Large B-cell Lymphoma
 - 4 with Follicular Lymphoma
- Both axi-cel and tisa-cel products were considered



Materials & Methods

- CAR-T cells were co-cultured with an *in vitro* model of CD19+ lymphoma, SU-DHL-8
 - For 1 and 3 hours
- Isolation of EVs and complete secretome (SECR) and administration to THP-1 cells
 - For 1, 3 and 24 hours
- Monocyte-to-macrophage transition (MMt) evaluation via morphological and molecular analysis
- EVs uptake evaluation via fluorescence microscopy



Materials & Methods



Morphological analysis

- Evaluation of cell structure
- Identification of cellular alteration



Molecular analysis

- Analysis of nucleic acids
- Study of gene expression



Cytokines expression

- Quantification of pro- and anti-inflammatory cytokines



Morphological analysis

- Evaluation of cell structure
- Identification of cellular alteration



Molecular analysis

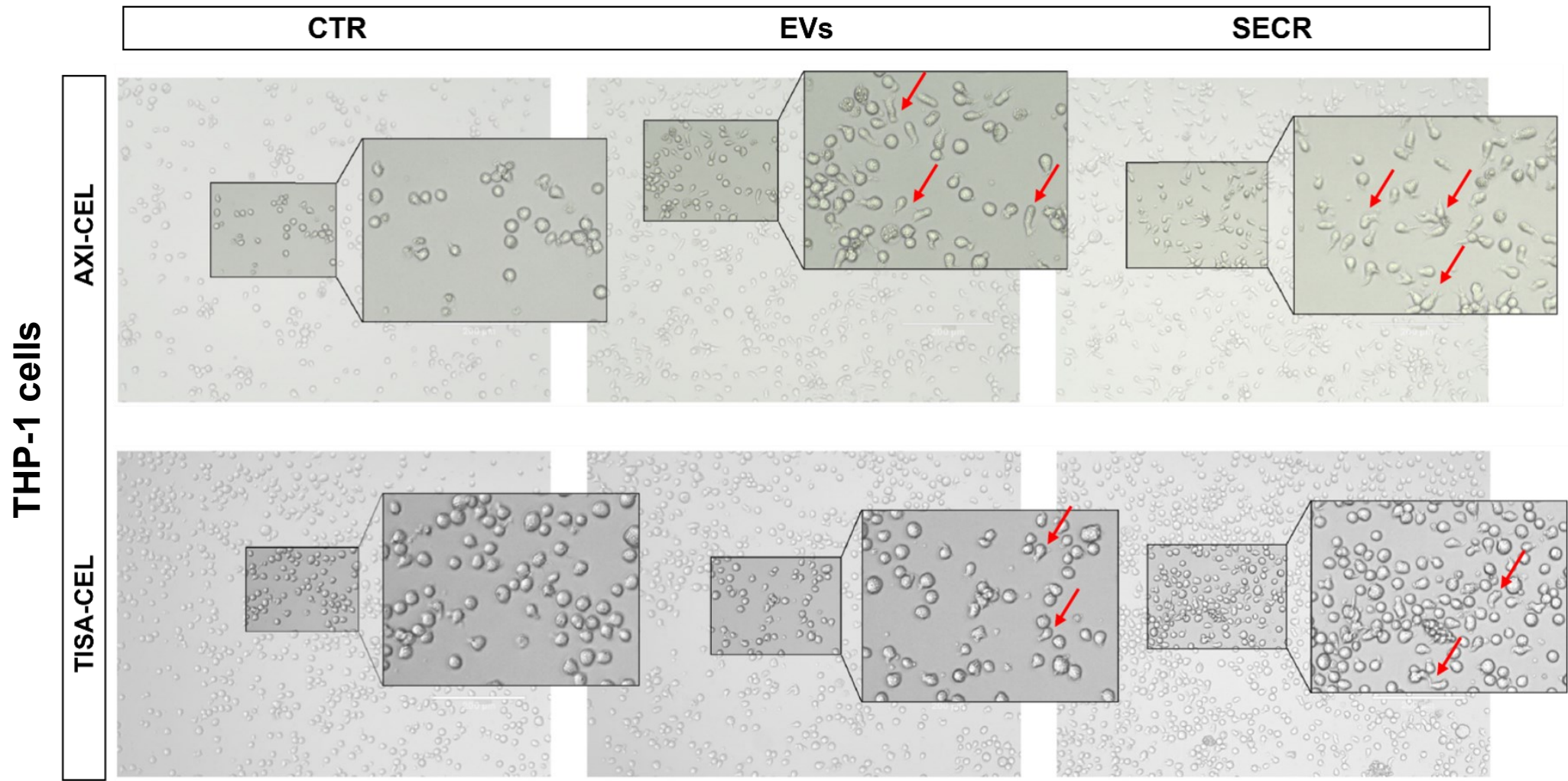
- Analysis of nucleic acids
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Cytokines expression

- Quantification of pro- and anti-inflammatory cytokines

Morphological changes





Morphological analysis

- Evaluation of cell structure
- Identification of cellular alteration



Molecular analysis

- Analysis of nucleic acids
- Study of gene expression



Cytokines expression

- Quantification of pro- and anti-inflammatory cytokines





Molecular analysis



Cytokines expression

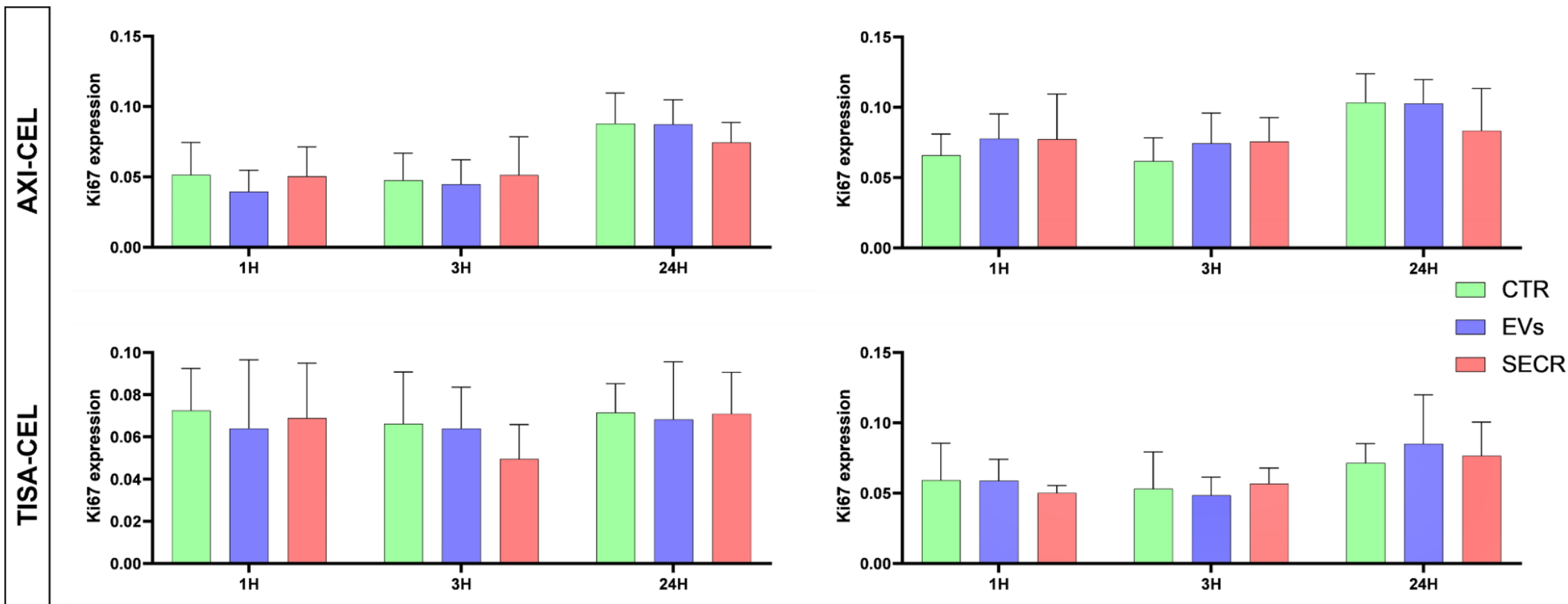
PPM1A expression



Ki67 expression

1h of co-culture

3h of co-culture





Morphological analysis

- Evaluation of cell structure
- Identification of cellular alteration



Molecular analysis

- Analysis of nucleic acids
- Study of gene expression



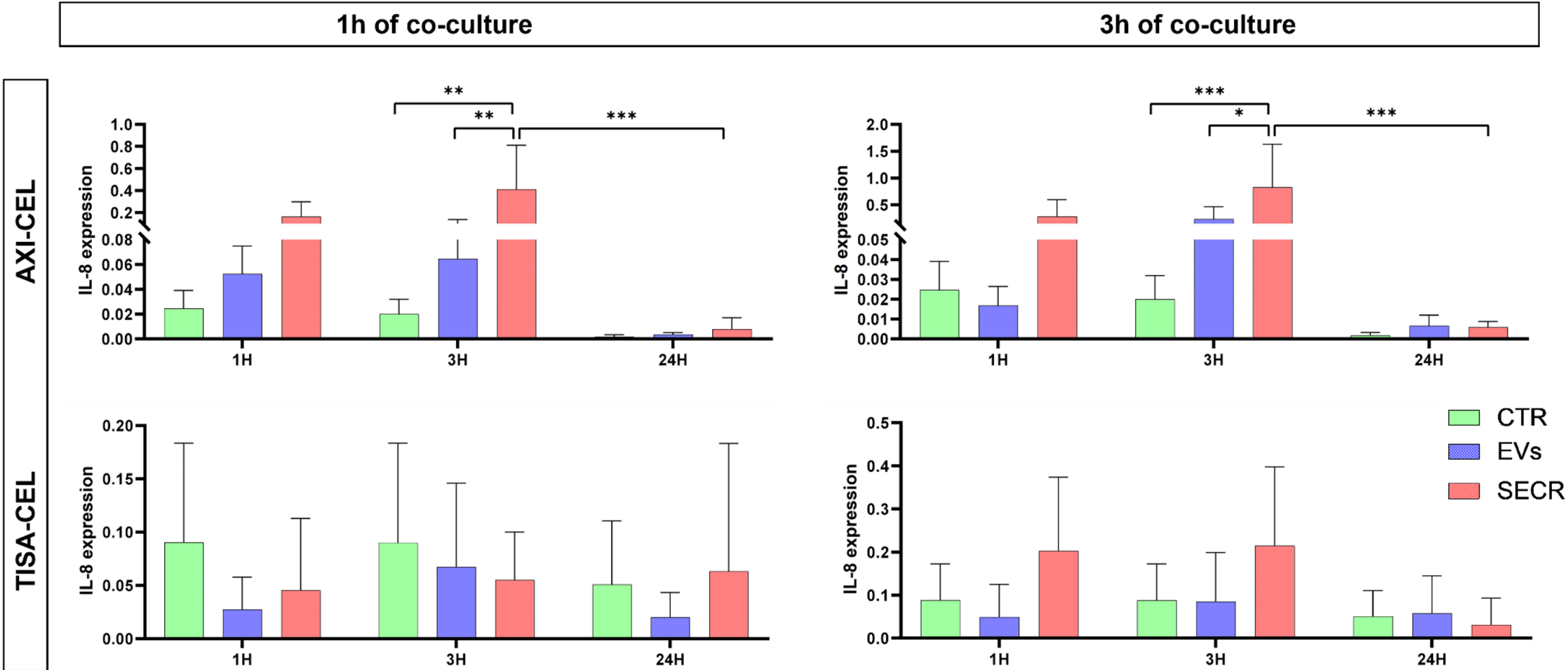
Cytokines expression

- Quantification of pro- and anti-inflammatory cytokines

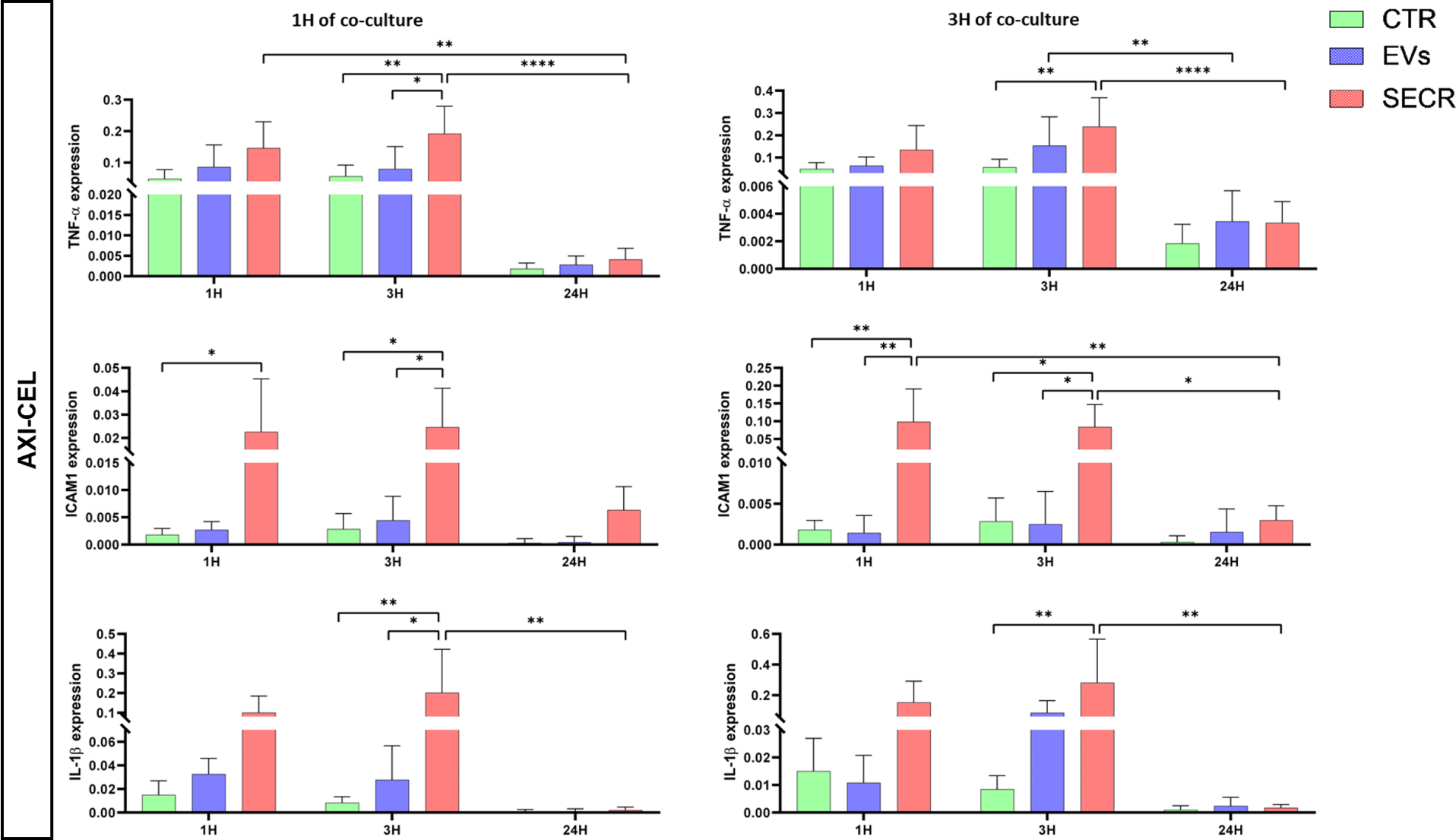
Cytokines expression

11 Cytokines:

- IL-8
- IL-6
- TNF-α
- IL-1β
- IL-10
- IL-2
- INF-γ
- ICAM1
- IL-17a
- IL-RN
- MMP9

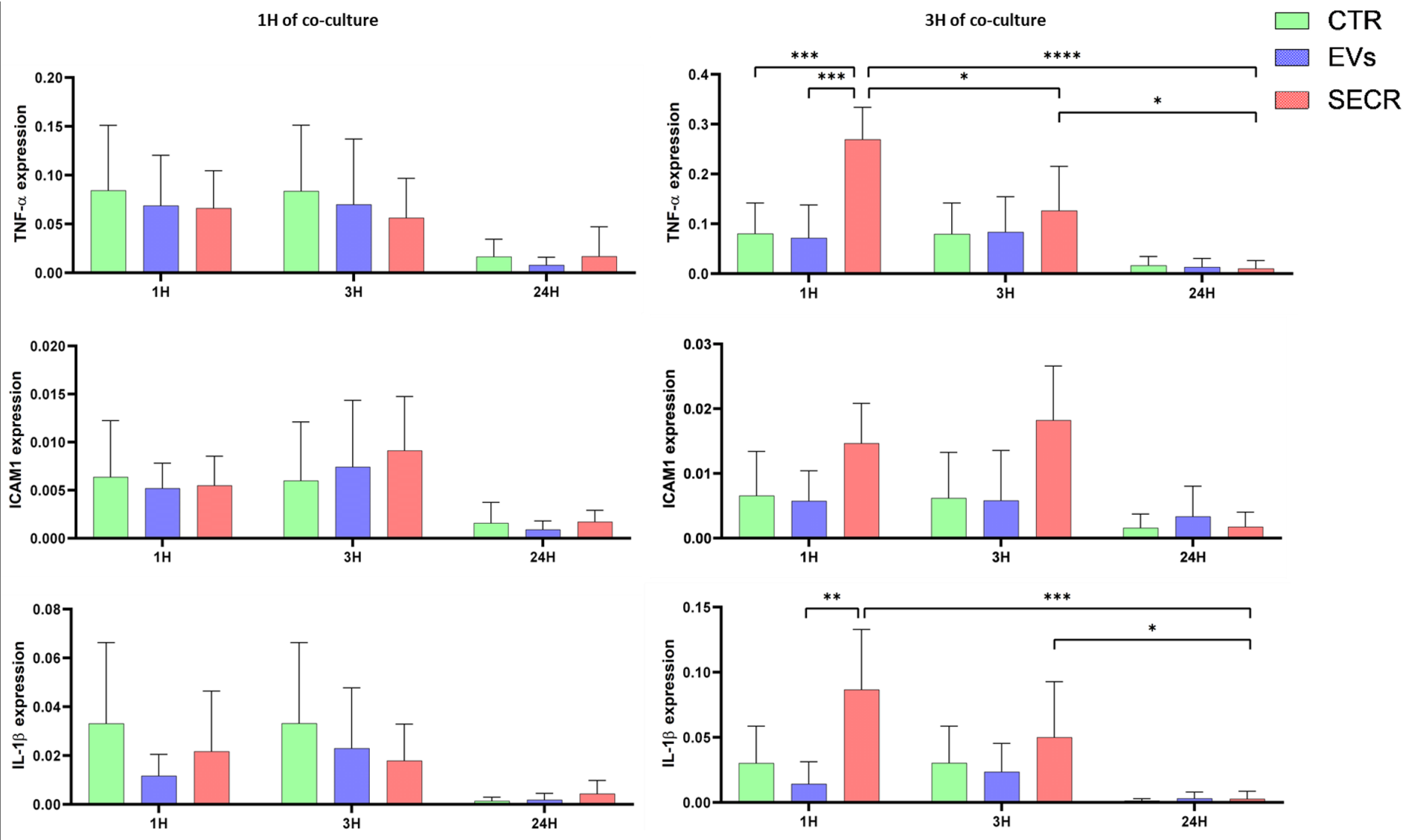


Cytokines expression



Cytokines expression

TISA-CEL





Morphological analysis

- Evaluation of cell structure
- Identification of cellular alteration



Molecular analysis

- Analysis of nucleic acids
- Study of gene expression

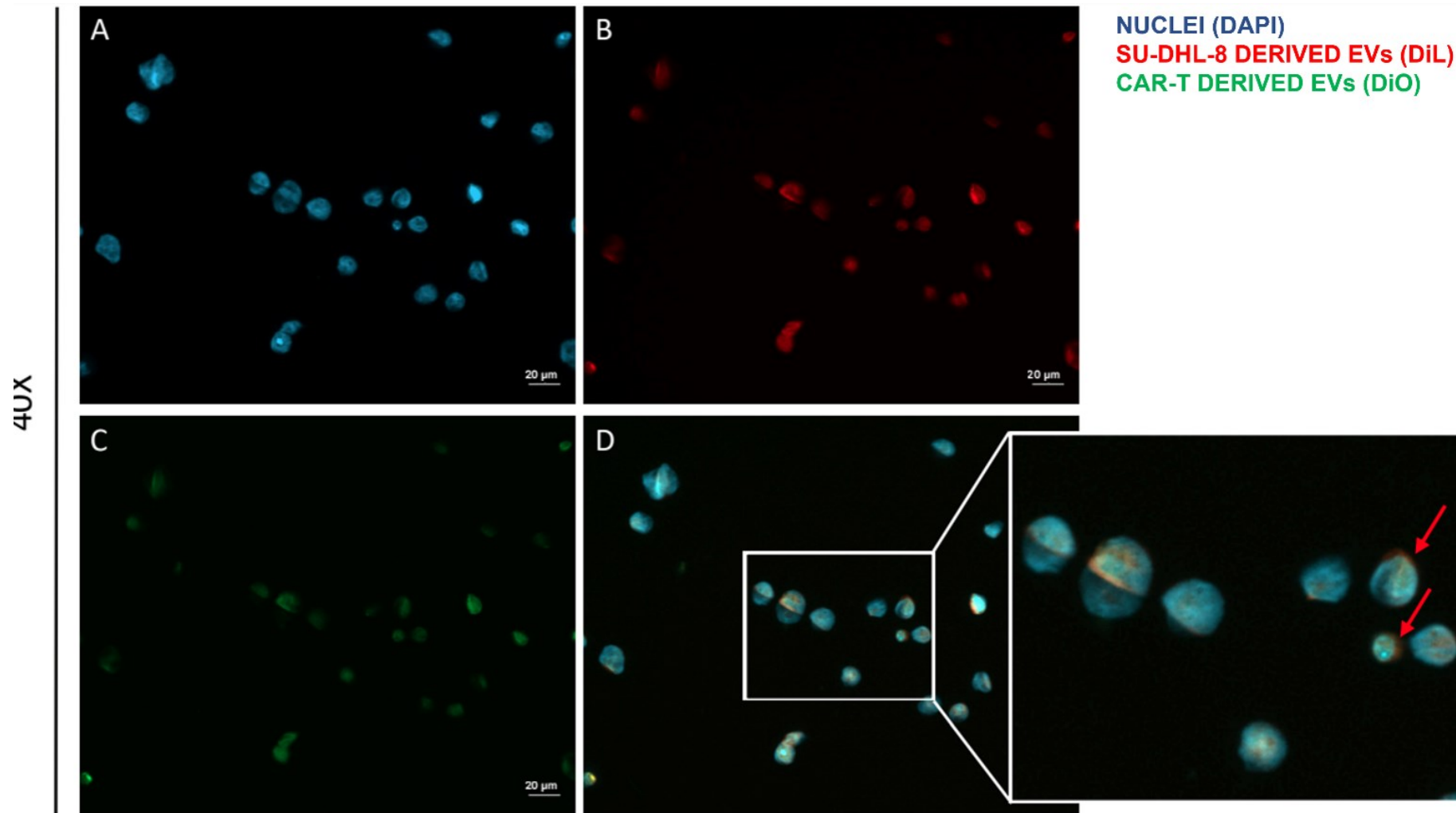


Cytokines expression

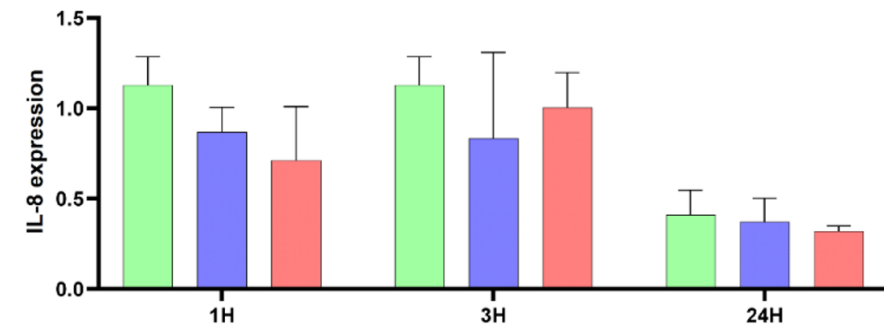
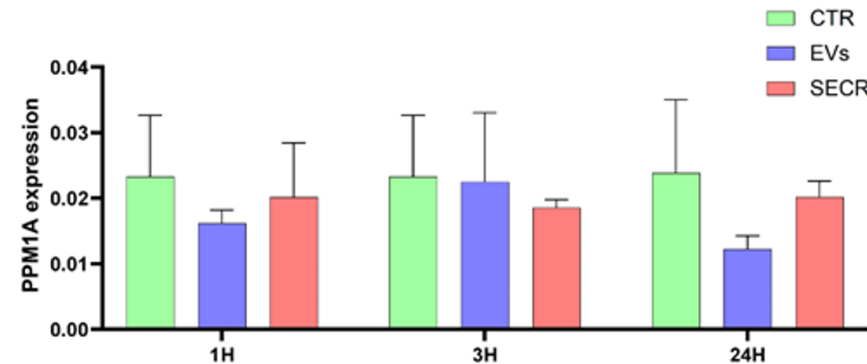
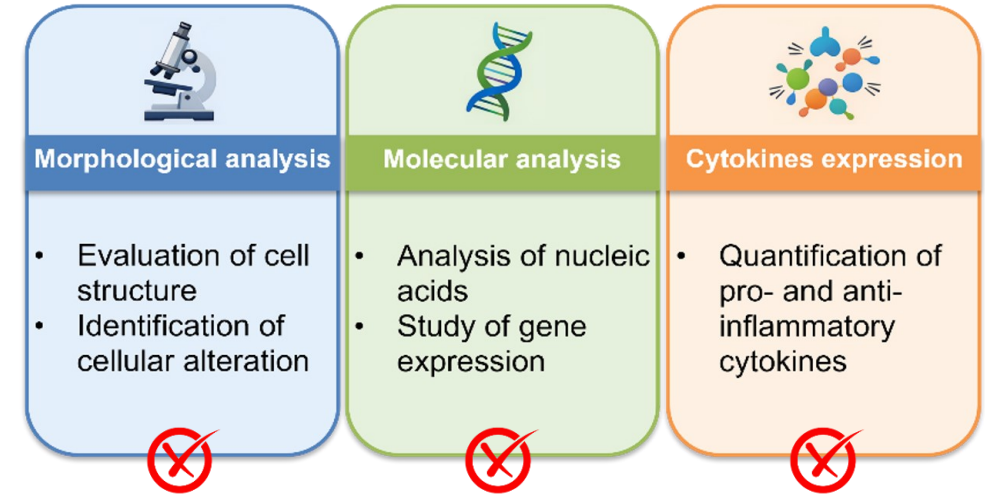
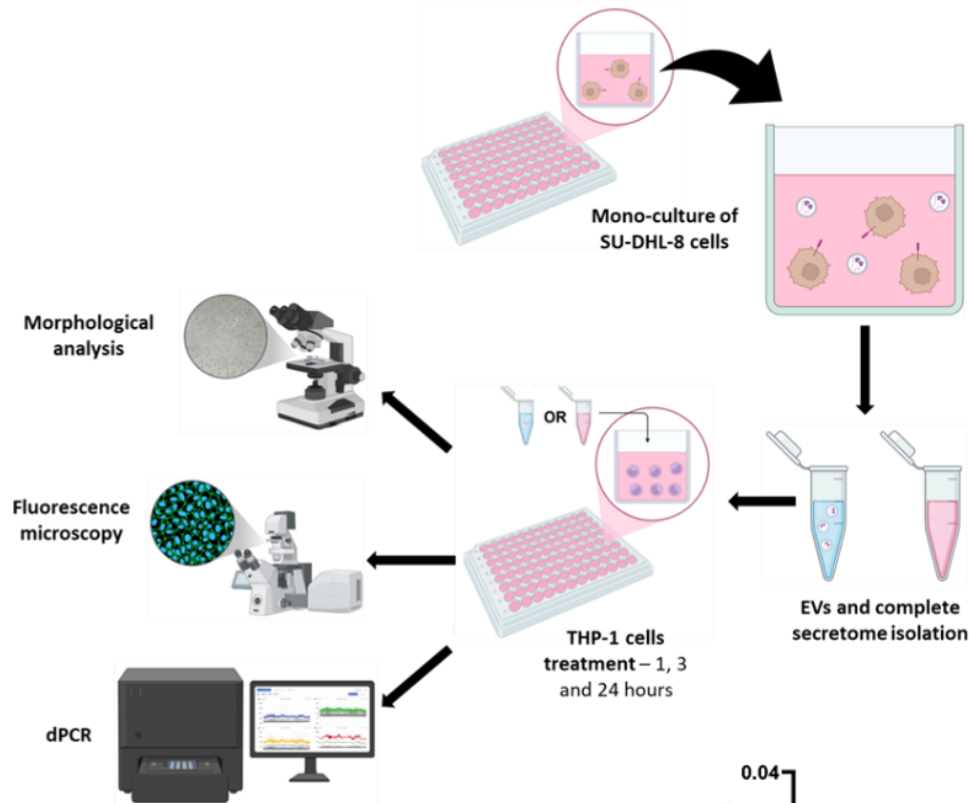
- Quantification of pro- and anti-inflammatory cytokines



EVs uptake



“Negative” controls – Naïve SU-DHL-8

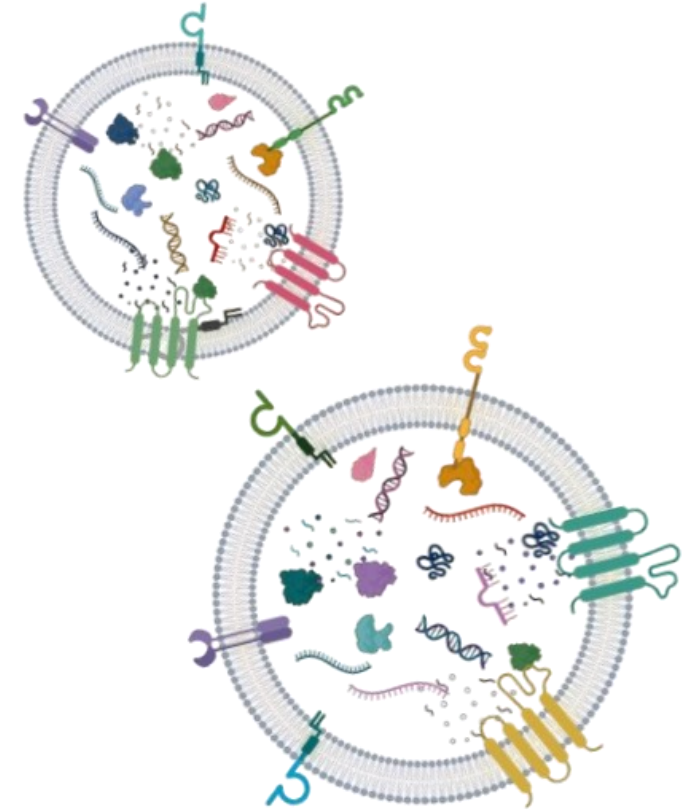


Conclusions

- CAR-T cells release factors capable of inducing MMt and pro-inflammatory activation of immune system;
- Key role of CAR-T-derived EVs/SECR in modulating innate immune responses;
- Identify potential targets to modulate immune activation and improve clinical outcomes in CAR-T-treated patients.

Future perspectives:

- EVs cargo characterization;
- Proteomic analysis;
- Plasma-derived EVs activity.





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Grazie per l'attenzione!