XIX CONGRESSO NAZIONALE SIES2026
Firenze | 4-6 marzo 2026 Palazzo degli Affari

XIX Congress of the Italian Society of Experimental Hematology ABSTRACT N. CO47

Stem Cell Transplantation, immunotherapy and cell therapy

EXPLORING THE ROLE AND CELLULAR ORIGIN OF EXTRACELLULAR VESICLES AS BIOMARKERS IN CAR-T CELL THERAPY

G. Lia[1|2], M. Dicataldo[1|2], A. Melis[1|2], F. Ferrando[1|2], I. Dogliotti[2], A. Busca[2], S. Ragaini[1], V. Granero[3], B. Botto[4], M. Clerico[5], M. Novo[4], F. Cavallo[6], L. Giaccone[6], B. Bruno[6]

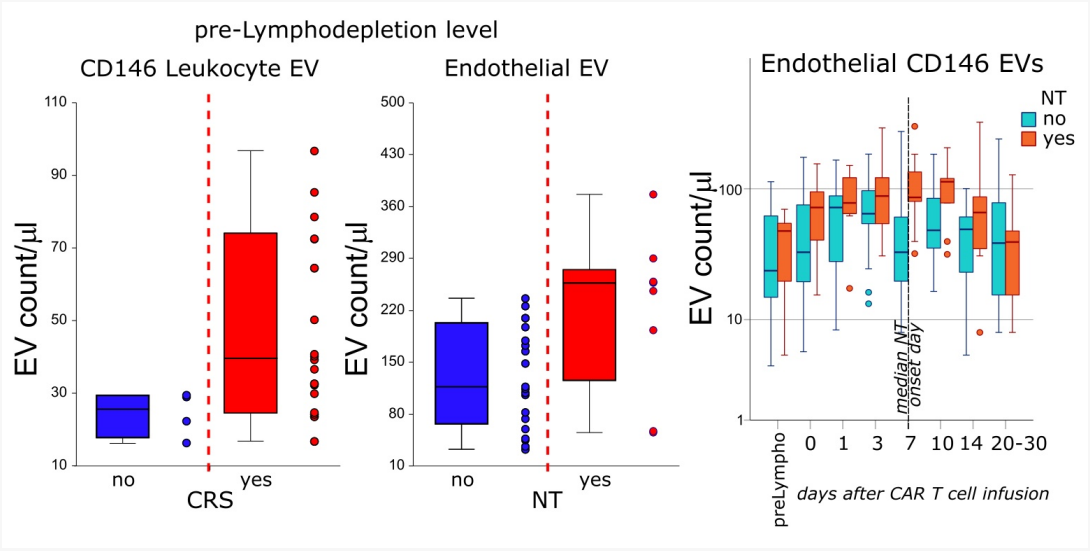
[1]Department of Molecular Biotechnology and Health Sciences, University of Torino; [2]Stem Cell Transplant Program, AOU Città della Salute e della Scienza di Torino;3 Blood Bank, Immunohaematology and Transfusion Medicine Center; A. O. U. Città della Salute e della Scienza di Torino; [4]Division of Hematology, AOU Città della Salute e della Scienza di Torino; [5]Division of Hematology U, A. O. U. Città della Salute e della Scienza di Torino; [6]Division of Hematology U, Department of Molecular Biotechnology and Health Sciences, University of Torino.

Introduction: CAR T-cell therapy represents a major advancement in the treatment of hematologic malignancies. However, common toxicities such as cytokine release syndrome (CRS), CAR-T neurotoxicity (NT), and prolonged cytopenia require careful monitoring. Several CAR T products, including axicabtagene ciloleucel (axi-cel), brexucabtagene autoleucel (brexu-cel), and tisagenlecleucel (tisa-cel), are approved for specific indications. Extracellular vesicles (EV) are emerging as valuable biomarkers reflecting inflammation and cellular stress. This study employed a patented polychromatic flow cytometry (PFC) protocol to investigate the kinetic changes of circulating EV following CAR T-cell therapy.

Methods: Thirty patients undergoing CAR T-cell therapy were enrolled. Most had diffuse large B-cell lymphoma (n=20); others follicular lymphoma (n=6), mantle cell lymphoma (n=3), or high-grade B-cell lymphoma (n=1). Blood samples were collected: before lymphodepletion (pre-Lym), and at 0, 1, 3, 7, 10, 14, and 20–30 days post-infusion. A few microliters of unprocessed blood were incubated in BD Trucount™ tubes for absolute particle count with phalloidin, LCD, and anti-: CD31, CD45, CD41a, and CD146. EV derived from leukocytes (LK), endothelial cells (EC), platelets, and activated (CD146) EC and LK were analyzed using BD FACSLyric™ flow cytometry. Associations between EV count and toxicity risk were assessed using logistic regression models (LRM). Mann–Whitney (U tests) were used for group comparisons. Biomarker predictivity was evaluated using univariate LRM and ROC curve analyses. Statistical analyses were performed with STATA 15 and SPSS 25.

Results: Among the patients, 21 received axi-cel, 6 tisa-cel, and 3 brexu-cel. CRS occurred in 26 patients (grade 1: n=21; grade 2: n=5), while NT was observed in 9 (grade 1: n=7; grade 2: n=2). Higher baseline levels of CD146 LK EV were found in patients who developed CRS, showing strong predictive value (AUROC 0.911). Eleven patients developed prolonged cytopenia and showed higher pre-Lym levels of total EVs, CD146 EV, and LK EV. U tests revealed that elevated pre-Lym CD146 LK EVs and their relative increase compared with total LK EV were associated with CRS (p=0.010 and p=0.033, respectively). LRM confirmed the correlation (Odds ratios [OR] 1.104; p=0.064). Pre-Lym EC EV and CD146 LK EV were also higher in NT patients (p=0.049 and p=0.04), though not confirmed by LRM. Between CAR T infusion and NT onset (median 7 days), CD146 EC EV increased significantly (U test p=0.003; LRM OR 1.012; p=0.044)[Figure].

Conclusions: This study highlights the potential of EV as biomarkers in CAR T-cell therapy. Elevated baseline CD146 LK EV correlated with CRS onset with strong predictive potential. Higher pre-Lym levels of CD146 EV were also associated with prolonged cytopenia. Further studies in larger cohorts are warranted. Future analyses will include EV profiling for immunological, inflammatory, and endothelial damage markers.


pre-Lymphodepletion level
CD146 Leukocyte EV
Endothelial EV
Endothelial CD146 EVs
EV count/µl
110
90
70
50
30
10
no
yes
CRS
500
430
360
290
220
150
80
10
NT
100
1
median NT onset day
preLympho
0
1
3
7
10
14
20-30
days after CAR T cell infusion