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Blinatumomab restores asparaginase activity in pediatric B-cell acute lymphoblastic leukemia patients with PEG-Asparaginase hypersensitivity

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Contribution: X.T. and X.F. designed the study and wrote the manuscript. X.T., Shi L., Y.W., and H.M. managed the patients and provided clinical data. Administrative support was provided by Si L. and F.W. L.Z.,W.X., D.W., and W.L. contributed to data verification and interpretation. Z.L., a student majoring in Statistics and Mathematics at the University of Toronto, was responsible for data analysis and language support.O.A. was responsible for clinical advice. All authors reviewed and approved the final manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Data-sharing statement

The datasets generated during the study are available from the corresponding author H.M. on reasonable request.

Trial registration

All patients were in the cohort of Chinese Children's Cancer Group (CCCG)-ALL-2020 trial (www.chictr.org.cn identifier: ChiCTR2000035264).

To the editor:

B-cell acute lymphoblastic leukemia (B-ALL) , the most common childhood leukemia, has achieved overall survival (OS) exceeding 90% , driven by advances in supportive care, risk stratification, central nervous system (CNS) prophylaxis, targeted therapies, immunotherapy, and intensified chemotherapy, including asparaginase. However, asparaginase therapy is often accompanied by hypersensitivity reactions (HSRs)(7.7%-27.3%)(1-3), leading to treatment disruptions. While desensitization or switching to *Erwinia*-derived asparaginase is an option(4), recent shortages and desensitization failures pose challenges. Notably, incomplete asparaginase therapy due to toxicity is linked to poorer outcomes in pediatric ALL(5).

CD19 is widely expressed on B cells and plasmablasts. CD19-targeted CAR T cell therapy (CART-19) is emerging as a promising treatment for autoimmune diseases, demonstrating efficacy in eliminating autoantibodies(6). Blinatumomab, a bispecific T-cell engager (BiTE) that targets CD19, is widely used in pediatric leukemia and has significantly improved survival rates(7, 8). Beyond its role in leukemia treatment, we hypothesized that blinatumomab might eliminate antibody-producing B lymphocytes, thereby allowing patients with asparaginase hypersensitivity to tolerate subsequent asparaginase therapy or restore enzymatic activity.

We conducted a retrospective cohort study at Shenzhen Children's Hospital between June 1, 2024, and February 28, 2025. This study was performed in compliance with relevant laws and institutional guidelines and was approved by Ethics Committee of Shenzhen Children's Hospital (No. 202108503). All patients provided informed consen . The median age at diagnosis was 6.1 years, and 6 out of 8 patients (75%) were male. Patients were stratified into the following risk groups: LR in 62.5% (5/8) and IR in 37.5% (3/8) . All patients were treated according to the CCCG-ALL-2020 protocol, receiving PEG-Asparaginase (PEG-ASP, Jiangsu Hengrui, China) at a dose of 2000 U/m² on days 6 and 26 of induction. Serum asparaginase activity (SAA) was monitored on days 7, 14, and 21 post-injection using a kit-based colorimetric method

at Kindstar Global (Supplementary Figure 1). Among the eight patients who experienced HSR after the first dose of PEG-ASP, 2 developed clinical allergy, while 6 exhibited silent inactivation. The clinical characteristics of the patients are summarized in Table 1.

In cases of clinical hypersensitivity or silent inactivation after the first dose , the second dose was replaced with six doses of *Erwinia (*20000 U/m², twice a week, sustained for three weeks*)* (Guangzhou Baiyunshan Mingxing, China). To mitigate the impact of PEG-ASP hypersensitivity, blinatumomab was incorporated following the VDLP and CAM regimens (**Supplementary Figure 1**). After completing induction and CAM chemotherapy, all patients received one cycle of blinatumomab (28 days, 15 ug/m^2/day), except for Patient 5, who received 14 days due to financial constraints. The consolidation phase included four courses of high-dose methotrexate (LR: 3 g/m², IR: 5 g/m²). During the IR interim chemotherapy and LR reinduction phase 1, PEG-ASP (2000 U/m²) was re-administered, with asparaginase activity monitored on days 7, 14, and 21 to assess enzyme activity and ensure adequate asparagine depletion (**Supplementary Figure 1**).

Post-blinatumomab treatment, a significant restoration of ASP activity was observed in 6 of 8 patients (75%). Patients P1–P6 demonstrated marked recovery of ASP activity, classified as the asparaginase activity restored (AAR) cohort, whereas patients P7 and P8 exhibited no recovery, constituting the asparaginase activity non-restored (AANR) group (**Figure 1A**). This dichotomous response highlights potential heterogeneity in ASP reactivation dynamics during blinatumomab therapy.

To further investigate the mechanisms underlying blinatumomab's ability to restore PEG-ASP activity, we conducted assays to evaluate the levels of anti-PEG, anti-ASP, anti-PEG-ASP antibodies (except for P6) (**Figure 1B-D**). We also evaluated the differential reduction in antibody levels post-blinatumomab treatment, expressed as a percentage of baseline (pre-treatment) values (**Figure 1E**). The AAR cohort exhibited a significantly greater decline in antibody titers compared to the AANR group ($p<$

0.05), suggesting an inverse relationship between antibody suppression and ASP activity restoration (**Figure 1B-E**), consistent with the established link between antibody positivity and reduced asparaginase activity (9). This association implies that blinatumomab-mediated modulation of humoral immunity may contribute to enzymatic reactivation, potentially influencing therapeutic outcomes in pediatric B-ALL.

Considering memory B cells and plasma cells critically contribute to asparaginase (ASP)-induced hypersensitivity by driving the production of neutralizing antibodies and sustaining immune-mediated allergic response, we analyzed the immune characteristics of B cells both in bone marrow and peripheral blood (except for P6). Bone marrow immunophenotypic profiling at initial diagnosis of B-ALL revealed substantial inter-individual heterogeneity within both AAR and AANR cohorts. Meanwhile, comparative analysis of peripheral blood lymphocyte subsets at the beginning of B-ALL diagnosis showed no statistically significant differences in overall distribution between AAR and AANR groups. However, a trend toward elevated B-cell proportions and absolute counts was observed in the AANR cohort (**Supplementary Figure 2**), suggesting a potential association between baseline B-cell enrichment and impaired rescue of ASP activity.

In addition, we conducted immunological evaluations of patients before and after the administration of blinatumomab (except for P6) by flow cytometry. Specific immunological assessments were performed as described previously(10). The heatmap illustrates the immune evaluation of B-ALL patients before (d0) and after (d14) blinatumomab treatment, stratified into AAR cohort and AANR cohort (**Figure 2A**). Hierarchical clustering revealed distinct immune profiles between the two groups. In the AAR cohort there was a notable increase in the percentage and absolute number of $CD56^{+}16^{+}$ NK cells, as well as in the $CD4^{+}$ naive T cell/CD4 ratio. Additionally, in the $CD8^{+}$ T cell subset, there was a significant difference in granzyme B^{+} (AAR: median 15%, Poor: median 5%, p <0.05) and perforin^{+} (Good:

median 30%, Poor: median 5%, p <0.05) expression between the two groups (**Figure 2B-E**). These data mechanistically link ASP activity restoration with multi-lineage immune potentiation, highlighting $CD8^+$T cell cytotoxic effector differentiation as hallmarks of therapeutic efficacy. Consistent with our study, previous research has reported that early activation markers, such as CD69, IL2RA, and TNFRSF18 are up-regulated within hours of treatment, with effector memory T (TEM) cells exhibiting heightened granzyme B expression and cytotoxic activity within 16~48 hours (11, 12). This mechanism underscores the critical role of blinatumomab in restoring ASP activity by redirecting $CD8^+$ T-cell-mediated cytotoxicity against $CD19^+$ B cells, thereby mitigating B-cell-driven immune responses.

These findings underscore the potential of blinatumomab as a novel selection by targeting the underlying immune mechanisms that drive hypersensitivity. Furthermore, our study emphasizes the importance of biomarker-driven strategies to identify patients who are most likely to benefit from blinatumomab therapy in the context of asparaginase hypersensitivity.

These findings raise important clinical considerations. Current strategies for managing PEG-ASP hypersensitivity(4, 13), including desensitization and *Erwinia* substitution, face limitations such as variable success rates, frequent dosing requirements, and drug shortages. However, in our study, two patients who experienced clinical hypersensitivity to PEG-ASP showed no signs of allergic reactions upon rechallenge after blinatumomab treatment. Notably, asparaginase activity was restored in 4 out of 6 patients (66.7%) with PEG-ASP silent inactivation. These findings suggest that blinatumomab is highly effective in managing PEG-ASP hypersensitivity and demonstrates remarkable efficacy, particularly in cases of silent inactivation, for which no highly effective treatments currently exist other than switching to *Erwinia*. In a small-scale study conducted by Chen et al, rituximab (anti-CD20) and bortezomib (anti-$CD138^+$ plasma cells) were able to successfully clear anti-asparagainase antibodies and restore asparaginase activity (14). This indirectly

suggests that in our study, patients who did not regain asparaginase activity, despite a slight decrease in antibody levels, might have antibodies derived from $CD19^-$ plasma cells which plays a significant role in antibody production (15). Compared with Chen's study, our approach has the advantage of not requiring additional agents such as rituximab or bortezomib for antibody clearance. Given that blinatumomab is now approved for frontline therapy and has been incorporated into multiple treatment regimens, Blinatumomab offers a viable strategy to salvage asparaginase activity by circumventing PEG-ASP hypersesitivity, therby providing an additional therapeutic alternative without exposing patients to extra cytotoxic agents. However, the small sample size is a limitation of this study, and further research with larger cohorts is needed to validate these findings.

We acknowledge the limitations of our study, including the small sample size and retrospective design. Larger prospective studies are warranted to validate these findings and further elucidate the mechanisms by which blinatumomab modulates humoral immunity in pediatric B-ALL.

In conclusion, our study is the first to provide compelling evidence that blinatumomab as a novel mechanism to salvage asparaginase activity by strategically bypassing PEG-ASP hypersensitivity, thereby establishing a pioneering therapeutic avenue to restore asparaginase efficacy in patients with PEG-ASP hypersensitivity. By reducing anti-asparaginase antibody titers and enhancing $CD8^+$ T cell responses, blinatumomab has the potential to facilitate continued asparaginase therapy, ultimately improving therapeutic outcomes for pediatric B-ALL patients. However, the mechanistic pathways linking humoral immune modulation (antibody suppression) and cellular cytotoxicity (granzyme B/perforin upregulation) remain undefined, warranting further investigation into the interplay between adaptive immunity and enzymatic reactivation in this therapeutic context. We urge further research to confirm these observations and explore the broader clinical applications of CD19-targeted immunotherapy in overcoming treatment-related toxicities in B-AL.

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Table 1 Clinical characteristics of B-ALL patients with PEG-ASP hypersenstivity

No	Gender	Age(years)	Risk Group	Type of hypersensitivity	CD19(%)	Days of blina
P1	M	7	LR	Silent inactiviation	84.9	28
P2	M	1.75	IR	Silent inactiviation	99.4	28
P3	M	2.58	LR	Clinical hypersensitivity	99.2	28
P4	F	9.08	LR	Silent inactiviation	99.8	28
P5	M	4.42	LR	Silent inactiviation	97.2	14
P6	F	13.58	IR	Silent inactiviation	90.7	28
P7	M	5.25	LR	Silent inactiviation	98.8	28
P8	M	6.92	IR	Clinical hypersensitivity	98	28

Note: LR: low risk; IR: intermediate risk; blina: blinatumomab.

Figure Legend

Figure 1. Impact of blinatumomab on asparaginase activity and antibody levels in pediatric B-ALL patients. (A) Longitudinal assessment of ASP activity in peripheral blood samples quantified by colorimetric method. Dashed line indicates the recommended level of asparaginase reflecting effective depletion of asparagine (≥100 IU/L). **(B-D)** ASP-specific antibody titers (Before and after blinatumomab) measured by enzyme-linked immunosorbent assay (ELISA). **(E)** Percentage reduction in ASP-specific antibody titers (relative to pre-treatment baseline) measured by enzyme-linked immunosorbent assay (ELISA). Data represent mean ± SD; Chromatographic ASP activity measurements were performed using a modified YSI biochemical system with enzymatic hydrolysis quantification, while anti-ASP IgG antibodies were detected via ELISA using recombinant ASP antigen-coated plates. ns: $p > 0.05$, *: $p < 0.05$.

Figure 2. Immune Assessment of Patients After Blinatumomab Administration

Heatmap representing the immune evaluation of B-ALL patients before (d0) and after (d14) blinatumomab treatment, stratified into the Asparaginase Activity Restored Group (AAR Group) and the Asparaginase Activity nonRestored Group (AANR Group). Immune cell subsets and markers were analyzed using multiparametric flow cytometry. The heatmap displays the log2 fold change of various immune parameters, with hierarchical clustering performed to group similar immune profiles. The color scale indicates the magnitude of change, with red representing upregulation and blue representing downregulation. The immune evaluation includes the percentage and absolute number of $CD56^+16^+$ NK cells, $CD4^+$ naive T cells, $CD8^+$ T cells, and other relevant immune markers. The heatmap provides a comprehensive overview of the immune landscape in B-ALL patients in response to blinatumomab treatment. **(B-D)** Boxplots showing the expression of granzyme B and perforin in different lymphocyte subsets in B-ALL patients after blinatumomab treatment, stratified into the AAR cohort (n=5) and AANR cohort (n=2). The boxplots display the median, interquartile range, and range of values. Statistical significance was determined using appropriate tests, with $p < 0.05$ considered significant. ns, not significant. **(E)** Representative flow cytometry histogram depicting granzyme B and perforin expression in $CD8^+$ T cells. ns: $p > 0.05$, *: $p < 0.05$.

Figure 1

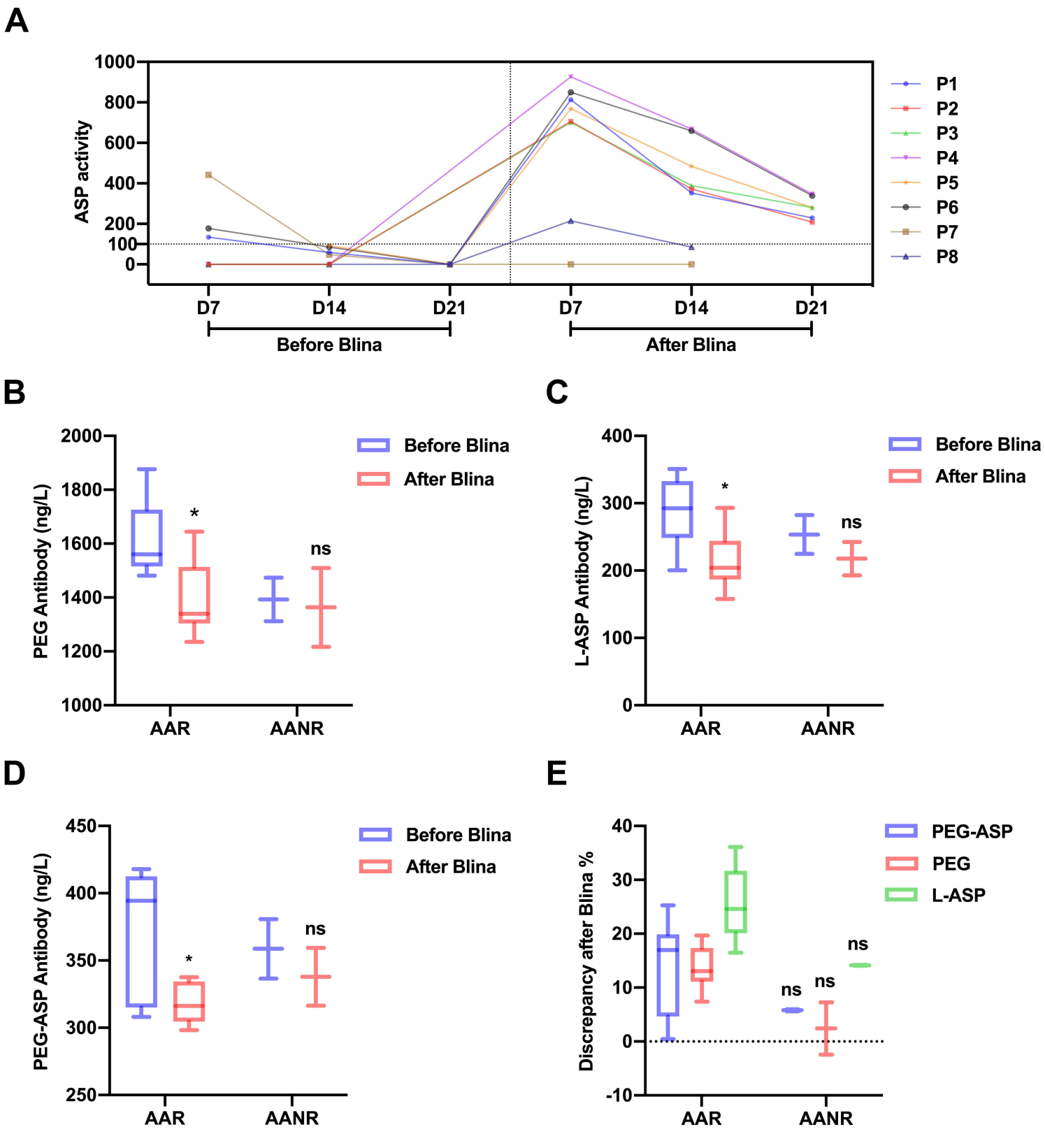
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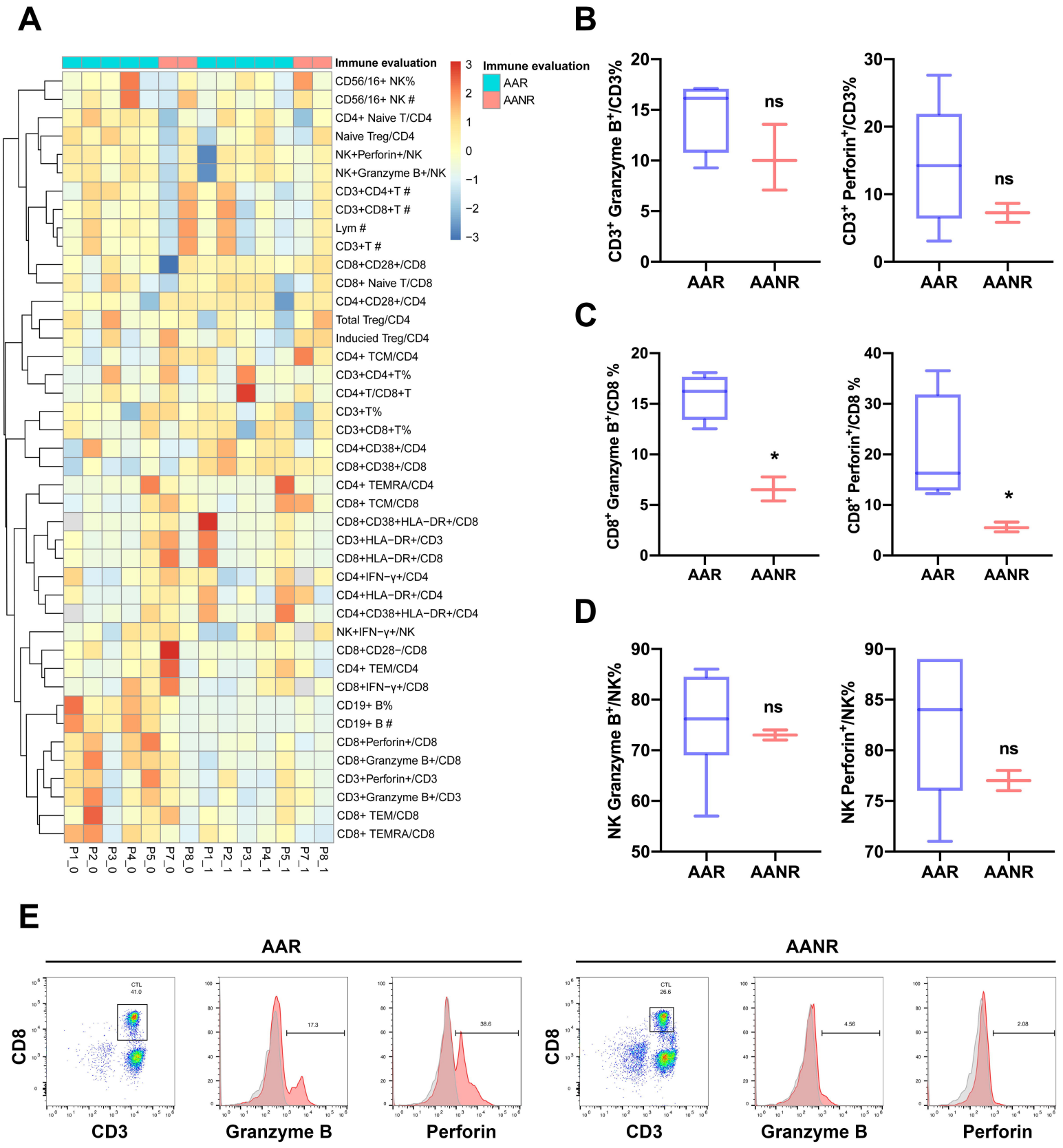
Figure 2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Naive Treg/CD4
NK+Perforin+/NK
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CD3+CD4+T #
CD3+CD8+T #
Lym #
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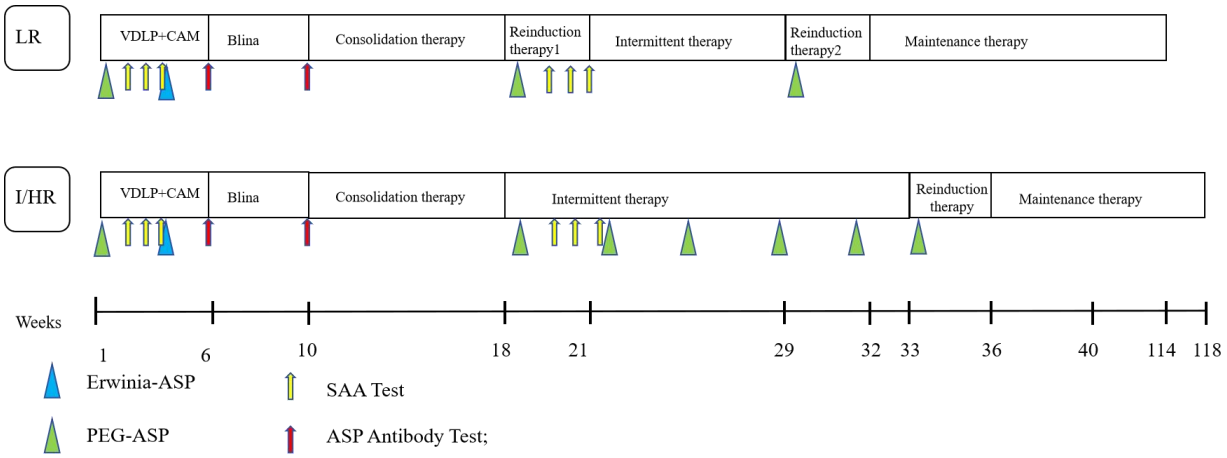
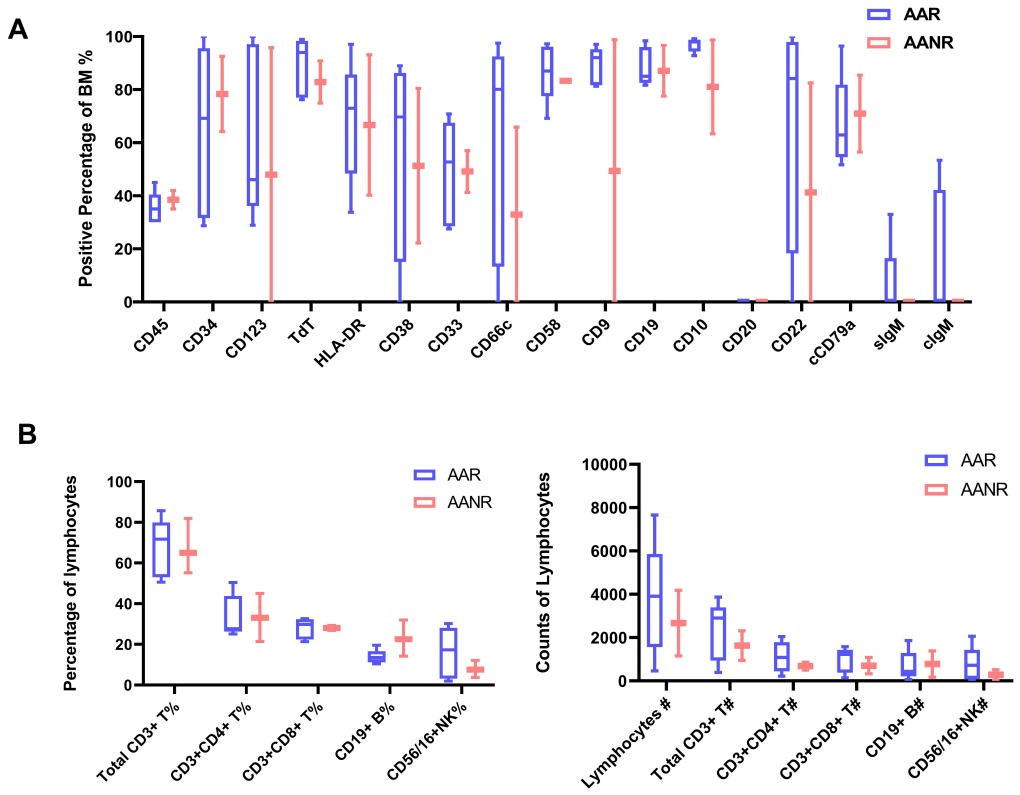
Supplementary Figures



Supplementary Figure 1. Schema of treatment protocols and SAA measurement point. SAA, serum asparaginase activity; LR, low risk; I/HR, intermediate/high risk; Blina, blinatumomab; Blue triangle, planned administration of Erwinia at a dose of 20000 U/m^2, with a total of six doses; Green triangle, planned administration of PEG-ASP at a dose of 2000 U/m^2; Yellow arrow, sampling timing of SAA; Red arrow, sampling timing of ASP antibody.



Supplementary Figure 2. Immune Assessment of Patient at the initial diagnosis of B-ALL both in bone marrow and pleura blood. (A) Analysis of diagnostic bone marrow samples from AAR and AANR pediatric cohorts by flow cytometry. Box plots depict the percentage distribution of key immunophenotypic markers (CD10, CD19, CD20, CD22, CD24, CD34, CD38, CD45, CD123, HLA-DR, and IgM) within leukemic blasts. Central lines represent medians, boxes span interquartile ranges (IQR), and whiskers extend to 1.5×IQR. Individual outliers are plotted as points. (B) Peripheral blood lymphocyte subset distribution at B-ALL diagnosis. Left panel: proportional representation of lymphocyte subsets in peripheral blood. Right panel: absolute counts (cells/μL) for corresponding subsets. Results showed as box plot construction.