A NORMALLY COMPOSED BUT FUNCTIONALLY SILENCED NK CELL COMPARTMENT DEFINES DOUBLE-REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA AND RATIONALIZES NK-ENGAGING THERAPIES

Richard I. Lewis, Alexander vom Stein, Sarah Krause and Michael Hallek

Department I of Internal Medicine, Faculty of Medicine, University of Cologne, University Hospital Cologne, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, Center for Molecular Medicine Cologne, CECAD Center of Excellence on Cellular Stress Responses in Aging-Associated Diseases, Cologne, Germany

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INTRODUCTION

The introduction of BTK inhibitors and BCL2 antagonists has revolutionized the treatment of chronic lymphocytic leukemia (CLL), yet an emerging population of patients is becoming refractory or intolerant to both drug classes. These double-refractory (2R) patients currently have no standard therapy and face a dismal prognosis. NK cells are emerging as critical effectors of antibody-dependent cellular cytotoxicity (ADCC) and novel NK-engaging strategies are now entering CLL trials. However, how the NK compartment evolves across the CLL treatment continuum, and specifically whether it is impaired in 2R patients, remains undefined.

AIM

To characterize the NK compartment of the tumor microenvironment including subset composition and functional capacity across the CLL treatment continuum, with particular focus on double-refractory CLL (2R) patients who have failed both BTK and BCL2 inhibitors. We hypothesized that 2R NK cells are functionally silenced but still persist even in the tumor microenvironment of heavily pretreated patients, providing a possible rationale for NK-engaging therapies reactivating them.

METHODS

STUDY DESIGN & PATIENTS

Single-center observational study of cryopreserved peripheral blood mononuclear cells (PBMCs) collected 2018 - 2026 from 13 CLL patients contributing 69 samples. Main analysis: 69 samples stratified by clinical state: treatment-naïve (n=4, 5 samples), Response or post-treatment remission (n=10, 32 samples), Progressive Disease/PD (n=9, 21 samples), and Double-Refractory after BTKi/BCL2i failure (2R, n=9, 11 samples. 9 patients contributed paired PD and Response samples, enabling within-patient comparison.

FLOW CYTOMETRY

PBMCs stained with fluorescent antibodies targeting:

NK subset markers: CD16, CD56 (defining CD16+CD56dim, CD16-CD56dim, CD56bright). Functional phenotype: CD107a (degranulation marker), CD159a/NKG2A (inhibitory checkpoint), CD314/NKG2D (activating receptor)

DATA PROCESSING & ANALYSIS

NK subset composition expressed as % of total NK cells (normalized to sum to 100%, independent of CLL burden). Functional markers expressed as % of parent NK subset. All comparisons based on per-patient means (one value per patient per group) to avoid pseudo-replication bias. Statistical testing: Kruskal-Wallis for omnibus comparisons across groups; Mann-Whitney U test for pairwise 2R vs. Response/PD comparisons. Significance: p<0.05 two-sided.

CONCLUSIONS

We identified a normally composed but functionally altered NK cell compartment in 2R-CLL patients. NK subset composition remained stable across all disease states (p>0.30), ruling out subset-selective depletion. In contrast, NK functional markers diverged sharply: NKG2A expression and CD107a decreased substantially in 2R versus Response patients (p<0.05), while NKG2D remained fully preserved (p>0.40). This suggests a “silenced-but-recoverable” phenotype with normal distributed NK cells but suppressed function. Thereby this could rationalize NK-engaging immunotherapy strategies precisely where BTKi and BCL2i have failed to reactivate the NK cell compartment.

RESULTS

Figure 1: NK Subset Composition (% of Total NK)

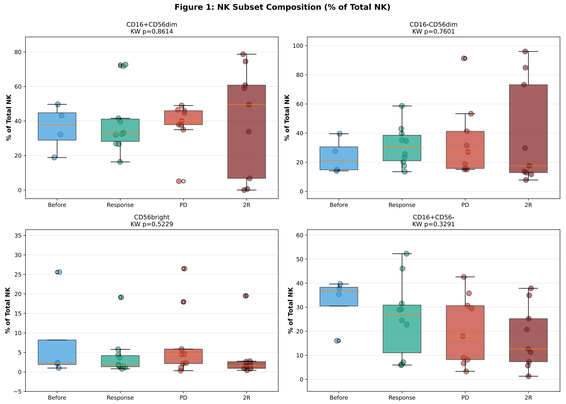

FIGURE 1: NK Subset Composition Across Disease States Boxplots with individual sample overlay showing frequency of NK subsets (% of total NK cells) defined by CD16 and CD56 expression across treatment-naïve (Before, n=4 patients), Response (n=10), PD (n=9), and 2R (n=9) states. Data shown as patient-level means; Kruskal-Wallis p>0.30 for all subsets. Key finding: NK subset composition remains stable; 2R does not reflect subset-selective depletion.

Figure 2: CD107a+ Degranulation (2R-Specific Collapse)

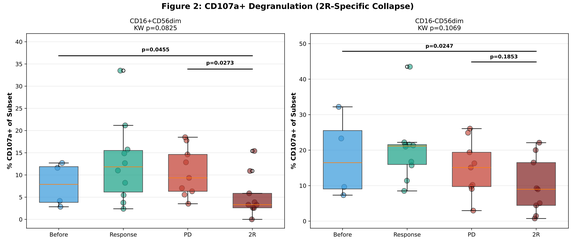

FIGURE 2: CD107a Expression Across Disease States Boxplots with individual sample overlay showing CD107a+ frequency (% of parent NK subset) on CD16+CD56dim and CD16-CD56dim NK cells across disease states (Before n=4, Response n=10, PD n=9, 2R n=9 patients). CD16+CD56dim: 3.3% in 2R vs 11.8% in Response (p=0.045); CD16-CD56dim: 9.0% vs 21.2% (p=0.025). Key finding: 2R NK cells show reduced degranulation markers despite normal subset composition.

Figure 3: NKG2A+ Expression (2R-Specific Downregulation)

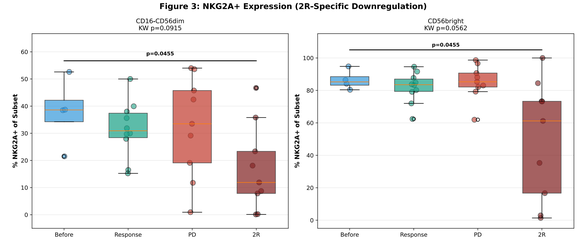

FIGURE 3: NKG2A Expression Across Disease States Boxplots with individual sample overlay showing NKG2A+ frequency (% of parent NK subset) on CD16-CD56dim and CD56bright NK cells across disease states (Before n=4, Response n=10, PD n=9, 2R n=9 patients). CD16-CD56dim: 11.9% in 2R vs 30.9% in Response (p=0.045); CD56bright: 61.2% vs 83.5% (p=0.045). Key finding: Inhibitory checkpoint is downregulated in 2R, suggesting functional silencing rather than classical checkpoint exhaustion.

Figure 4: NKG2D+ Expression (Preserved Across States)

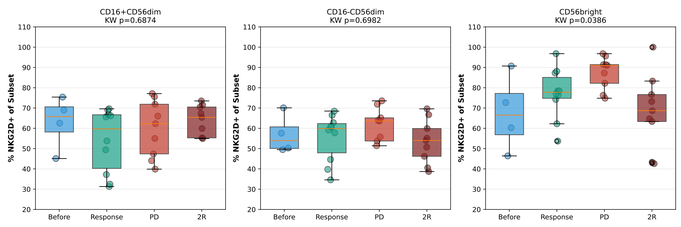

FIGURE 4: NKG2D Expression Across Disease States Boxplots with individual sample overlay showing NKG2D+ frequency (% of parent NK subset) on CD16+CD56dim, CD16-CD56dim, and CD56bright NK cells across disease states (Before n=4, Response n=10, PD n=9, 2R n=9 patients). Kruskal-Wallis p>0.40 for all subsets. Key finding: Activating receptor remains preserved in 2R despite CD107a reduction, providing a rationale for NK-engaging immunotherapy where BTKi and BCL2i have failed.

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CONTACT INFORMATION

Dr. Richard Lewis, M.D.
Kerpener Strasse 62
D-50937 Cologne, Germany
E-Mail: richard.lewis@uk-koeln.de

REFERENCES

1. Lewis RI, vom Stein AF, Hallek M. Targeting the tumor microenvironment for treating double-refractory chronic lymphocytic leukemia. Blood. 2024;144(7):601-614.
2. Sportoletti P, De Falco F, Del Papa B, et al. NK cells in chronic lymphocytic leukemia and their therapeutic implications. Int J Mol Sci. 2021;22(13):6665.
3. Hallek M, Al-Sawaf O. Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures. Am J Hematol. 2021;96(12): 1679-1705.