XIX CONGRESSO NAZIONALE SIES 2026

MONOCYTE ACTIVATION BY CAR-T CELLS: ROLE OF EXTRACELLULAR VESICLES AND SECRETOME IN IMMUNE MODULATION

Dr. Besjana Xhahysa
Università degli Studi di Brescia
besjana.xhahysa@unibs.it

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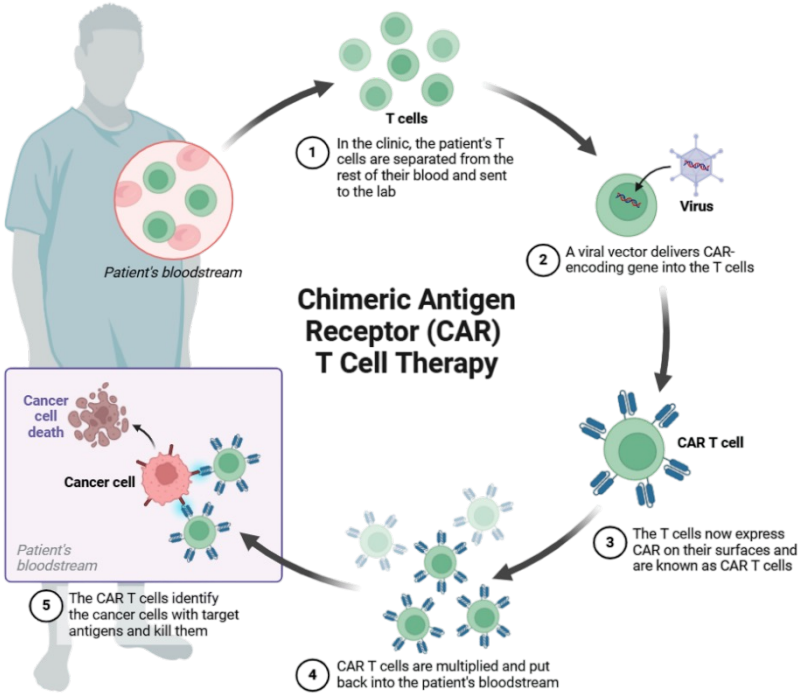
Palazzo degli Affari

Disclosures of Besjana Xhahysa

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

Introduction

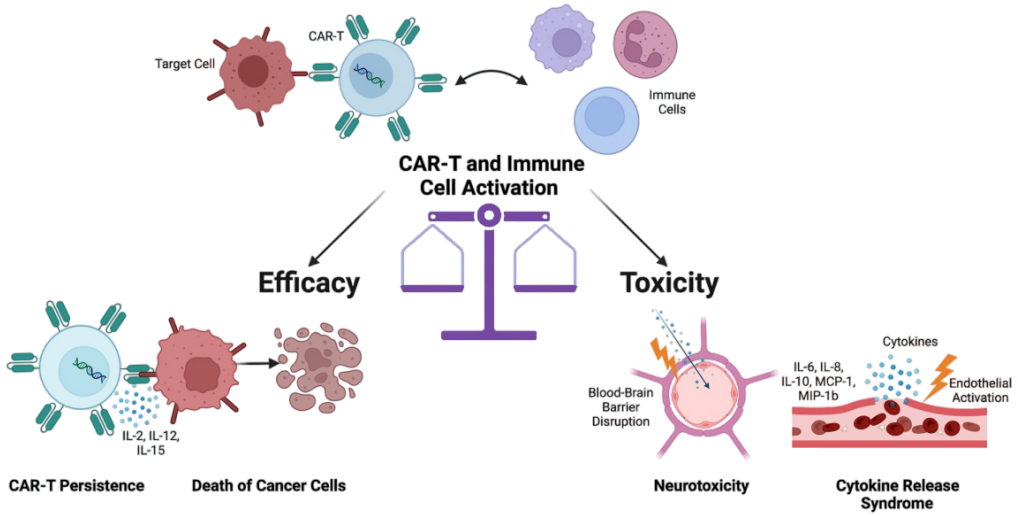
- Chimeric Antigen Receptor-T (CAR-T) cell therapy has transformed treatment of hematological malignancies



BioRender

Introduction

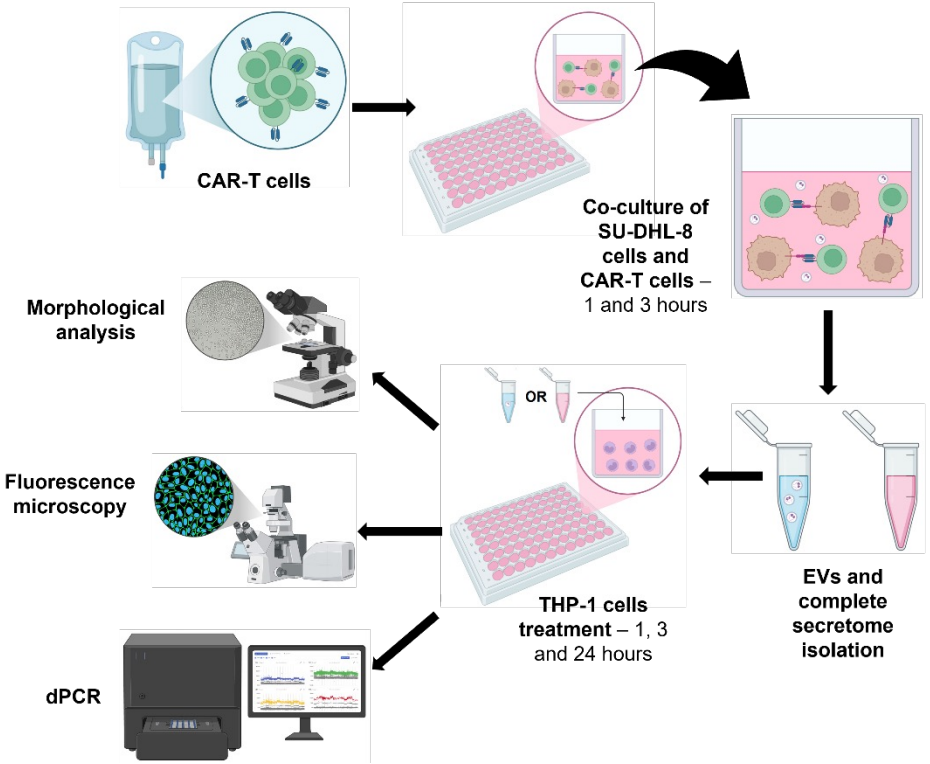
- Chimeric Antigen Receptor-T (CAR-T) cell therapy has transformed treatment of hematological malignancies
- Accompanied by varying types and grades of immune-mediated toxicities
  - Still not fully elucidated
  - The monocyte-macrophage lineage appears to play a significant role
- We investigated how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



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

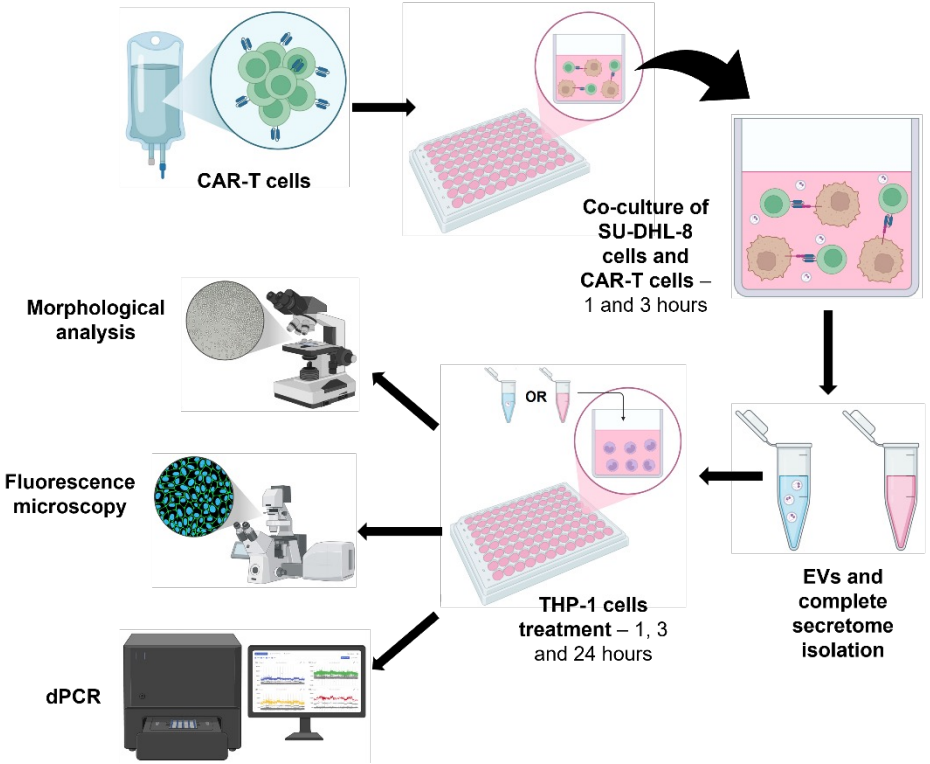
Material and Methods

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



Material and 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



Material and 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

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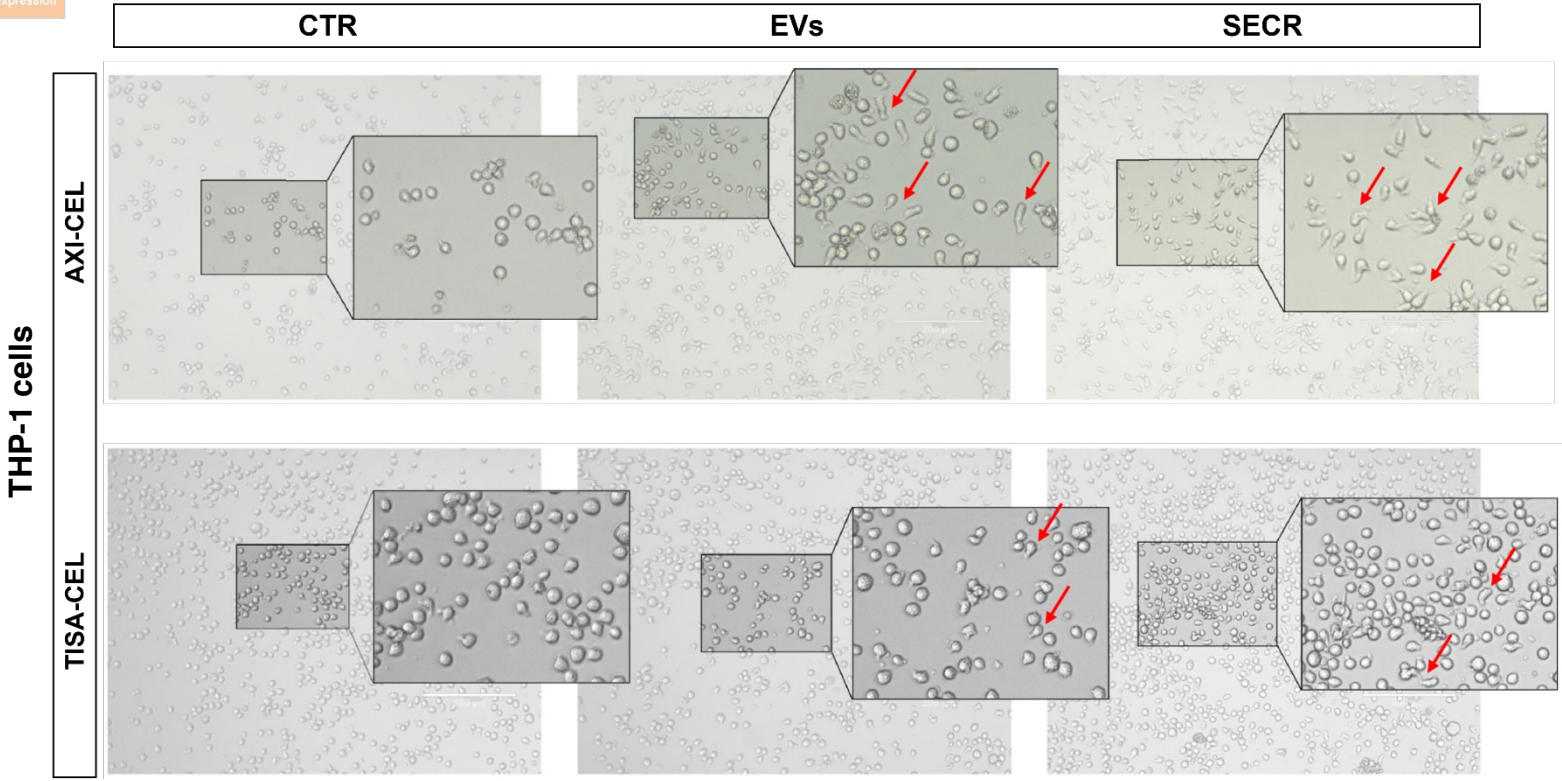
Morphological analysis | Molecular analysis | Cytokines expression

Morphological changes



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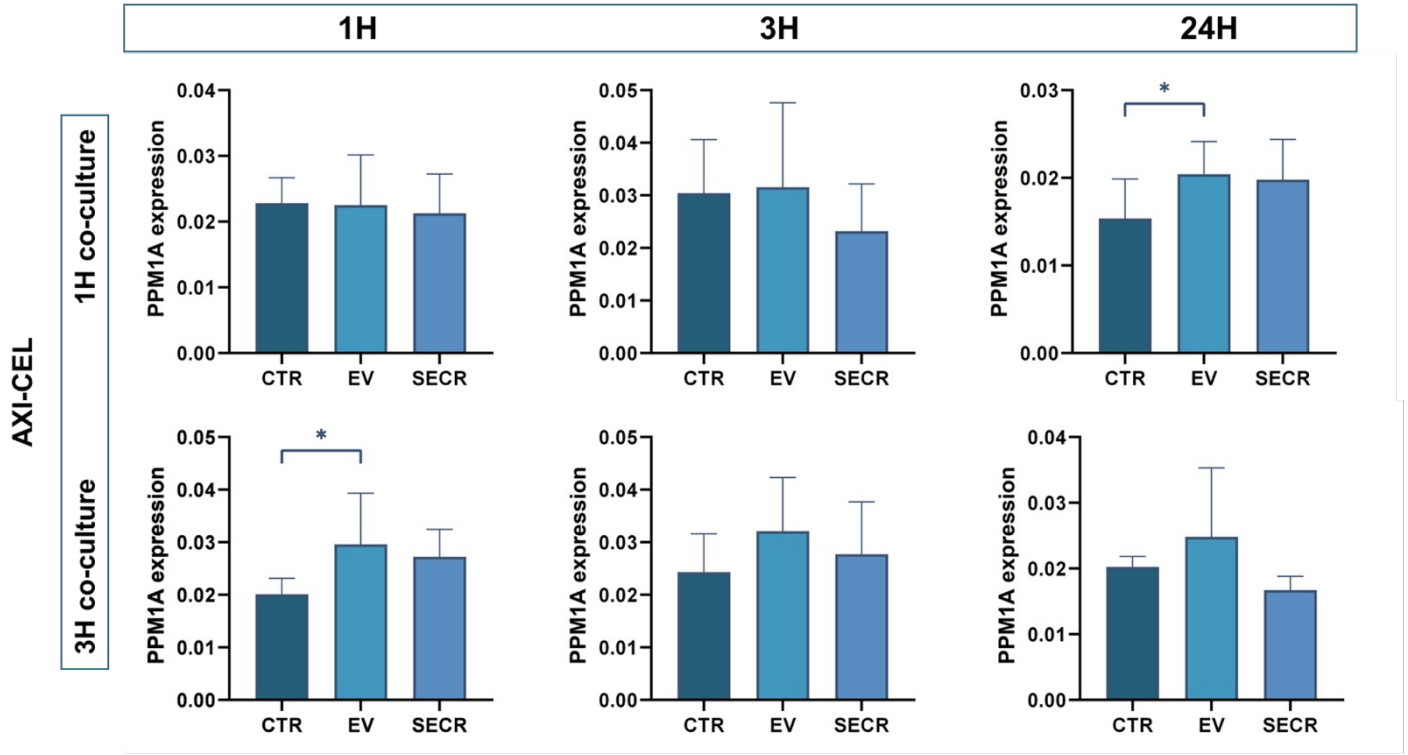
Morphological analysis | Molecular analysis | Cytokines expression

PPM1A expression



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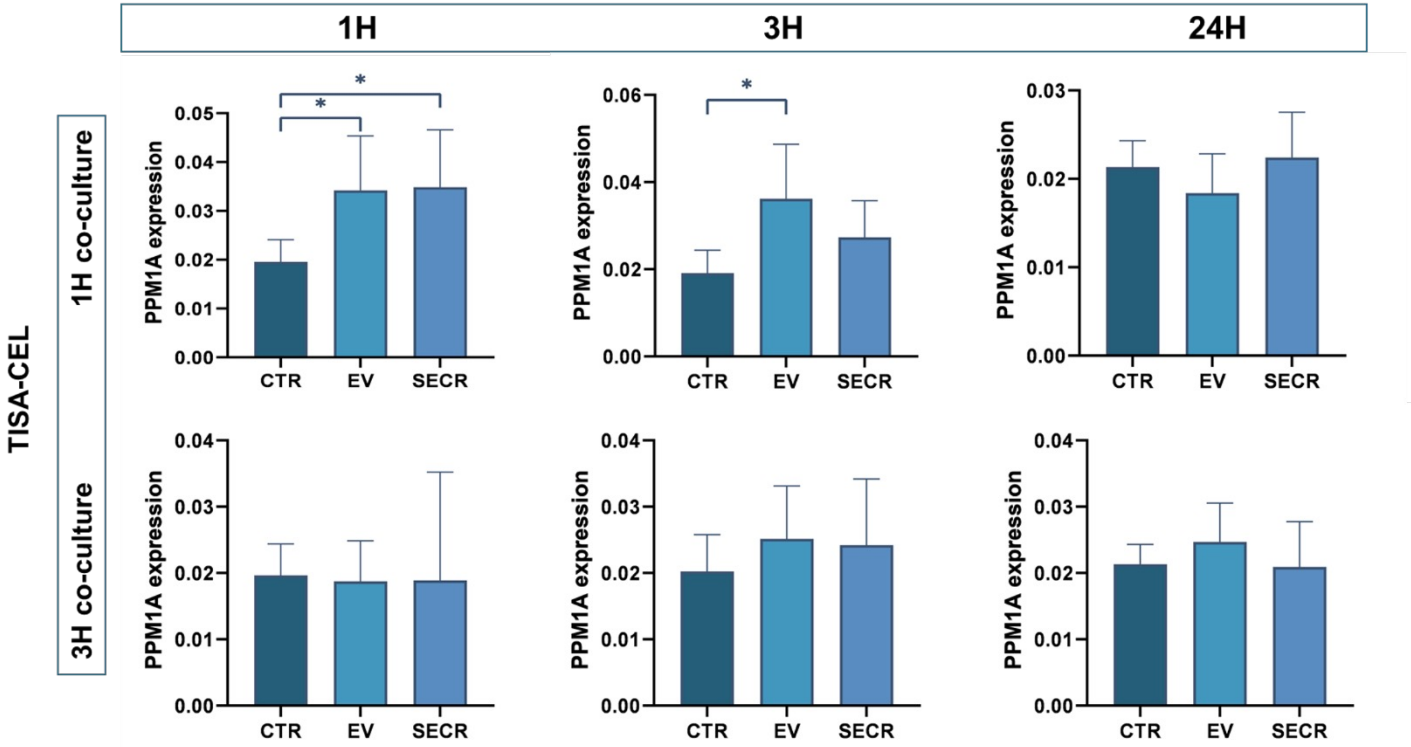
Morphological analysis | Molecular analysis | Cytokines expression

PPM1A expression



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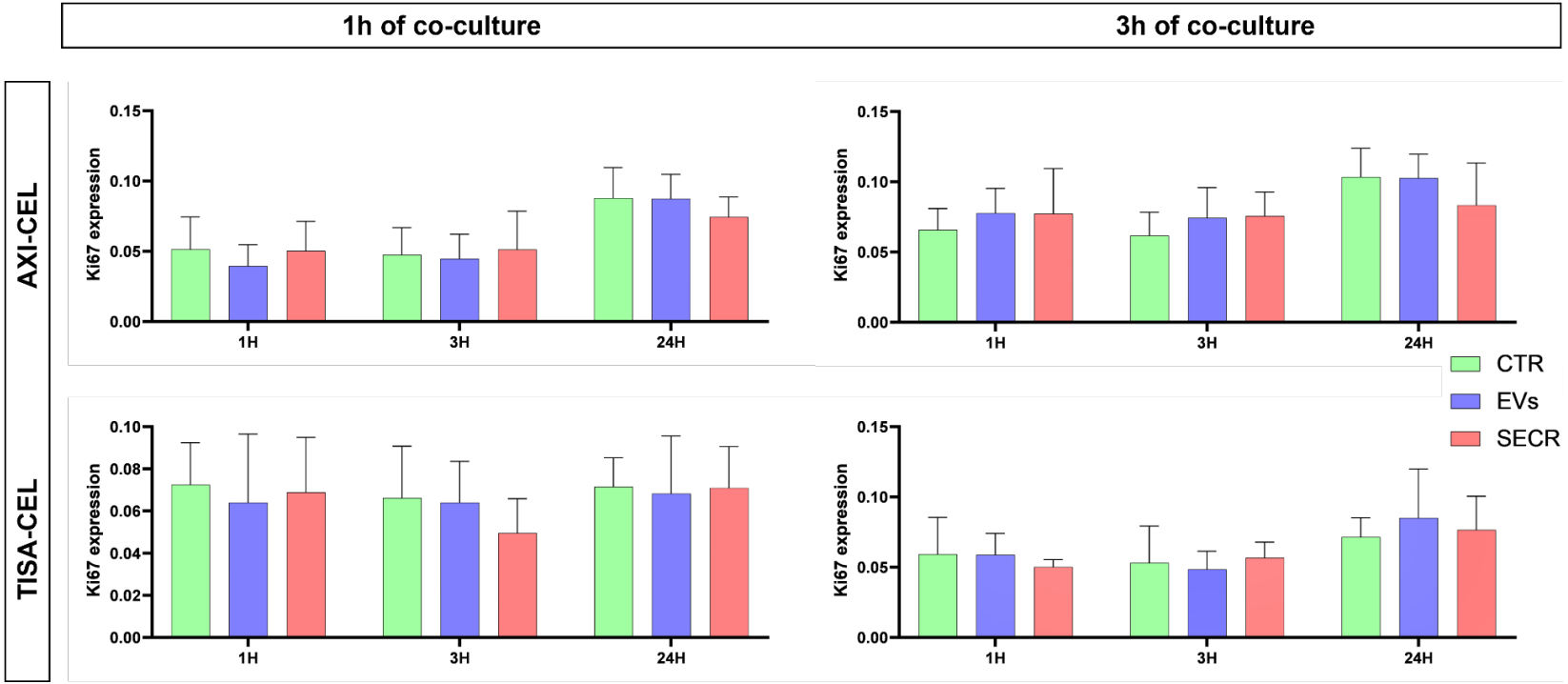
Morphological analysis | Molecular analysis | Cytokines expression

Ki67 expression



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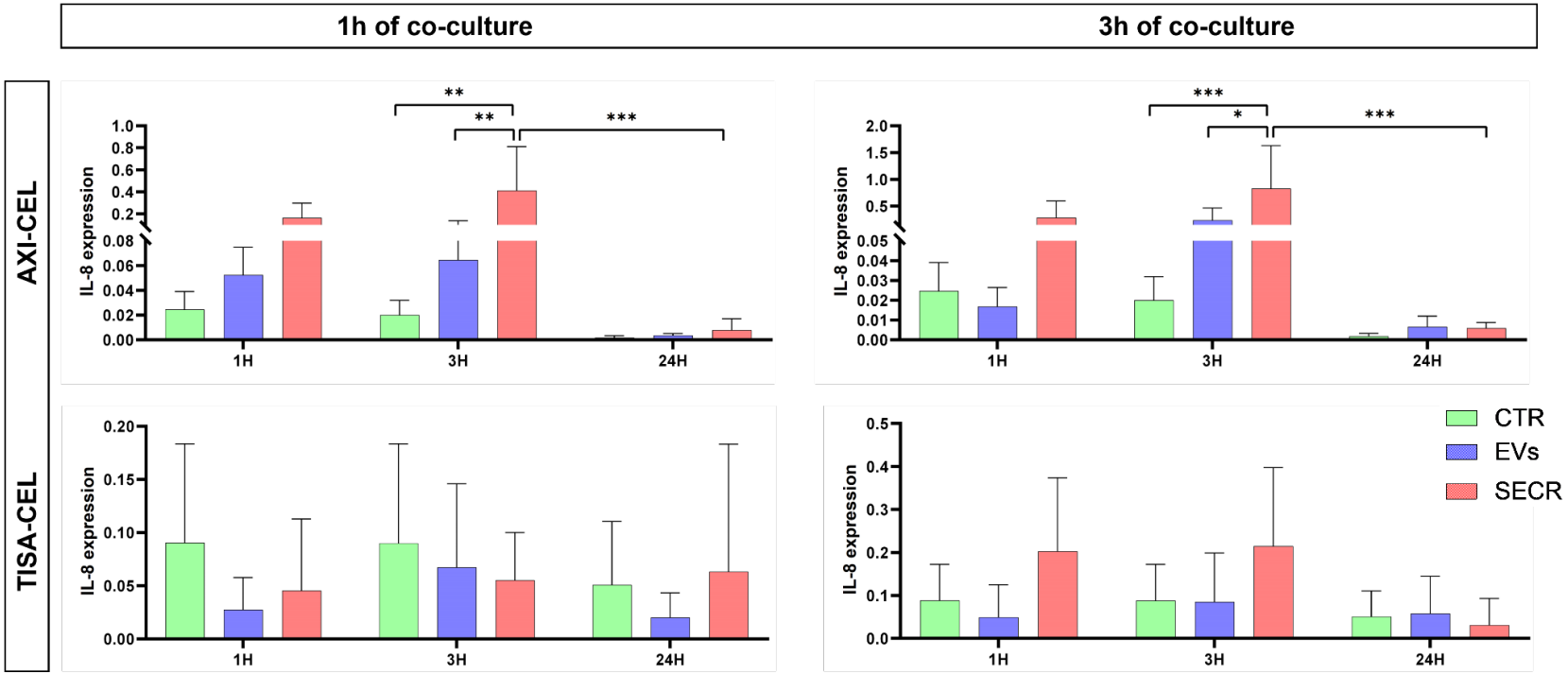
Morphological analysis | Molecular analysis | Cytokines expression

Cytokines expression

11 Cytokines:

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



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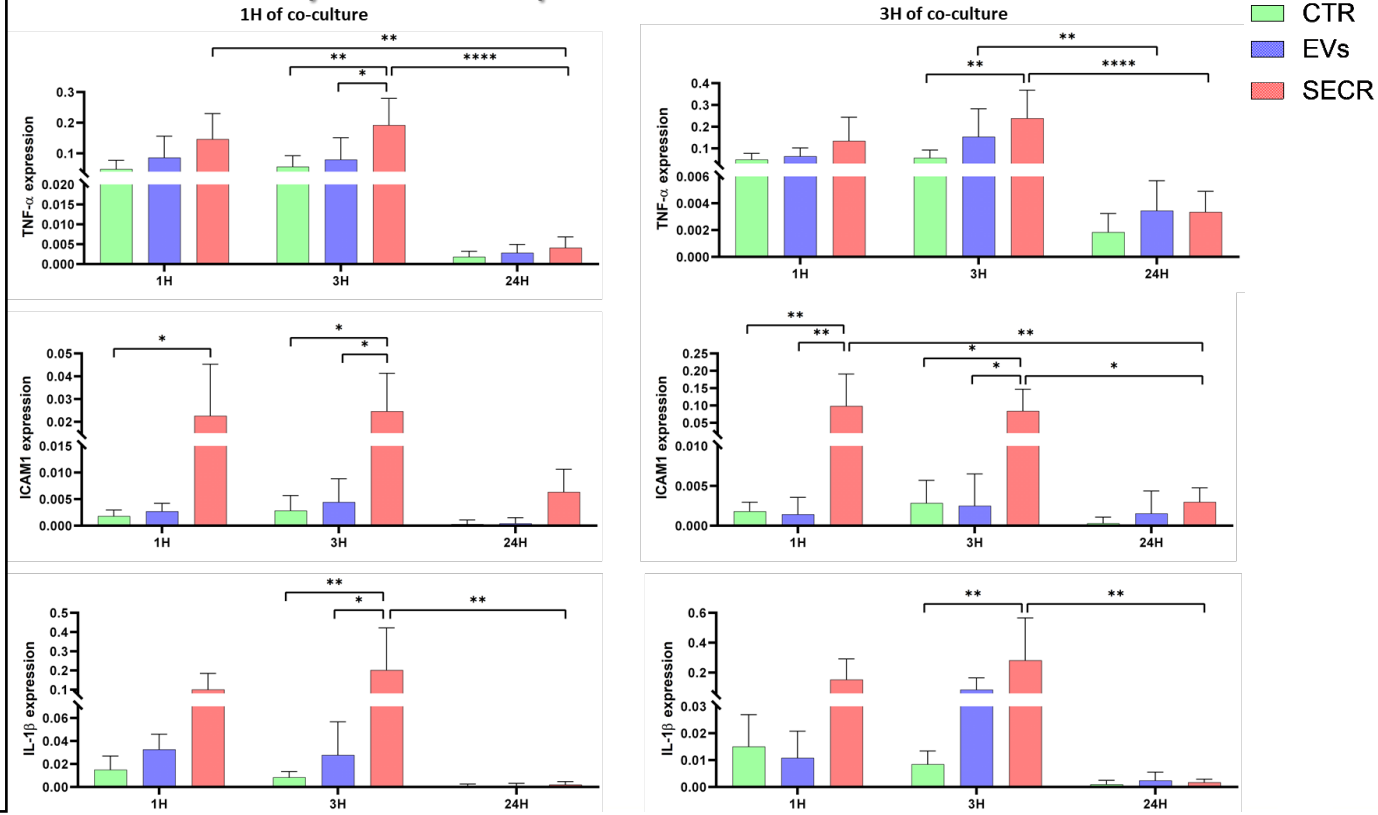
Morphological analysis | Molecular analysis | Cytokines expression

Cytokines expression

AXI-CEL



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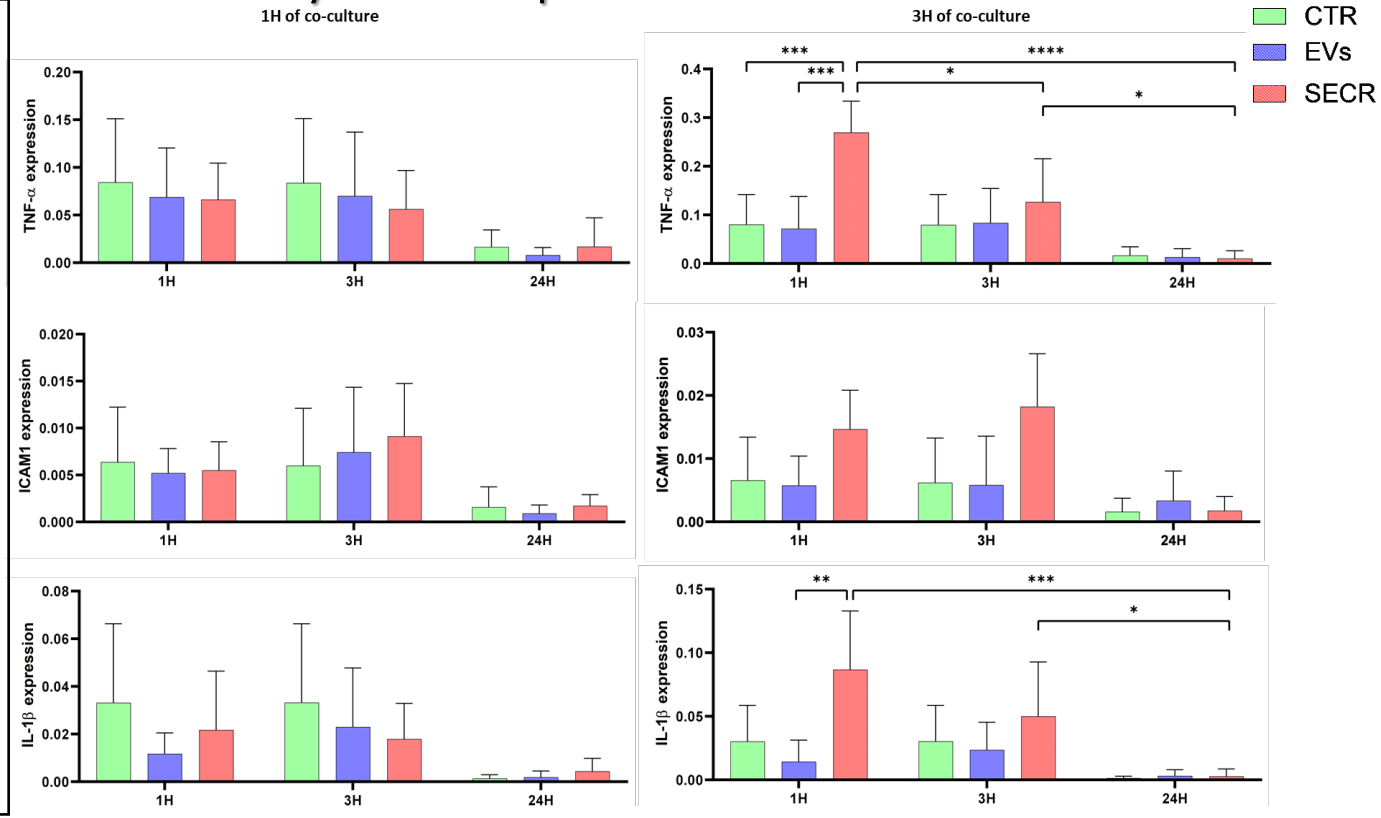
Morphological analysis | Molecular analysis | Cytokines expression

Cytokines expression

TISA-CEL



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Cytokines expression

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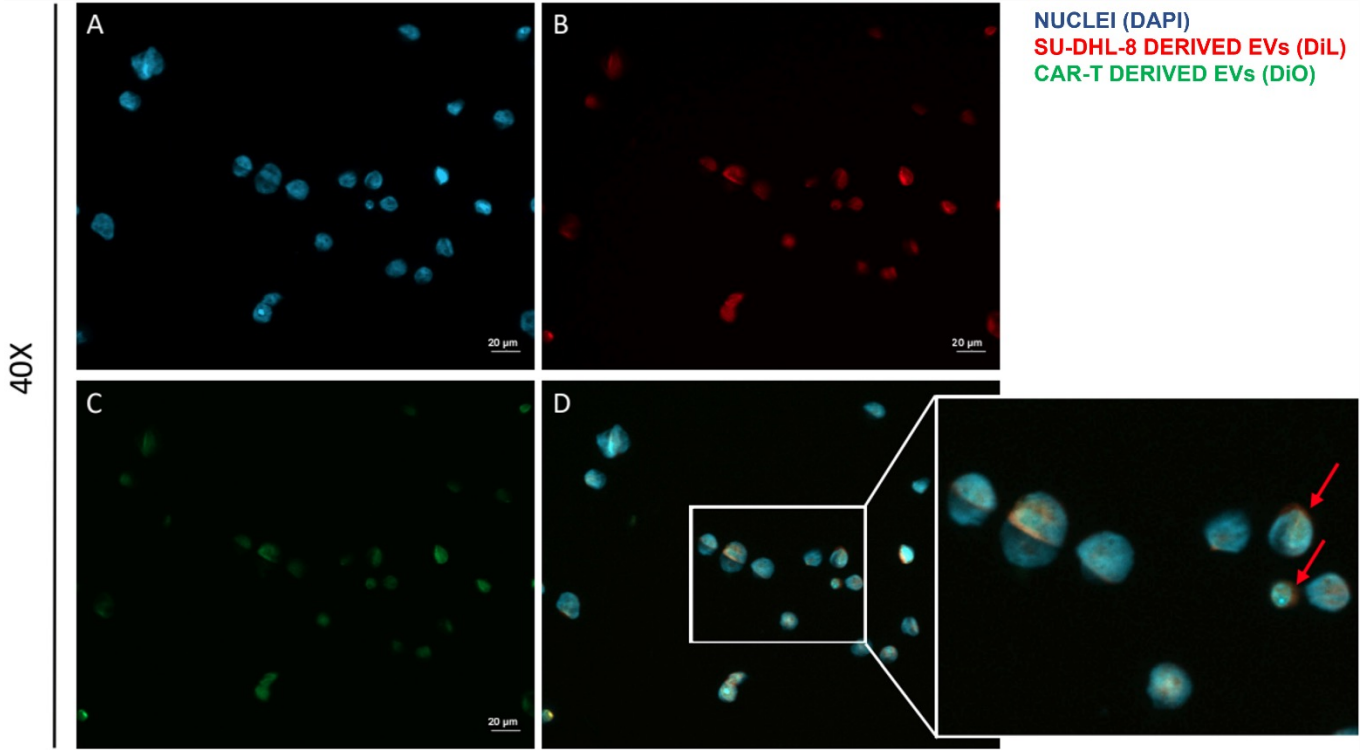
EVs uptake



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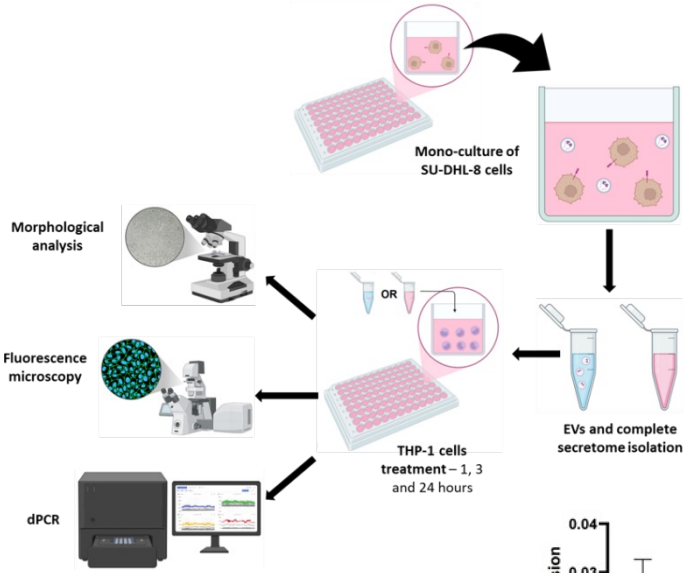
“Negative” controls – Naïve SU-DHL-8



Morphological analysis	Molecular analysis	Cytokines expression
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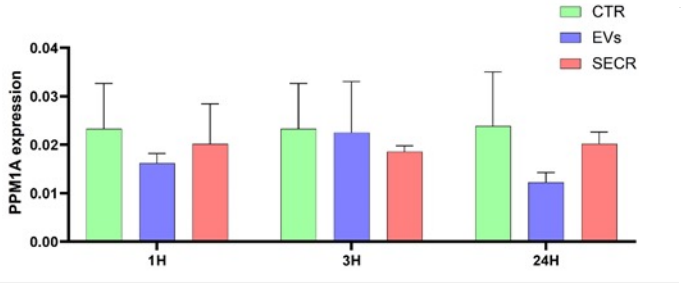

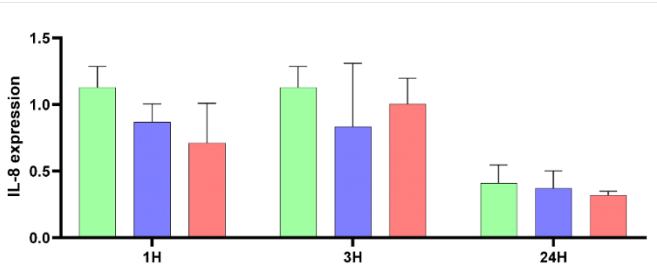

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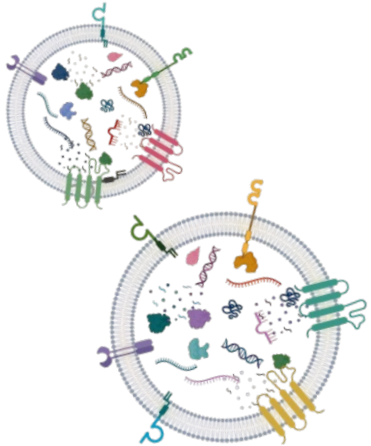
Conclusions

- 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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