Pre-treatment circulating microRNA signatures predict outcomes of anti-CD19 CAR T-cell therapy

Chimeric antigen receptor (CAR) T-cell therapy has been one of the major therapeutic advances of the last decade, substantially improving the prognosis of several relapsed/refractory hematologic malignancies. Anti-CD19 CAR T-cell products, including axicabtagene ciloleucel (axi-cel), tisagenlecleucel (tisa-cel), and lisocabtagene maraleucel (liso-cel) have been approved for relapsed/refractory large B-cell lymphoma (LBCL), primary mediastinal B-cell lymphoma (PMBCL), and transformed follicular lymphoma (tFL).[1,2] Nevertheless, 50-60% of patients eventually relapse and only a minority achieve durable remissions.[3] In addition, CAR T-cell therapy may be complicated by severe toxicities, most notably cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).[4,5] Reliable biomarkers able to predict both efficacy and toxicity before infusion are, therefore, urgently needed. Several pre-infusion variables, including tumor burden, response to prior therapy, and baseline inflammatory status, have been associated with outcome.[6,7] More recently, plasma CAR$^+$ extracellular vesicles measured within 24 hours after infusion were shown to predict ICANS onset.[8] MicroRNA (miRNA) are small non-coding RNA that regulate gene expression post-transcriptionally and are involved in cancer biology, inflammation, and immune regulation.[9] Because circulating miRNA are highly stable in plasma and can be readily quantified, they represent attractive non-invasive biomarkers[10,11] and may reflect both tumor- and immune-related biological processes relevant to CAR T-cell therapy outcomes. Here, we profiled the circulating miRNome at the pre-lymphodepletion (PLD) time point in patients with relapsed/refractory B-cell non-Hodgkin lymphoma (NHL) treated with anti-CD19 CAR T cells, to investigate whether plasma miRNA could predict toxicity and clinical outcome.

We conducted a prospective observational study in 53 patients with relapsed/refractory B-cell NHL treated at the IRCCS Azienda Ospedaliero-Universitaria di Bologna (IRCCS AOUBO), with approved anti-CD19 CAR T-cell products. The study was approved by the institutional ethics committee and was registered at clinicaltrials.gov (identifiers: NCT04892433, NCT05807789). It was carried out in accordance with the principles of the Declaration of Helsinki.

Plasma miRNA profiling was performed by small-RNA sequencing, starting from 5 μL of RNA for library preparation, using QIAseq miRNA Library Kit (Qiagen). Library pools were diluted to 1.5 pM and sequenced using NextSeq 500 platform (Illumina). Differentially expressed miRNA were identified using a log fold change >1. P<0.05 was considered statistically significant (Wald test). Unsupervised hierarchical cluster analysis of normalized intensity values was performed using GeneSpring GX software (Agilent Technologies).

Correlation between variables was assessed using Spearman rank coefficient; P<0.05 was considered statistically significant. Group comparisons were performed using Fisher exact test for categorical variables and the Kruskal-Wallis test for continuous variables. For toxicity analyses, patients were stratified according to CRS (grade <2 *vs*. ≥2) and ICANS (presence *vs*. absence; grade <2 *vs*. ≥2). To reduce dimensionality and account for class imbalance, weighted LASSO regression was applied for variable selection.[12] Variables most frequently selected were included in multivariable logistic regression models together with significant clinical co-variates. Model performance was evaluated by accuracy, sensitivity, specificity, and precision, using leave-one-out cross-validation (LOOCV). For response prediction, logistic regression models were developed for 3- and 12-month overall response rate (ORR). To address the high dimensionality of miRNA data, LASSO-based variable selection and repeated training-validation splits were used to identify the most stable predictors, which were then tested in a final model. Time-to-event outcomes (progression-free survival [PFS], time-to-progression [TTP], overall survival [OS]) were analyzed using Kaplan-Meier estimates and log-rank tests. Cut-off values for stratifying patients according to miR-542-5p levels were determined using *surv_cutpoint* R function.

The study cohort comprised 53 patients: 46 (87%) with LBCL and 7 (13%) with PMBCL. (See *Online Supplementary Table S1* for patients' characteristics). Twenty-eight patients (53%) received axi-cel and 25 (47%) tisa-cel, after a median of two previous lines of therapy. CRS of any grade occurred in 45 patients (85%), including 16 (30%) with grade ≥2 CRS. Patients with grade ≥2 CRS were more frequently female (P=0.043) and had higher PLD C-reactive protein (CRP) levels (P=0.027). ICANS developed in 18 patients (34%), including 14 with grade ≥2 events; 2 patients died from grade 5 ICANS. ICANS was significantly associated with female sex (P=0.022), PMBCL histotype (P=0.004), ECOG performance status ≥1 (P=0.013), and elevated CRP levels at PLD (P=0.006). Median onset was one day for CRS and five days for ICANS. Among 49 evaluable patients, the ORR was 61% at three months and 39% at 12 months. No prophylactic corticosteroids were administered to axi-cel-treated patients.

Small-RNA sequencing identified 573 circulating miRNA detectable in at least one sample. Unsupervised clustering of the PLD miRNome revealed 3 patient clusters (Figure 1).

One cluster was enriched for patients who failed bridging therapy and for those who subsequently developed grade ≥2 CRS or ICANS, suggesting that baseline circulating miRNA patterns reflect disease biology associated with unfavorable outcomes.

To identify biomarkers of CRS, we compared the PLD miRNome of patients who developed grade ≥2 CRS (N=16) with that of patients with grade <2 CRS (N=37). Twenty-five miRNA were differentially expressed (Figure 2A, *Online Supplementary Table S2*). After weighted LASSO selection and multivariable logistic regression including sex and PLD CRP, low miR-136-5p and high miR-4511 were independently associated with grade ≥2 CRS. The resulting model achieved 81% accuracy.

Comparison of patients who developed ICANS (N=18) with those who did not (N=35) identified 33 differentially expressed miRNA (Figure 2B, *Online Supplementary Table S2*). After weighted LASSO selection and multivariable analysis including sex, histology, ECOG, and PLD CRP, high levels of miR-1290, PMBCL histology, and PLD CRP were associated with ICANS. This model predicted ICANS with 87% accuracy. Moreover, when stratified by ICANS grading, patients with

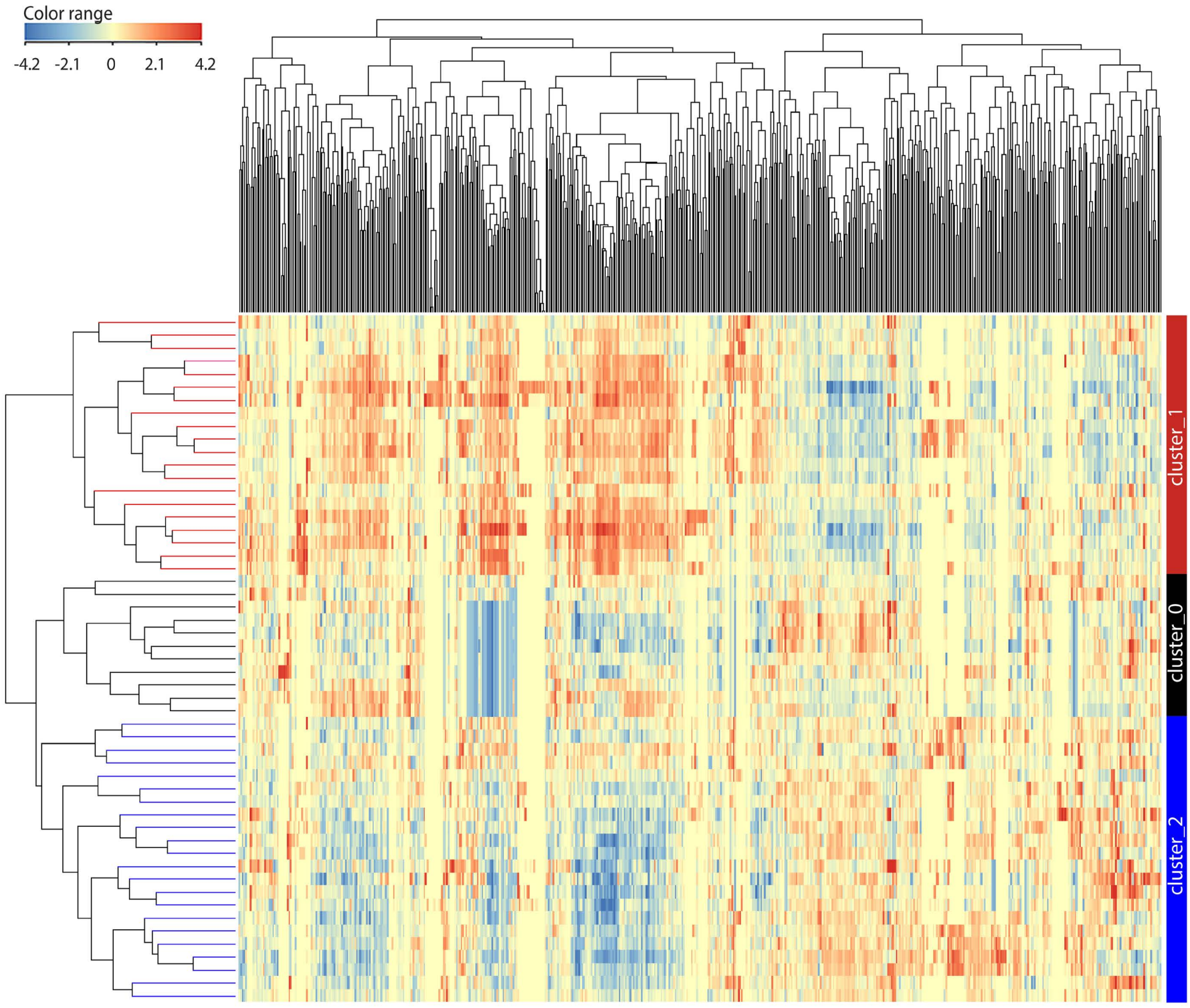


Figure 1. Hierarchical clustering of patients according to the pre-lymphodepletion circulating miRNA profile. Heatmap showing unsupervised hierarchical clustering of 53 patients with relapsed/refractory non-Hodgkin lymphoma (rows) based on circulating miRNA profiles obtained by small-RNA sequencing of pre-lymphodepletion (PLD) plasma samples. A total of 573 circulating miRNA detected across samples were included in the analysis. Clustering was performed using Pearson centered correlation and complete linkage. Colors represent normalized miRNA expression levels scaled to the mean expression across samples, ranging from blue (low expression) to red (high expression). Dendrogram branches identify three major patient clusters: cluster_1 (red), cluster_0 (gray), and cluster_2 (blue).

severe manifestations (grade ≥2, N=14) had significantly higher levels of circulating miR-1290 at PLD compared to patients with grade <2 ICANS symptoms (N=39) (median levels: 2.3 *vs*. 0; Kruskal-Wallis, *P*<0.0007).

We next investigated whether PLD miRNA could predict response at three and 12 months. These time points were selected to capture both initial and sustained treatment responses. Weighted LASSO was applied to miRNA and clinical variables. A 3-variable model including CAR T-cell product, let-7d-3p, and miR-542-5p, predicted 3-month response with 75% accuracy (Table 1). A second model including miR-143-3p, miR-542-3p, and miR-542-5p predicted 12-month response with 73% accuracy (Table 1). Using an optimized miR-542-5p cut-off value of 5.5, patients with elevated PLD miR-542-5p levels showed inferior PFS, TTP, and OS (*Online Supplementary Figure S1*), and these levels were positively correlated with lactate dehydrogenase (r=0.37, *P*=0.006), circulating cell-free DNA (r=0.29, *P*=0.04), and polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) (r=0.34, *P*=0.08).

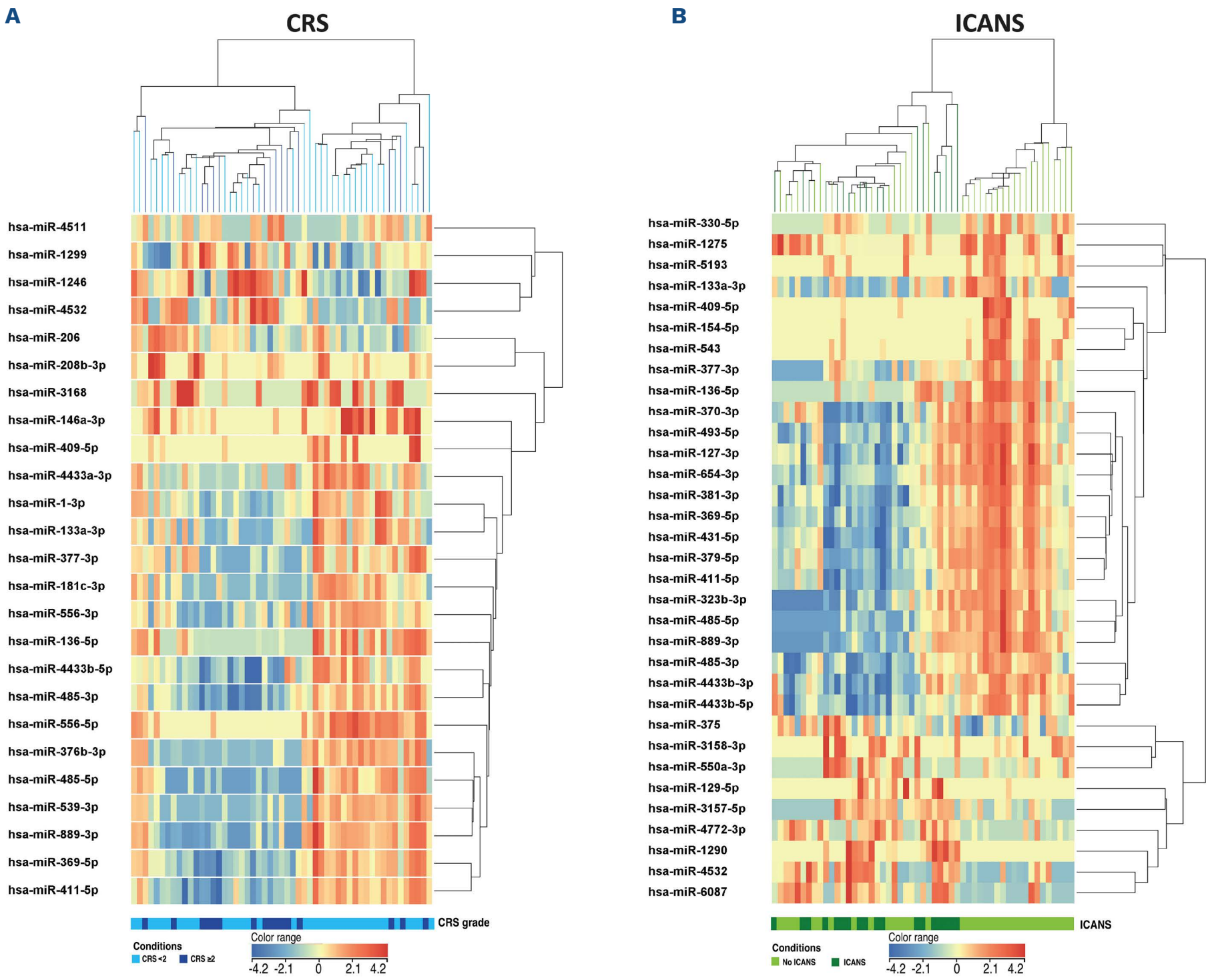


Figure 2. Differentially expressed circulating miRNA associated with toxicity after chimeric antigen receptor T-cell therapy. (A) Heatmap of the 25 differentially expressed (DE) miRNA (rows) identified by comparing pre-lymphodepletion (PLD) circulating miRNA profiles of patients who developed grade ≥2 cytokine release syndrome (CRS) (N=16) with those with grade 0-1 CRS (N=37). Columns represent individual chimeric antigen receptor T-cell (CAR T)-treated patients. Expression values are normalized and mean-centered across samples; blue indicates lower and red higher expression levels. Patients with grade ≥2 CRS are indicated in dark blue; patients with grade <2 CRS are shown in light blue. (B) Heatmap of the 33 DE miRNA (rows) identified by comparing PLD circulating miRNA profiles of patients who developed immune effector cell-associated neurotoxicity syndrome (ICANS) (N=18) with those who did not (N=35). Expression values are normalized and mean-centered across samples; blue indicates lower and red higher expression levels. Patients with ICANS are indicated in dark green; patients without ICANS are shown in light green.

Table 1. Response predictors.

3-month follow-up prediction			
Predictors	**Co-efficient**	***P***	**Accuracy**
Product	-591.077	0.00473	75%
hsa-let-7d-3p	-0.03498	0.00616	
hsa-miR-542-5p	0.72076	0.01632	
12-month follow-up prediction			
Predictors	**Co-efficient**	***P***	**Accuracy**
hsa-miR-143-3p	0.0009494	0.05869	73%
hsa-miR-542-3p	0.0201877	0.25701	
hsa-miR-542-5p	0.3405031	0.04141	

To our knowledge, this is the first comprehensive analysis of circulating plasma miRNA in patients receiving anti-CD19 CAR T-cell therapy. Our findings show that PLD miRNA profiles are associated with both treatment-related toxicity and therapeutic efficacy. Specifically, low miR-136-5p and high miR-4511 identified patients at increased risk of grade ≥2 CRS. We also identified miR-1290 as a predictor of ICANS together with CRP and PMBCL histology, supporting the role of inflammatory and endothelial pathways in neurotoxicity. These findings further suggest a broader relevance of circulating miRNA signatures across CAR T-cell–related toxicities, including hematologic toxicities, although this requires further investigation.

The most clinically relevant observation of our study is the identification of miRNA-based models able to predict response before lymphodepletion, and thus before CAR T-cell infusion. In both the 3- and 12-month models, miR-542-5p emerged as the most consistent adverse biomarker. Elevated PLD miR-542-5p identified patients with inferior response and survival and negative prognostic markers, including PMN-MDSC, circulating cell-free DNA, and LDH, suggesting that this circulating miRNA captures both tumor burden and myeloid-driven immunosuppression,[13-15] both of which critically affect CAR T-cell expansion and persistence.

Overall, our data suggest that circulating miRNA may integrate biological information on inflammation, endothelial stress, tumor burden, and immune suppression more effectively than individual clinical variables, thus supporting their potential clinical utility. These findings support the potential use of circulating miRNA as non-invasive biomarkers for patient stratification prior to CAR T-cell therapy. In addition, these signatures may provide insights into the biological mechanisms underlying treatment response and toxicity, potentially informing future therapeutic strategies.

The main limitations of this study are the relatively small sample size and the lack of external validation. Therefore, our findings should be considered exploratory and hypothesis-generating, and require validation in larger, independent cohorts. In addition, the implementation of circulating miRNA-based biomarkers across different centers may be limited by technical challenges, including assay standardization, reproducibility, and inter-laboratory variability, which will require further harmonization before clinical use. Larger prospective studies will be required to validate these findings and determine the clinical utility of circulating miRNA biomarkers in patients undergoing anti-CD19 CAR T-cell therapy.

Authors

Maria Naddeo,[1*] Roberta Roncarati,[2*] Francesco De Felice,[1,3*] Elena Zuin,[4] Ilaria Pace,[3] Francesco Iannotta,[1] Noemi Laprovitera,[1] Beatrice Fontana,[3] Giorgio Durante,[1] Irene Salamon,[1] Gianluca Storci,[1] Serena De Matteis,[1] Francesca Ricci,[1] Salvatore Nicola Bertuccio,[1] Daria Messelodi,[1] Margherita Ursi,[1,3] Marcello Roberto,[1,3] Francesco Barbato,[1,3] Federica Ardizzoia,[1,3] Enrica Tomassini,[1] Barbara Sinigaglia,[1] Luca Zazzeroni,[1] Elisa Dan,[1] Renee Concetta Duardo,[1] Francesca Vaglio,[1] Marta Tassoni,[1,3] Cinzia Pellegrini,[1] Marianna Gentilini,[1] Enrico Maffini,[1] Beatrice Casadei,[1] Gabriele Sales,[4] Pier Luigi Zinzani,[1,3] Massimiliano Bonafé,[1,3] Manuela Ferracin[1,3#] and Francesca Bonifazi[1#]

[1]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna; [2]Istituto di Genetica Molecolare "Luigi Luca Cavalli-Sforza" – Consiglio Nazionale delle Ricerche (CNR), Bologna; [3]Department of Medical and Surgical Sciences (DIMEC) University of Bologna, Bologna and [4]Department of Biology, University of Padova, Padova, Italy

MN, RR and FDeF contributed equally as first authors.

#MF and FBo contributed equally as senior authors.

Correspondence:
M. FERRACIN - manuela.ferracin@unibo.it
M. BONAFÈ - massimiliano.bonafe@unibo.it

https://doi.org/10.3324/haematol.2025.300294

Received: December 24, 2025.
Accepted: April 16, 2026.
Early view: April 23, 2026.

©2026 Ferrata Storti Foundation
Published under a CC BY-NC license

Disclosures

PLZ reports scientific advisory boards for Secura Bio BIO, Celltrion, Gilead, Janssen-Cilag, BS, Servier, Sandoz, MSD, TG Therap., Takeda, Roche, EUSA Pharma, Kiowa Kirin, Novartis, ADC Therap., Incyte, and Beigene; consultancy for EUSA Pharma, MSD, and Novartis; and speaker's bureau for Celltrion, Gilead, Janssen-Cilag, BMS, Servier, MSD, TG Therap., Takeda, Roche, EUSA Pharma, Kiowa Kirin, Novartis, Incyte, and Beigene. FB reports scientific advisory boards and speaker fees for Neovii, EOVII, Novartis, OVARTIS, Kite, Gilead,

Pfizer, Celgene, and MSD. All the other authors have no conflicts of interest to disclose.

Contributions

MB, MF and FBo are responsible for the study concept. RR, NL, BF and GD are responsible for the generation of smallRNA sequencing data. MN, FDF, EZ, IP, FI and GSa are responsible for the analysis and interpretation of smallRNA sequencing data. FDF, FI, IS, GSt, MU, MR, FBa, FA, BS, LZ, ED, CP, MG, EM and BC collected the clinical samples, and compiled the clinical information and omics sequencing data. MN, SDM, FR, FV and MT are responsible for the FACS analysis and cytokine dosage. SNB, DM, ET and RCD gave technical support. MN and FDeF wrote the original draft. MN, FDF, RR, MB, MF and FBo wrote, reviewed and edited the manuscript. PLZ, MB, MF and FBo supervised the study.

Acknowledgments

The authors thank AIL Bologna ODV, the Italian Association for Research on Leukemia, Lymphoma and Myeloma, for the support of the Laboratory of Immunobiology of Transplant and Cellular Therapies, IRCCS AOU di Bologna, Bologna, Italy, led by FBo.

Funding

The work reported in this publication was funded by NextGenerationEU through the Italian Ministry of University and Research under PNRR - M4C2-I1.3 Project HEAL ITALIA - Health Extended Alliance for Innovative Therapies, Advanced Lab-research, and Integrated Approaches of Precision Medicine (PE0000019, to FBo), CUP F33C22000500006. The views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them.

Data-sharing statement

The sequencing data presented in this study have been submitted to the Sequence Read Archive (SRA) online repository. The samples can be retrieved at https://www.ncbi.nlm.nih.gov/sra using the accession numbers SAMN39307284-SAMN39307344.

References

1. Neelapu SS, Dickinson M, Munoz J, et al. Axicabtagene ciloleucel as first-line therapy in high-risk large B-cell lymphoma: the phase 2 ZUMA-12 trial. Nat Med. 2022;28(4):735-742.
2. Schuster SJ, Tam CS, Borchmann P, et al. Long-term clinical outcomes of tisagenlecleucel in patients with relapsed or refractory aggressive B-cell lymphomas (JULIET): a multicentre, open-label, single-arm, phase 2 study. Lancet Oncol. 2021;22(10):1403-1415.
3. Ruella M, Korell F, Porazzi P, Maus MV. Mechanisms of resistance to chimeric antigen receptor-T cells in haematological malignancies. Nat Rev Drug Discov. 2023;22(12):976-995.
4. Penack O, Koenecke C. Complications after CD19+ CAR T-Cell therapy. Cancers (Basel). 2020;12(11):3445.
5. Karschnia P, Jordan JT, Forst DA, et al. Clinical presentation, management, and biomarkers of neurotoxicity after adoptive immunotherapy with CAR T cells. Blood. 2019;133(20):2212-2221.
6. Vercellino L, Di Blasi R, Kanoun S, et al. Predictive factors of early progression after CAR T-cell therapy in relapsed/refractory diffuse large B-cell lymphoma. Blood Adv. 2020;4(22):5607-5615.
7. Nastoupil LJ, Jain MD, Feng L, et al. Standard-of-care axicabtagene ciloleucel for relapsed or refractory large B-cell lymphoma: results from the US Lymphoma CAR T Consortium. J Clin Oncol. 2020;38(27):3119-3128.
8. Storci G, De Felice F, Ricci F, et al. CAR+ extracellular vesicles predict ICANS in patients with B cell lymphomas treated with CD19-directed CAR T cells. J Clin Invest. 2024;134(14):e173096.
9. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215-233.
10. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008;105(30):10513-10518.
11. Ferracin M, Negrini M. Micromarkers 2.0: an update on the role of microRNAs in cancer diagnosis and prognosis. Expert Rev Mol Diagn. 2015;15(10):1369-1381.
12. Tibshirani R. Regression shrinkage and selection via the Lasso. J R Stat Soc Series B Stat. 1996;58(1):267-288.
13. Jain MD, Zhao H, Wang X, et al. Tumor interferon signaling and suppressive myeloid cells are associated with CAR T-cell failure in large B-cell lymphoma. Blood. 2021;137(19):2621-2633.
14. Jaeger U, Bishop MR, Salles G, et al. Myc expression and tumor-infiltrating T cells are associated with response in patients (Pts) with relapsed/refractory diffuse large B-cell lymphoma (r/r DLBCL) treated with tisagenlecleucel in the Juliet Trial. Blood. 2020;136(Suppl 1):48-49.
15. Tumino N, Weber G, Besi F, et al. Polymorphonuclear myeloid-derived suppressor cells impair the anti-tumor efficacy of GD2. CAR T-cells in patients with neuroblastoma. J Hematol Oncol. 2021;14(1):191.