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Elevated CD10⁻ neutrophils correlate with non-response and poor prognosis of CD19 CAR T-cell therapy for B-cell acute lymphoblastic leukemia

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Abstract

Background The primary challenges in CD19-specific chimeric antigen receptor T-cell (CD19 CAR T) therapy for patients with refractory/relapsed B-cell acute lymphoblastic leukemia (r/r B-ALL) are non-response and relapse; it is urgent to reveal these mechanisms. Neutrophils play a critical role in the immunosuppressive tumor microenvironment (TME), which can hinder CAR T efficacy. Our previous research identified a subset of immunosuppressive neutrophils with a special phenotype ($CD14^{-}CD10^{-}CD45^{-}HLA\text{-}DR^{-}SSC^{++}$, termed $CD10^{-}$ neuts), which suppress T cell function. Therefore, we speculate that $CD10^{-}$ neuts may also influence CAR T efficacy, and this study aims to clinically validate this hypothesis.

Methods We enrolled 44 patients with r/r B-ALL undergoing CD19 CAR T therapy and 47 healthy controls (HCs). Peripheral blood samples were obtained prior to CAR T infusion to detect $CD10^{-}$ neuts levels by flow cytometry. Key parameters included the percentage of $CD10^{-}$ neuts in neutrophils ($CD10^{-}$ neuts/neutrophils), in all nucleated cells ($CD10^{-}$ neuts/nucleated cells), and the absolute count of $CD10^{-}$ neuts. We analyzed the correlations between these indicators and therapeutic response, relapse-free survival (RFS), overall survival (OS), and CAR T cell persistence time.

Results $CD10^{-}$ neuts levels were significantly elevated in patients with r/r B-ALL compared to HCs. Additionally, non-responding patients exhibited higher $CD10^{-}$ neuts levels than those in remission. Specifically, $CD10^{-}$ neuts/neutrophils, $CD10^{-}$ neuts/nucleated cells, and absolute $CD10^{-}$ neuts count were 64.44% vs. 25.43% ($p=0.004$), 28.61% vs. 9.81% ($p=0.018$), and 766.1/μL vs. 152.9/μL ($p=0.04$), respectively. Among these indices, only $CD10^{-}$ neuts/neutrophils emerged as an independent risk factor for CAR T response (OR = 19.8, $p=0.013$), relapse (HR = 4.704, $p=0.004$), and survival (HR = 6.417, $p=0.001$). Patients with $CD10^{-}$ neuts/neutrophils ≥ 21.57% demonstrated significantly shorter RFS and OS compared to those with lower levels ($p=0.001$; $p=0.0002$). Furthermore, $CD10^{-}$ neuts/neutrophils were negatively correlated with the persistence time of CAR T cells.

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Conclusions As one of the key factors in the TME, abnormally elevated $CD10^-$ neuts correlate with CAR T therapy resistance. Targeting these neutrophils could enhance the effectiveness of CAR T treatment.

Keywords $CD10^-$ neutrophils, CAR T, B-cell acute lymphocytic leukemia, Response, Prognosis

Background

Chimeric antigen receptor T-cell (CAR T) therapy, with response rates ranging from 62 to 93% in children and young adults with refractory/relapsed B-cell acute lymphoblastic leukemia (r/r B-ALL), has become a mainstream treatment for hematologic malignancies [1–3]. Nonetheless, long-term remission is not assured, as fewer than 30% of patients sustain remission beyond 3 years [4–6]. Our previous research indicated an 80.9% remission rate in 47 patients with r/r B-ALL treated with CD19 CAR T therapy, with high levels of regulatory T cells (Tregs) and active extramedullary disease (EMD) identified as independent risk factors for poor prognosis [3, 7]. Additionally, some scholars consider that target antigen escape, inadequate CAR T cell persistence, and the complex tumor microenvironment (TME) contribute to adverse outcomes [7–11]. However, the role of neutrophils in the TME remains insufficiently explored and infrequently reported.

Neutrophils, the most prevalent circulating leukocytes in human blood, play a pivotal role in the TME [12–14]. Due to their high heterogeneity, neutrophils exhibit both tumor-promoting and anti-tumor functions [15, 16]. They can directly kill cancer cells and enhance anti-tumor immunity. However, other studies indicate that neutrophils may also promote cancer cell proliferation, metastasis, and angiogenesis and suppress anti-tumor T cell responses. Recently, we identified a special subset of neutrophils in patients with non-Hodgkin lymphoma (NHL), which has a distinctive immune phenotype with $CD14^-CD10^-CD45^-HLA\text{-}DR^-SSC^{++}$ (termed $CD10^-$ neuts). These neutrophils exhibit MDSC-like biological and functional characteristics, including an immature morphology and the ability to suppress T cell function, and are associated with disease progression and poor prognosis [17, 18]. Based on these observations, it is hypothesized that $CD10^-$ neuts may hinder the efficacy of CAR T therapy. This study is the first to investigate the impact of $CD10^-$ neuts on the clinical outcomes of CAR T therapy.

To investigate this, the peripheral blood (PB) levels of $CD10^-$ neuts were assessed in patients with r/r B-ALL prior to CAR T-cell infusion to examine their association with CAR T treatment response, prognosis, and CAR T cell persistence time. Three indicators— $CD10^-$ neuts/neutrophils, $CD10^-$ neuts/nucleated cells, and the absolute count of $CD10^-$ neuts—were used to reflect $CD10^-$ neuts levels. Elevated levels of $CD10^-$ neuts were detected in patients with r/r B-ALL, and high $CD10^-$ neuts/neutrophils were correlated with poor CAR T cell treatment response, adverse prognosis, and diminished CAR T cell persistence time.

Methods

Study design

This prospective observational pilot study was an extension of the previous phase II clinical trial (ClinicalTrials.gov number: NCT02735291). Approval for the study protocol was granted by the institutional review board at the Second Affiliated Hospital of Anhui Medical University (SHAMU). All participants provided written informed consent in accordance with the principles of the Declaration of Helsinki prior to enrollment.

Participants

A total of 47 patients with r/r B-ALL underwent CD19 CAR T-cell infusion, with participant enrollment and screening detailed in a previous report [3]. However, three patients were not assessed for $CD10^-$ neuts prior to CAR T-cell infusion, resulting in 44 patients being included in this study. A control group of 47 age-matched healthy individuals (HCs) was also included.

Detection of $CD10^-$ neuts

Sample preparation

PB samples (2–5 ml) were collected from patients with r/r B-ALL on the same day as CAR T treatment, anticoagulated with EDTA, and analyzed for $CD10^-$ neuts within 4 h. The samples were incubated with FITC-conjugated CD14-specific monoclonal antibodies (mAbs), ECD-conjugated HLA-DR mAbs, APC-conjugated CD33 mAbs, PE-conjugated CD10 mAbs, and PC7-conjugated CD45 mAbs, along with their appropriate isotype controls. After a 15-min incubation in the dark at room temperature, red blood cells were lysed with ammonium chloride solution, and the samples were analyzed immediately using a flow cytometer (FC-500) with CXP 2.0 software (Beckman Coulter, USA).

The aforementioned mAbs specific to human surface antigens were sourced from Beckman Coulter Immunotech (Miami, FL, USA), including FITC-labeled CD14 (clone 116), PE-labeled HLA-DR (clone B8.12.2), APC-labeled anti-CD33 (clone 13B8.2), PC7-labeled CD45 (clone J.33), and the respective isotype controls.

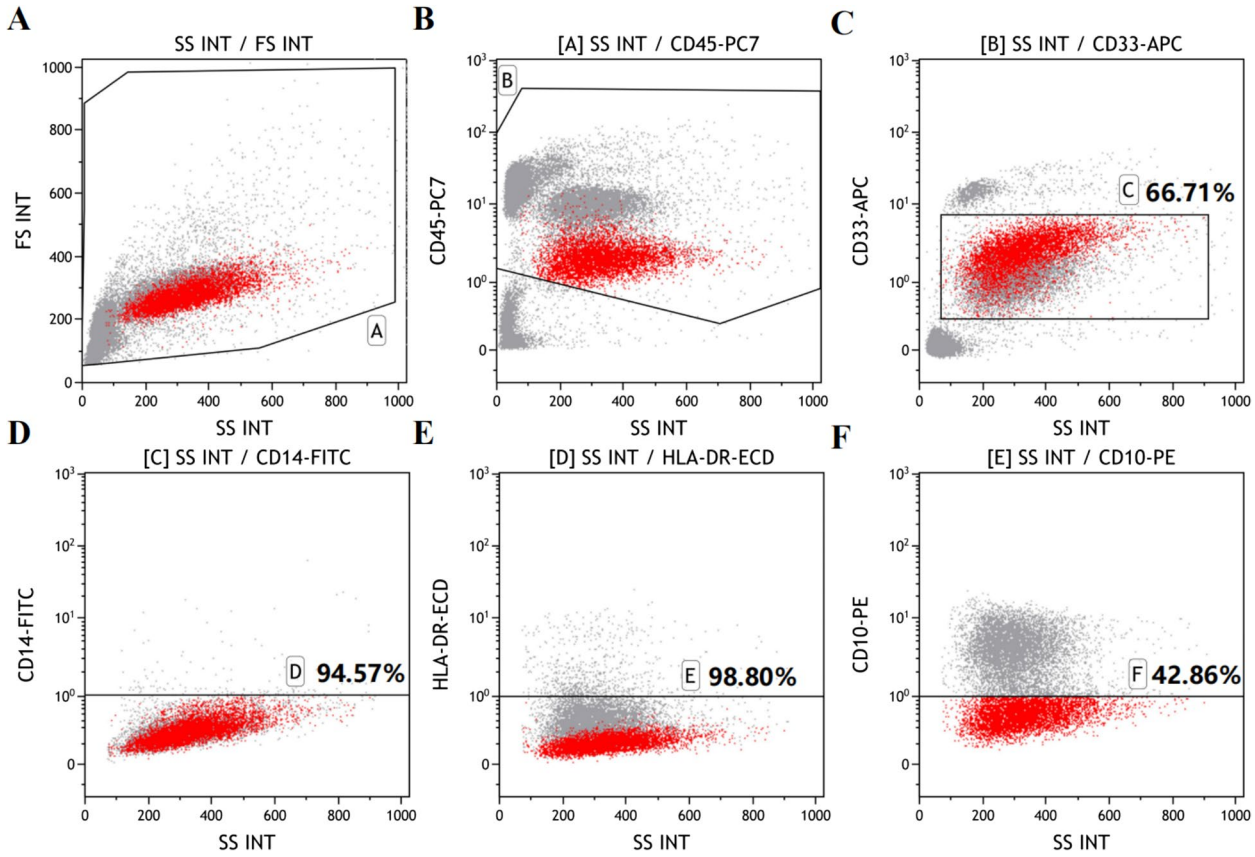


Fig. 1 Detection of $CD10^-$ neuts in peripheral blood by flow cytometry. $CD10^-$ neuts were defined as $CD10^-CD14^-HLA\text{-}DR^-CD33^+CD45^+SSC^+$ cells. The analysis method is outlined as follows: **A** FSC and SSC were used to exclude residual red blood cells and debris. **B** All nucleated cells (Gate B) were selected from Gate A based on SSC and CD45 expression. **C** $CD33^+CD45^+SSC^+$ cells (Gate C) were isolated from Gate B. **D** $CD14^-CD33^+CD45^+SSC^+$ cells (Gate D) were selected from Gate C. **E** $CD14^-CD33^+CD45^+HLA\text{-}DR^-$ SSC^+ cells (Gate E) were selected from Gate D. **F** $CD10^-CD14^-HLA\text{-}DR^-CD33^+CD45^+SSC^+$ cells, representing $CD10^-$ neuts, were selected from Gate E. The percentage of $CD10^-CD14^-HLA\text{-}DR^-CD33^+CD45^+SSC^+$ cells in all neutrophils (Gate C) represented $CD10^-$ neuts/neutrophils; the percentage of $CD10^-CD14^-HLA\text{-}DR^-CD33^+CD45^+SSC^+$ cells in nucleated cells (Gate B) represented $CD10^-$ neuts/nucleated cells; the absolute count of $CD10^-$ neuts was calculated as $CD10^-$ neuts/nucleated cells × WBC count from the same-day routine blood tests. WBC, white blood cells

Flow cytometry detection and analysis

The $CD10/CD14/HLA\text{-}DR/CD33/CD45$ antibody panel, combined with forward scatter (FSC) and side scatter (SSC), and multi-parameter flow analysis was used to identify the cell population with the $CD10^-CD14^-HLA\text{-}DR^-CD33^+CD45^+SSC^+$ phenotype as $CD10^-$ neuts. Detailed methods for detection and analysis are described in Fig. 1A–F.

Clinical data

Demographic information and relevant clinical history were collected, including age, sex, diagnosis data, chemotherapy cycles, cytogenetic or molecular abnormalities (Additional file 1: Table S1), and history of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Additionally, white blood cell (WBC), neutrophil, and lymphocyte counts were determined prior to CAR T-cell infusion. Tregs were assessed by flow cytometry as described in our previous research [3].

Detection of persistence time of CD19 CAR T cells in vivo

Expansion and persistence of CD19 CAR T cells in PB samples were measured using real-time quantitative reverse transcription PCR (qRT-PCR), as previously described by our research group [3].

Therapeutic evaluation and follow-up of patients

Bone marrow aspiration and related examinations were performed between day 14 and day 28 post-infusion to evaluate response and remission status. Patients were categorized according to their treatment response as follows: complete remission or complete remission with incomplete hematologic recovery (CR/CRi), and non-response (NR). The criteria for efficacy assessment were

outlined in our recent study [3]. Relapse-free survival (RFS) was defined as the duration from remission to relapse or the last observation. Overall survival (OS) was calculated from the date of CD19 CAR T-cell infusion to the date of death or the last observation.

Statistical analysis

All statistical analyses were performed using SPSS software (version 22.0). Graphs were created with GraphPad Prism (version 8.0) and R (version 4.0.3). The Kolmogorov–Smirnov test assessed normality. Quantitative data are presented as medians with interquartile ranges [M (Q25, Q75)]. Non-normally distributed or heterogeneous data were analyzed with the nonparametric Mann–Whitney or Kruskal–Wallis tests. Categorical variables are presented as counts and percentages (n (%)), and comparisons were made using the chi-squared test or Fisher's exact test. Cutoff values for continuous variables associated with treatment response, recurrence, and mortality were established through receiver operating characteristic (ROC) analysis and the area under the curve (AUC). A cutoff value was selected if AUC ≥ 0.65 and $p < 0.05$; otherwise, the median value was applied. OS and RFS were estimated using the Kaplan–Meier (KM) method, with significance assessed via a two-tailed log-rank test. Independent risk factors influencing CAR T therapy response were identified through binary logistic regression for both univariate and multivariate analyses. The Cox proportional hazards model was used for univariate and multivariate Cox regression to identify independent risk factors for RFS and OS. Statistically significant factors from the univariate logistic analysis ($p < 0.05$) were incorporated into the multivariate regression analysis. Spearman's correlation analysis was conducted to evaluate the relationship between $CD10^-$ neuts and CD19 CAR-T cell persistence time. Statistical significance was set at $p < 0.05$.

Results

Elevation of $CD10^-$ neuts in PB from patients with r/r B-ALL

A total of 47 HCs and 44 patients with r/r B-ALL were evaluated for $CD10^-$ neuts levels. Baseline characteristics of both groups were presented in Table 1, revealing no significant differences in sex or age ($p = 0.68$ and 0.58, respectively). The $CD10^-$ neuts/neutrophils and $CD10^-$ neuts/nucleated cells in patients with r/r B-ALL were 34.94% (17.19%, 55.51%) and 12.18% (6.16%, 25.54%), significantly higher compared to HCs, which were 3.07% (2.09%, 5.08%) and 1.32% (0.89%, 2.45%) ($p < 0.0001$ for both, Fig. 2A, B). Furthermore, the absolute count of $CD10^-$ neuts in patients was also elevated compared to HCs (227.4 [49.31, 601.4]/μL vs 82.79 [45.77, 140.9]/μL), $p = 0.002$, Fig. 2C).

Table 1 Baseline characteristics of r/r B-ALL patients and healthy controls

Characteristics	r/r B-ALL patients ($n = 44$)	Healthy controls ($n = 47$)	*p* value
Sex (male,%)	21 (47.8%)	22 (46.8%)	0.68
Age, years	21 (9, 39)[a]	23 (7, 40)[a]	0.58
White blood cells (10^9/L)	1.42 (0.94, 3.02)[a]	5.67 (4.85, 7.02)[a]	< 0.0001
Neutrophils (10^9/L)	0.91 (0.21, 2.26)[a]	3.08 (3.49, 4.12)[a]	< 0.0001
Lymphocytes (10^9/L)	0.39 (0.17, 0.73)[a]	3.08 (2.59, 4.12)[a]	< 0.0001
Tregs(%)	6.31 (4.38, 8.45)[a]		
Previous chemotherapy—no. (%)			
≥ 10	18 (40.9%)		
< 10	26 (59.1%)		
Previous allo-HSCT—no. (%)			
Yes	9 (20.5%)		
No	35 (79.5%)		
Primary refractory disease—no. (%)	2 (4.5%)		
Relapse—no. (%)			
1 time	24 (54.5%)		
≥ 2 times	18 (40.9%)		
Percentage of blasts in bone marrow ≥ 20%—no. (%)	25 (56.8%)		
High-risk cytogenetic/molecular factors—no. (%)	26 (59.1%)		
Active extramedullary diseases—no. (%)	10 (22.7%)		

[a] After the Kolmogorov–Smirnov test was used for normality test, the data were all non-normal distribution, so presented as medians and quartiles (M (Q25, Q75))

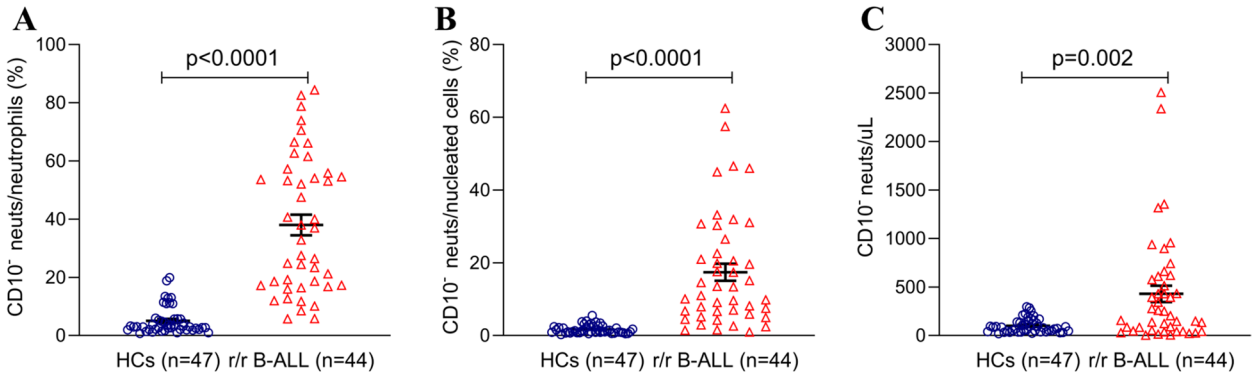


Fig. 2 Elevation of CD10⁻ neuts in peripheral blood from patients with r/r B-ALL. **A**–**C** The proportion of CD10⁻ neuts/neutrophils, CD10⁻ neuts/nucleated cells, and the absolute count of CD10⁻ neuts were significantly higher in patients with r/r B-ALL ($n=44$) compared to healthy controls (HCs, $n=47$). HCs: healthy controls, r/r B-ALL: refractory/relapsed B-cell acute lymphoblastic leukemia patients

The levels of CD10⁻ neuts correlate with the non-response to CD19 CAR T-cell therapy

The overall remission rate following CD19 CAR T-cell therapy infusion was 81.8% (36/44). The proportions of CD10⁻ neuts/neutrophils, CD10⁻ neuts/nucleated cells, and the absolute count of CD10⁻ neuts were significantly lower in the CR/CRi group compared to the NR group (25.43% vs 64.44%, $p=0.004$; 9.81% vs 28.61%, $p=0.018$; 152.9/μL vs 766.1/μL, $p=0.04$, Fig. 3A–C). ROC analysis revealed superior predictive value for CD10⁻ neuts/neutrophils (AUC = 0.816, $p=0.006$) over CD10⁻ neuts/nucleated cells (AUC = 0.774, $p=0.016$) and the absolute count of CD10⁻ neuts (AUC = 0.733, $p=0.046$) in forecasting response to CAR T-cell therapy (Fig. 3D, F). Based on the optimal cutoff value for CD10⁻ neuts determined by the ROC analysis, patients were stratified into high and low groups. Results showed that lower levels of CD10⁻ neuts/neutrophils (< 53.86%, $n=31$, $p=0.002$), CD10⁻ neuts/nucleated cells (< 20.20%, $n=30$, $p=0.008$), and the absolute count of CD10⁻ neuts (< 918.16/μL, $n=38$, $p=0.006$) were associated with higher CR/CRi rates compared to the high group (Table 2).

To assess whether CD10⁻ neuts levels were independent predictors of CAR T treatment response, univariate and multivariate binary logistic regression analyses were performed, incorporating CD10⁻ neuts levels and other clinical variables. Univariate analysis identified high CD10⁻ neuts/neutrophils, CD10⁻ neuts/nucleated cells, absolute count of CD10⁻ neuts, Tregs, and active EMD as predictors of poor treatment response. In multivariate analysis, CD10⁻ neuts/neutrophils (OR = 19.8, 95% CI 1.894–206.965, $p=0.013$) and active EMD (OR = 17.268, 95% CI 1.645–181.307, $p=0.018$) remained significant predictors (Fig. 3G).

The levels of CD10⁻ neuts correlate with poor RFS

The median RFS was 10.6 months (95% CI 4.87–16.33), with 1-year, 2-year, and 3-year RFS rates of 50% (18/36), 27.8% (10/36), and 25% (9/36), respectively. Ten patients (27.8%) who underwent bridging to allo-HSCT were excluded from this analysis. The predictive accuracy for relapse within 1 year following CR/CRi after CAR T-cell therapy was moderate for CD10⁻ neuts/neutrophils (AUC = 0.778, $p=0.022$) and CD10⁻ neuts/nucleated cells (AUC = 0.745, $p=0.043$), whereas the absolute count of CD10⁻ neuts (AUC = 0.690, $p=0.12$) showed no significant predictive value (Fig. 4A–C). Patients were stratified into low and high groups based on the respective cutoff values. KM analysis revealed that the median RFS was significantly shorter in patients with high CD10⁻ neuts/neutrophils (≥ 21.57%, $n=16$, $p=0.001$) compared to those in the low group (Fig. 4D). However, RFS did not significantly differ between the high and low groups based on CD10⁻ neuts/nucleated cells (≥ 11.6%, $n=14$, $p=0.059$) or the absolute count of CD10⁻ neuts (≥ 227.4/μL, $n=13$, $p=0.32$) (Fig. 4E, F).

To further assess whether CD10⁻ neuts levels were independent risk factors for relapse after CAR T-cell therapy, Cox regression analysis was conducted. In the univariate analysis, high CD10⁻ neuts/neutrophils and Tregs were identified as potential prognostic factors for RFS, with CD10⁻ neuts/neutrophils remaining a significant predictor in the multivariate analysis (HR = 4.704, 95% CI 1.658–13.343, $p=0.004$) (Fig. 4G).

The levels of CD10⁻ neuts correlate with poor OS

The median OS for patients was 23.6 months (95% CI 5.3–41.8), with 1-year, 2-year, and 3-year OS rates of 59.1% (26/44), 50.0% (22/44), and 34.1% (15/44), respectively. As in the RFS analysis, the ten patients bridged

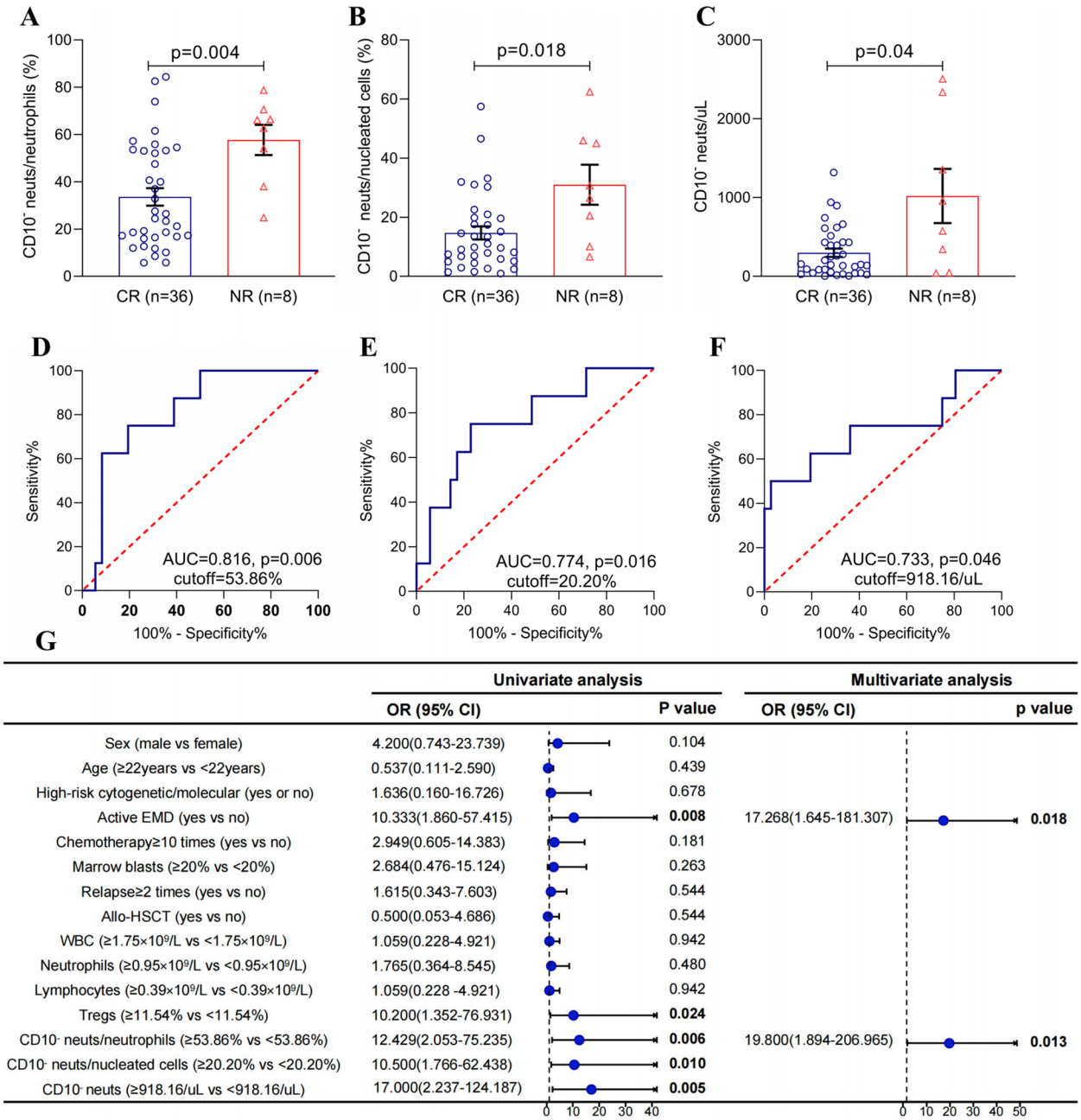


Fig. 3 Correlation of CD10⁻ neuts levels with non-response to CD19 CAR T-cell therapy. **A**–**C** The proportion of CD10⁻ neuts/neutrophils, CD10⁻ neuts/nucleated cells, and the absolute count of CD10⁻ neuts were higher in the NR group ($n=8$) compared to the CR/CRi group ($n=36$). **D**–**F** ROC curve analysis for predicting clinical response using CD10⁻ neuts/neutrophils, CD10⁻ neuts/nucleated cells, and the absolute count of CD10⁻ neuts. **G** Univariate and multivariate logistic regression analysis of independent factors influencing clinical response based on clinical and laboratory indicators. The cutoff values for age, WBC, neutrophils, and lymphocytes were set at the median. CR/CRi, complete remission or complete remission with incomplete hematological recovery; NR, non-response; OR, odds ratio; 95% CI, 95% confidence interval; WBC, white blood cell

to allo-HSCT were excluded. CD10⁻ neuts/neutrophils (AUC = 0.941, $p=0.0008$) and CD10⁻ neuts/nucleated cells (AUC = 0.774, $p=0.038$) demonstrated strong predictive value for survival status at the end of follow-up, whereas the absolute count of CD10⁻ neuts (AUC = 0.708, $p=0.11$) showed no such association (Fig. 5A–C). Based on their respective cutoff values, patients were divided into low and high groups. KM analysis revealed that the median OS in patients for high CD10⁻ neuts/neutrophils (≥ 21.57%, $n=24$, $p=0.0002$) and CD10⁻ neuts/nucleated

Table 2 Comparison of treatment response between high/low CD10⁻ neuts levels

Variable	Cutoff value[a]	CR/CRi	NR	Total	*p* value
CD10⁻ neuts/neutrophils[b]	< 53.86%	29 (93.5%)	2 (6.5%)	31	0.002
	≥ 53.86%	7 (53.8%)	6 (46.2%)	13	
CD10⁻ neuts/nucleated cells[c]	< 20.20%	28 (93.3%)	2 (6.7%)	30	0.008
	≥ 20.20%	8 (57.1%)	6 (42.9%)	14	
Count of CD10⁻ neuts in peripheral blood	< 918.16/μL	34 (89.5%)	4 (10.5%)	38	0.006
	≥ 918.16/μL	2 (33.3%)	4 (66.7%)	6	

[a] The cutoff value of CD10⁻ neuts levels determined by the ROC curve

[b] The percentage of CD10⁻ neuts in neutrophils

[c] The percentage of CD10⁻ neuts in all nucleated cells

cells (≥ 17.48%, $n=16$, $p=0.014$) was significantly shorter compared to the low group (Fig. 5D, E). However, no significant OS difference was observed between low and high groups based on the absolute count of CD10⁻ neuts (≥ 268.9/μL, $n=17$, $p=0.076$) (Fig. 5F).

Univariate and multivariate analyses were performed to identify independent factors influencing OS in patients with r/r B-ALL undergoing CD19 CAR T-cell therapy. Univariate analysis showed that high CD10⁻ neuts/neutrophils, CD10⁻ neuts/nucleated cells, Tregs, and active EMD were associated with poor OS prognosis. Multivariate analysis identified CD10⁻ neuts/neutrophils (HR = 6.417, 95% CI 2.178–19.228, $p=0.001$) and active EMD (HR = 2.812, 95% CI 1.146–5.639, $p=0.048$) as independent prognostic factors for OS (Fig. 5G).

The levels of CD10⁻ neuts correlate with the persistence time of CAR T cells

CD19 CAR T cells isolated from PB were quantified via qRT-PCR, and their persistence was assessed. Spearman's correlation analysis evaluated the relationship between CD10⁻ neuts levels and the persistence time of CD19 CAR T cells. As illustrated in Fig. 6, CD10⁻ neuts/neutrophils showed a significant negative correlation with the persistence time of CD19 CAR T cells ($r=-0.479$, $p=0.024$). A similar trend was noted for CD10⁻ neuts/nucleated cells, although the association did not achieve statistical significance ($r=-0.397$, $p=0.067$). No significant correlation was found between the absolute count of CD10⁻ neuts and the persistence time of CD19 CAR T cells ($r=0.175$, $p=0.437$).

Discussion

CAR T-cell therapy has shown considerable promise in treating hematological malignancies, achieving notable advances. However, long-term follow-up data and large-scale trials highlight treatment failure and relapse as persistent challenges. The TME presents significant obstacles to the effectiveness of CAR T-cell therapy. Neutrophils, the predominant myeloid cells in human blood, play a pivotal role in the TME, with their impact on progression being influenced by their polarization status [13, 14]. In particular, the tumor-promoting function of neutrophils is gaining increasing recognition. Despite this, the role of immunosuppressive neutrophils in affecting CAR T-cell therapy efficacy remains underexplored.

In our previous studies, EMD and higher Tregs were identified as independent high-risk factors for poor OS and RFS, respectively [3, 7]. In the present study, EMD remains an independent risk factor for OS. This is the first study to identify CD10⁻ neuts/neutrophils as independent risk factors for CAR T treatment response and OS. Additionally, CD10⁻ neuts/neutrophils were recognized as an independent predictor for RFS, while Tregs, previously identified as an independent risk factor, remained significant only in univariate analysis. The discrepancy in Tregs results between this and previous studies may stem from differences in sample sizes and the longer follow-up period in this study, or from potential interactions between CD10⁻ neuts and Tregs, as both represent immunosuppressive cell populations that could influence multivariate analysis results.

Additionally, high-risk cytogenetic/molecular abnormalities are commonly associated with poor prognosis in B-ALL. However, our study did not show a significant impact of these abnormalities on RFS and OS in patients receiving CAR T treatment, consistent with our previous findings [3, 7]. It is important to note that other studies have shown that certain mutations, such as TP53, negatively affect the efficacy of CAR T therapy [19, 20]. This discrepancy may be attributed to the high heterogeneity and complexity of cytogenetic/molecular abnormalities in B-ALL, as well as the small sample size in our study, which could limit the statistical power. To better understand the true relationship and interaction between cytogenetic/molecular abnormalities in leukemia and the effect of CAR T therapy,

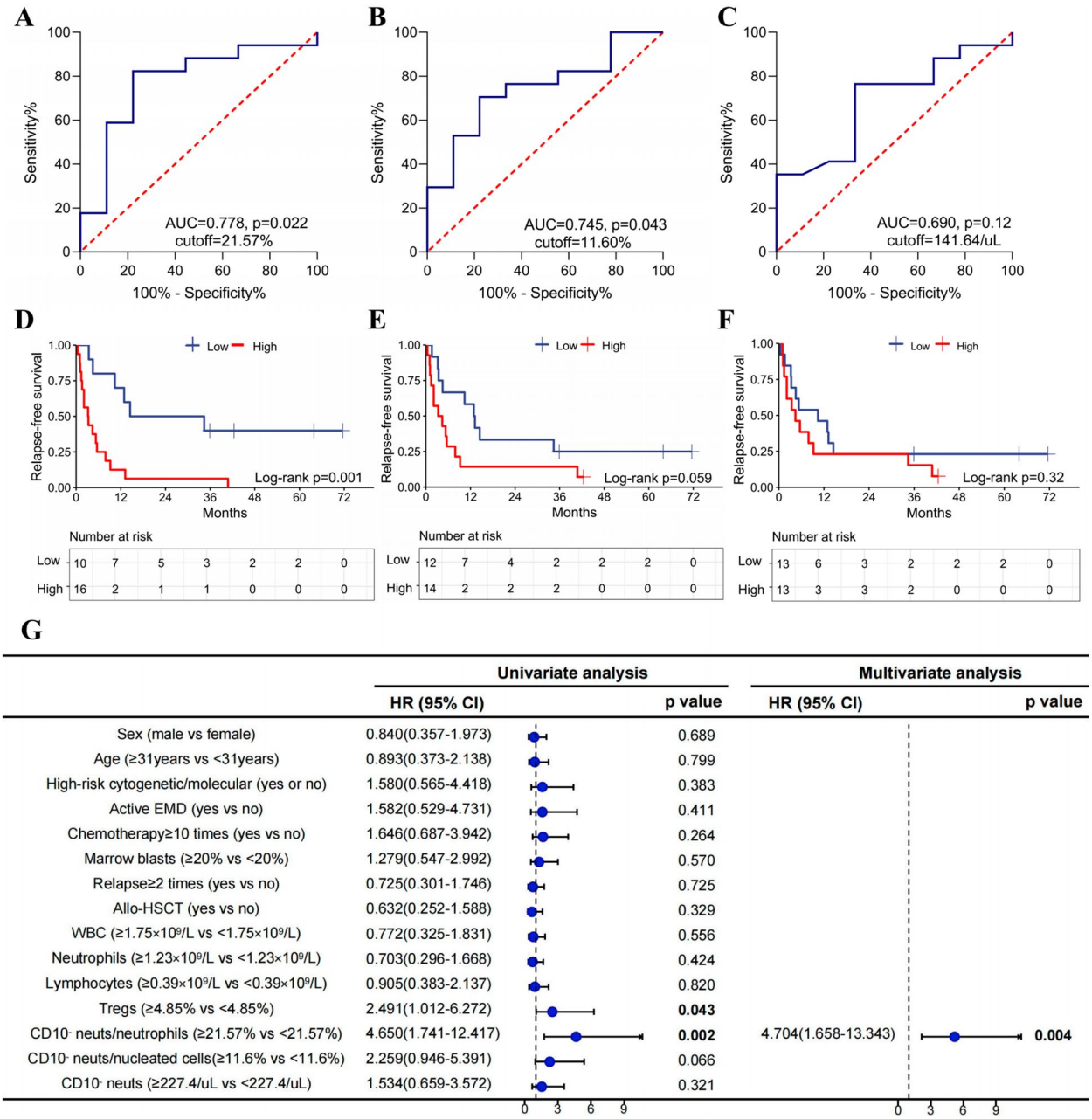
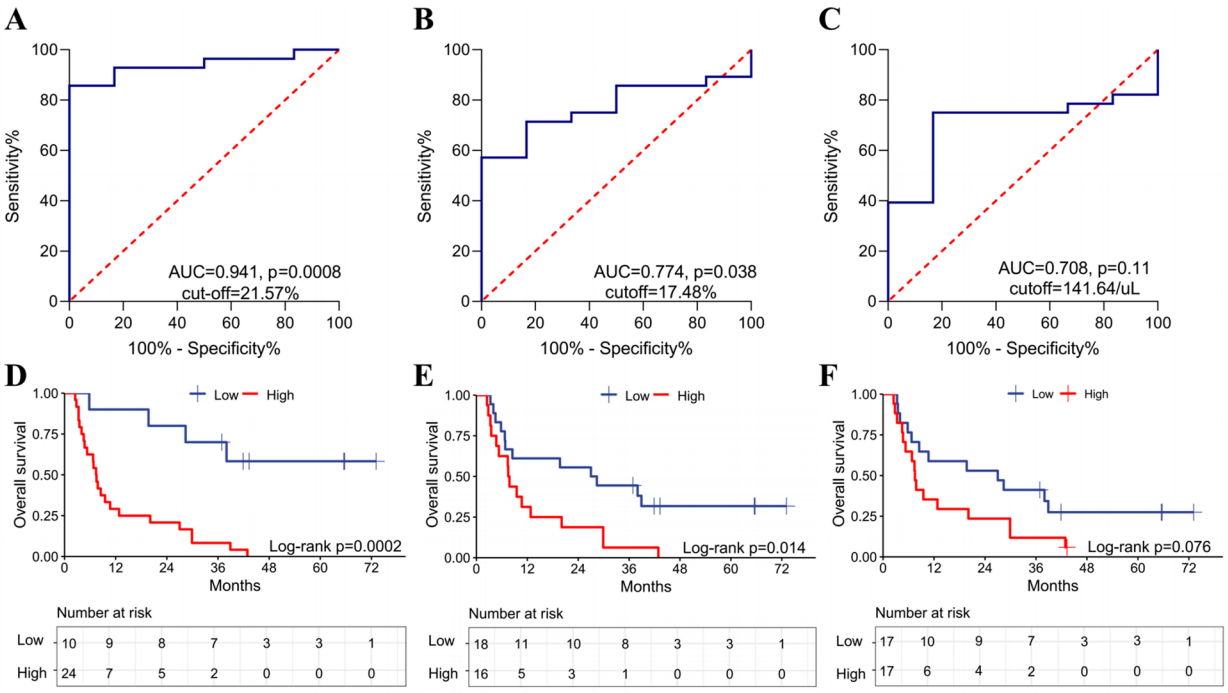


Fig. 4 Correlation between CD10⁻ neuts levels and poor RFS. **A**–**C** ROC curve analysis for predicting relapse using CD10⁻ neuts/neutrophils, CD10⁻ neuts/nucleated cells, and the absolute count of CD10⁻ neuts. **D**–**F** KM analysis of RFS based on high and low groups defined by the cutoff value of CD10⁻ neuts/neutrophils, CD10⁻ neuts/nucleated cells, and the absolute count of CD10⁻ neuts. **G** Univariate and multivariate Cox regression analysis of independent factors influencing RFS based on clinical and laboratory indicators. The cutoff values for age, WBC, neutrophils, and lymphocytes were set at the median. RFS, relapse-free survival; HR, hazard ratio; 95% CI, 95% confidence interval; WBC, white blood cells

future studies should include more patients, preferably from multiple centers, for a more comprehensive and detailed analysis.

CAR T cell expansion and persistence in vivo are essential for sustained remission, with the post-infusion levels of CAR T cells serving as a key predictor of response durability [11, 21]. Clinical studies have shown that CAR T cell persistence at 6 months correlates with reduced recurrence rates and extended progression-free survival [22]. Additionally, early CAR T cell proliferation following infusion has been associated with sustained therapeutic responses [23]. In our study, levels of CD10⁻ neuts



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	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	p value
Sex (male vs female)	0.950(0.452-1.996)	0.950		
Age (≥24years vs <24years)	0.795(0.374-1.690)	0.551		
High-risk cytogenetic/molecular (yes or no)	1.999(0.735-5.438)	0.383		
Active EMD (yes vs no)	2.465(1.110-5.474)	**0.027**	2.812(1.146-5.639)	**0.048**
Chemotherapy≥10 times (yes vs no)	2.103(0.963-4.592)	0.264		
Marrow blasts (≥20% vs <20%)	1.509(0.713-3.193)	0.282		
Relapse≥2 times (yes vs no)	1.019(0.476-2.183)	0.961		
Allo-HSCT (yes vs no)	0.613(0.259-1.453)	0.266		
WBC (≥1.75×109/L vs <1.75×109/L)	0.744(0.347-1.597)	0.744		
Neutrophils (≥1.03×109/L vs <1.03×109/L)	0.667(0.312-1.426)	0.296		
Lymphocytes (≥0.39×109/L vs <0.39×109/L)	0.772(0.359-1.661)	0.508		
Tregs (≥5.65% vs <5.65%)	3.127(1.298-7.535)	**0.011**		
$CD10^-$ neuts/neutrophils(≥21.57% vs <21.57%)	6.417(2.178-19.228)	**0.001**	6.417(2.178-19.228)	**0.001**
$CD10^-$ neuts/nucleated cells(≥17.48% vs <17.48%)	2.535(1.174-5.478)	0.175		
$CD10^-$ neuts(≥268.9/uL vs <268.9/uL)	1.953(0.916-4.161)	0.083		

Fig. 5 Correlation between $CD10^-$ neuts levels and poor OS. **A**–**C** ROC curve analysis for predicting survival using $CD10^-$ neuts/neutrophils, $CD10^-$ neuts/nucleated cells, and the absolute count of $CD10^-$ neuts. **D**–**F** KM analysis of survival based on high and low groups defined by the cutoff value of $CD10^-$ neuts/neutrophils, $CD10^-$ neuts/nucleated cells, and the absolute count of $CD10^-$ neuts. **G** Univariate and multivariate Cox regression analysis of independent factors influencing OS based on clinical and laboratory indicators. The cutoff values for age, WBC, neutrophils, and lymphocytes were set at the median. OS, overall survival; 95% CI, 95% confidence interval; WBC, white blood cells

were inversely correlated with the persistence time of CD19 CAR T cells, suggesting that immunosuppressive $CD10^-$ neuts may hinder CAR T cell retention. In contrast, Marini et al. reported that $CD10^-$ neuts from granulocyte colony-stimulating factor (G-CSF)-mobilized donors exhibited T-cell stimulatory properties, despite their immature morphology, which contrasts with our findings [24]. This discrepancy may be explained by differences in both the source and methodology used in the studies. Specifically, Marini et al. isolated $CD10^-$ neuts from healthy donors through G-CSF stimulation and density gradient centrifugation, whereas our study

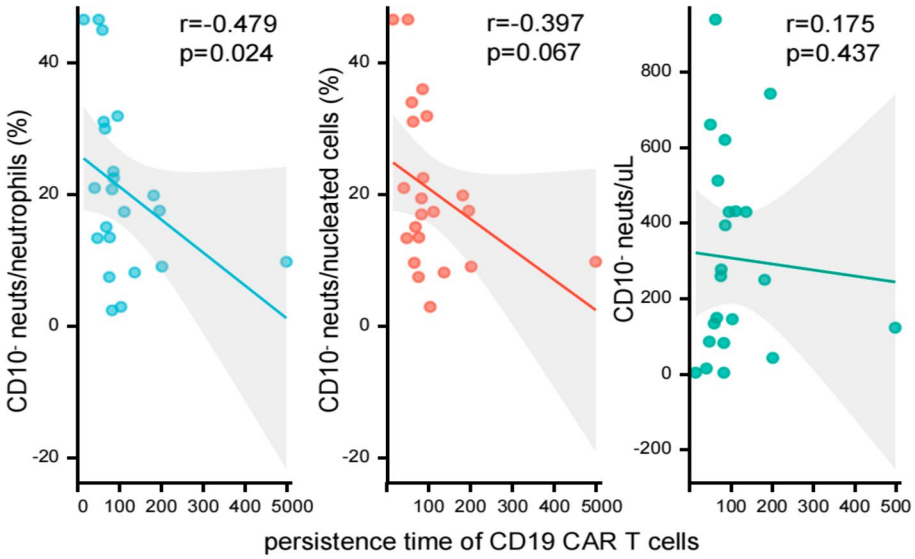

Fig. 6 Correlation between CD10⁻ neuts levels and the persistence time of CAR T cells

focused on CD10⁻ neuts from patients in a pathological state, derived from the entire neutrophil population in PB. Different physiological states contribute to the heterogeneity of neutrophils, and variations in detection methods may also influence results.

Our previous study demonstrated that CD10⁻ neuts in patients with NHL exhibited characteristics typical of MDSCs, including an immature morphology and the ability to suppress T cell activity [18]. One study reported that the early accumulation of MDSCs following focal radiotherapy was a common phenomenon across various solid tumor models, where these cells inhibit both CAR T cell infiltration and therapeutic efficacy. Moreover, MDSC blockade is essential when combining CAR T therapy with focal radiotherapy in solid tumor treatment [25]. Further research showed that CXCR2 blockade, in combination with focal radiotherapy, effectively hindered MDSC trafficking to tumors, improving both CAR T cell intratumoral infiltration and therapeutic outcomes [25, 26]. A previous study also indicated that CD10⁻ neuts exert their immunosuppressive effects primarily through arginase 1 (Arg1), which impairs T cell function in NHL [18]. Although CAR T cells can target and activate independently of conventional T-cell receptor signaling, their metabolic profile remains unchanged. Similar to conventional T cells, CAR T cells require sufficient L-arginine levels to maintain their metabolic functions and proliferative capacity [27–29]. This suggests that CD10⁻ neuts may influence the longevity of CAR T cells through Arg1, thereby impacting the overall efficacy of CAR T therapy.

The infusion of CAR T cells involves complex interactions between the cells and immunosuppressive neutrophils. Tumor cell killing by CAR T cells generates large quantities of cytokines, such as IL-6 [30, 31], which in turn promote the proliferation and activation of suppressive myeloid cells [32]. This study, however, primarily focuses on the analysis of CD10⁻ neuts levels prior to CAR T-cell infusion. The absence of longitudinal measurements of CD10⁻ neuts levels limits a comprehensive assessment of their impact on CAR T cell persistence and obstructs a deeper understanding of their dynamic interactions with CAR T-cells. Given that post-infusion CD10⁻ neuts levels are influenced by various factors, future research should address these limitations by incorporating larger sample sizes and analyzing additional variables to offer a more comprehensive and accurate understanding.

Conclusions

In summary, this study identifies a distinct subset of immunosuppressive neutrophils in the TME and their role in CAR T therapy resistance. Elevated levels of CD10⁻ neuts correlate with poor therapeutic outcomes in CD19 CAR T therapy for patients with r/r B-ALL, likely due to their influence on CAR T cell retention. Targeting and inhibiting the expansion and functional activity of CD10⁻ neuts may enhance the host's anti-tumor immune response and improve the tumor-killing efficacy of CAR T cells.

Abbreviations

CD19 CAR T	CD19-specific chimeric antigen receptor T-cell
B-ALL	B-cell acute lymphoblastic leukemia
TME	Tumor microenvironment
MDSCs	Myeloid-derived suppressor cells
CD10⁻ neuts	CD10⁻ neutrophils

HCs	Healthy controls
RFS	Relapse-free survival
OS	Overall survival
Tregs	Regulatory T cells
EMD	Extramedullary diseases
NHL	Non-Hodgkin lymphoma
PB	Peripheral blood
allo-HSCT	Allogeneic hematopoietic stem cell transplantation
WBC	White blood cell
qRT-PCR	Real-time quantitative reverse transcription PCR
CR/CRi	Complete remission or complete remission with incomplete hematologic recovery
NR	Non-response
ROC	Receiver operating characteristic
AUC	Area under the curve
KM	Kaplan-Meier

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-03968-5.

Additional File 1: Table S1: Cytogenetic/molecular abnormalities in patients with r/r B-ALL.

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Authors' contributions
JLZ, ZMZ, JZ and XL designed the study and wrote the manuscript. FRA, YWL, FW and YP collected and analyzed the data. JLZ, YYD, XYJ, MX and HX analyzed the data and made figures. HPW and HX performed the flow cytometry. ZMZ, HPW and XL verified the data and edited the manuscript. The corresponding author had final responsibility for the decision to submit the manuscript for publication. All authors read and approved the final manuscript.

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Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the institutional review boards at SHAMU (the Medical Ethics Committee and the Academic Committee at SHAMU) (No. SL-YX: 2015–06-01). All participants provided written informed consent in accordance with the principles of the Declaration of Helsinki prior to enrollment.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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