RESEARCH LETTER

blood advances

TO THE EDITOR:

Impact of Perforin 1 A91V germ line background in patients with aggressive B-NHL treated with R-CHOP

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Rituximab has significantly improved the treatment of aggressive B-cell non-Hodgkin lymphoma (B-NHL) across all risk groups.[1,2] Besides complement-dependent cytotoxicity (CDC),[3,4] its effect is primarily mediated by antibody-dependent cellular cytotoxicity (ADCC) involving neutrophils, macrophages,[5,6] and, in particular, natural killer (NK) and $CD16^+$ T cells as effector cells.[7,8] ADCC can be influenced by variables including the abundance and binding capacity of Fc gamma receptor (FcγR),[5,9] specific constellations of killer cell immunoglobulin-like receptors (KIRs),[10] and vitamin D deficiency,[11] which contributes to NK-cell anergy.[12]

NK and cytotoxic T-cell (CTL) cytotoxicity is mediated by the release of lytic perforin/granzyme–containing granules, direct secretion of cytotoxic effector cytokines such as interferon gamma, and the expression of death receptor ligands such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) or Fas ligand (FASL).[13]

To date, several genetic predispositions to ineffective NK cell and CTL killing are known, including variations in genes contributing to susceptibility to familial forms of hemophagocytic lymphohistiocytosis (fHLH), which can result in inefficient viral clearance and potentially fatal cytokine storm conditions. In this context, mutations affecting perforin 1 *(PRF1)*, with A91V as the most frequent variant associated with fHLH, can be associated with presynaptic and postsynaptic NK/CTL dysfunction. Importantly, perforinopathies have also been reported as a risk factor for lymphoma development.[14,15]

In this post hoc analysis, we aimed to investigate the potential influence of the *PRF1* A91V variant, associated with impaired cytotoxic immune function, on outcome and rituximab-mediated cellular cytotoxicity in patients treated with (immuno-) chemotherapy for aggressive B-NHL.

Blood samples from patients included in the RICOVER-60 trial (NCT0052936/EU-20243) were genotyped for a single nucleotide polymorphism (rs35947132) resulting in the *PRF1* A91V variant. RICOVER-60 randomized older patients (aged 61-80 years) with aggressive B-cell lymphoma in a 2 × 2 factorial design to 6 vs 8 cycles of cyclophosphamide, doxorubicin, vincristine, plus oral prednisone or prednisolone-14 (CHOP-14), with or without rituximab.[16] Blood samples from the NHL-B2 (CHOP

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Original data are available from the corresponding author, Lorenz Thurner (lorenz.thurner@uks.eu), on request.

The full-text version of this article contains a data supplement.

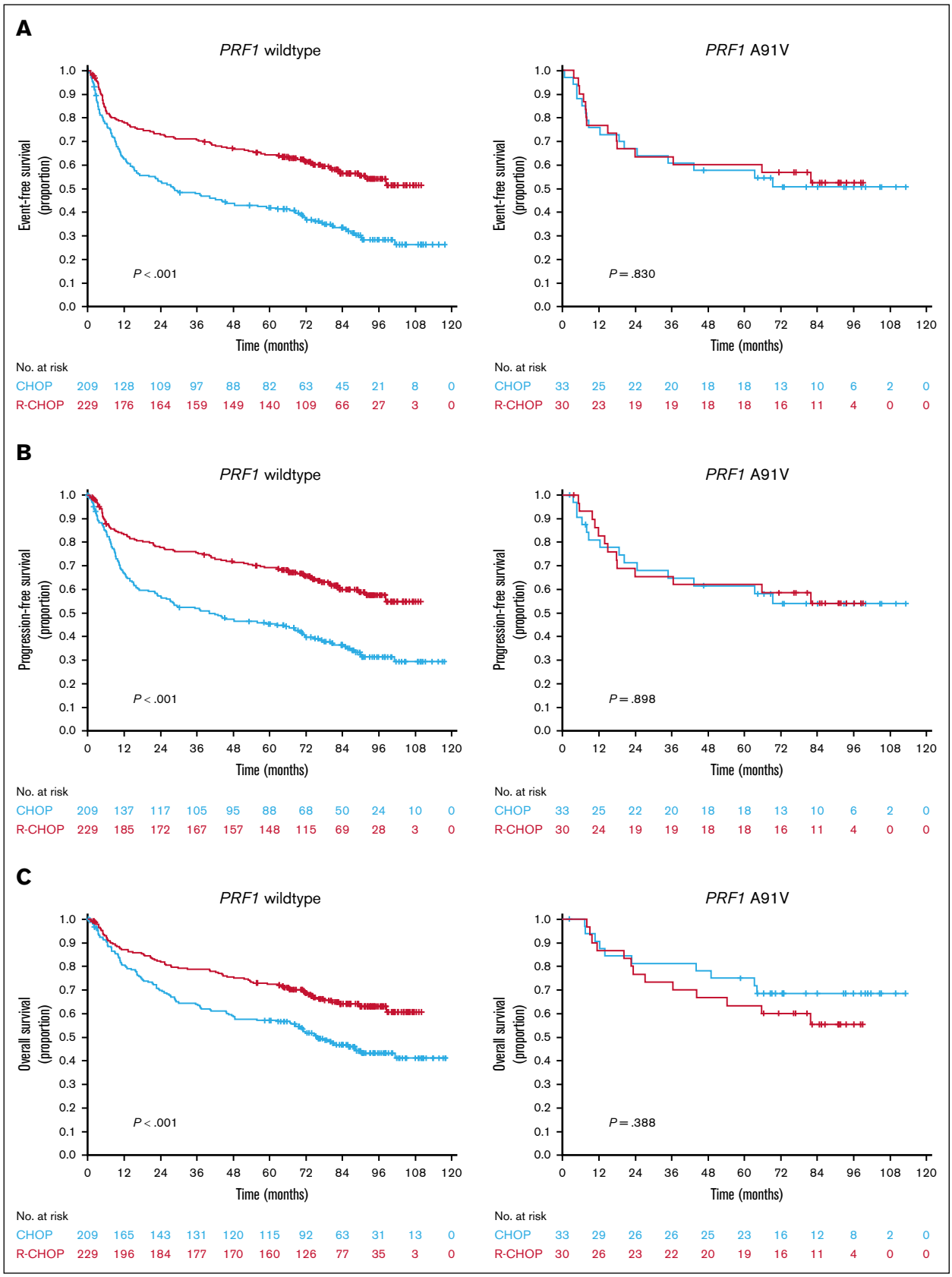

Figure 1. Kaplan-Meier survival curves for 501 patients in the RICOVER-60 trial. Survival outcomes according to *PRF1* wild-type (left) or *PRF1 A91V* (right) status are shown for EFS (A), PFS (B), and OS (C) treated with CHOP alone (blue) or R-CHOP (red).

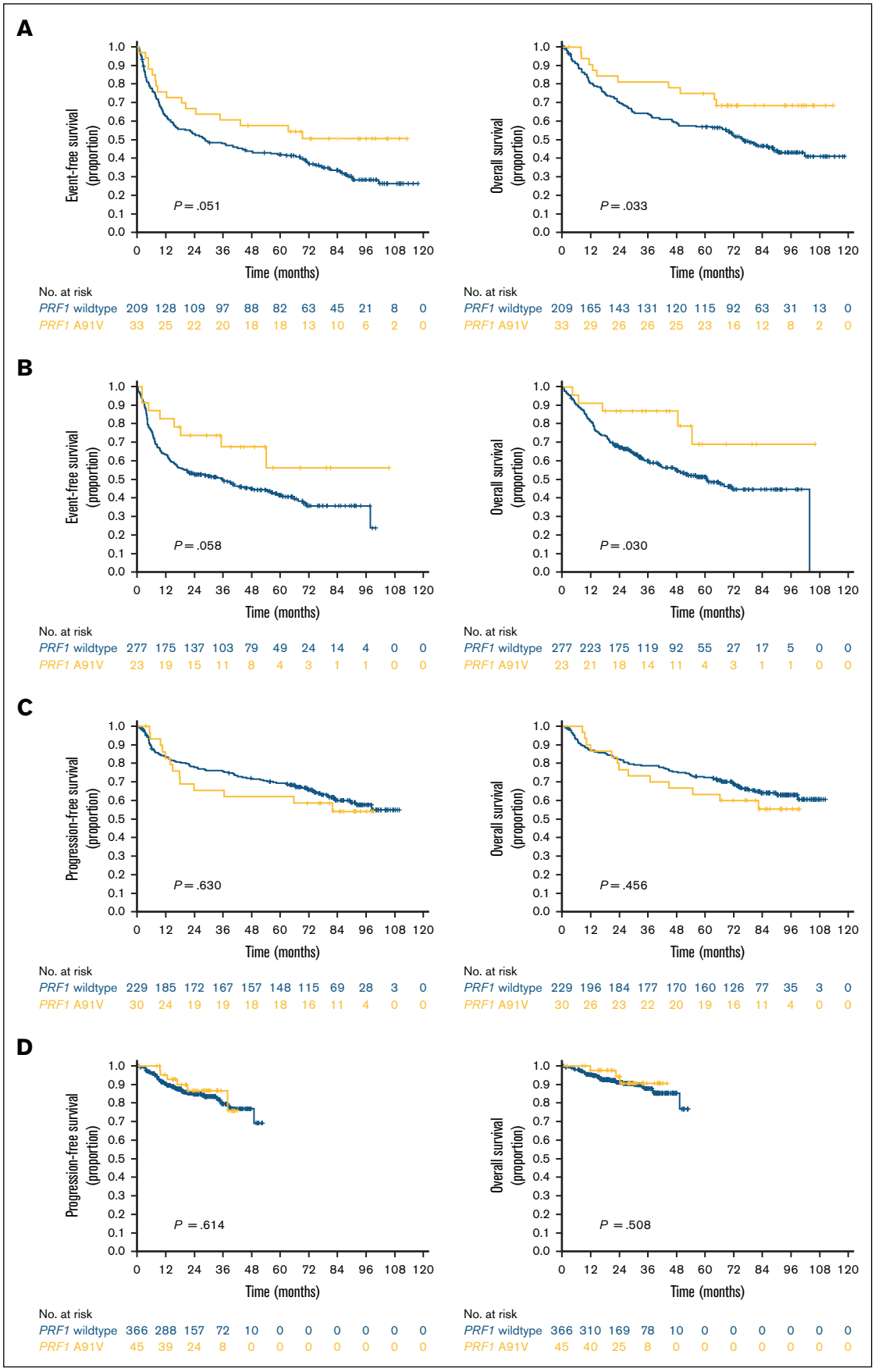

Figure 2.

only treatment) and OPTIMAL >60 trials (CHOP with rituximab) served as independent validation cohorts (supplemental Figure 1).[17,18] Throughout, genomic DNA was isolated from whole blood using the QIAamp DNA Blood Kit (Qiagen). Variant tests were performed using TaqMan (Applied Biosystems) single nuclear polymorphism genotyping assays (C_25600964).

Of 1222 older patients (aged 61-80 years) enrolled in the RICOVER-60 trial, blood samples were available from 501 patients (rituximab, cyclophosphamide, doxorubicin, vincristine, plus oral prednisone or prednisolone [R-CHOP], n = 259; CHOP, n = 242), who were representative of the full trial cohort (supplemental Table 1). Among those, 60 (12%) were heterozygous (G/A) and 3 (0.6%) homozygous (A/A) for the *PRF1* A91V risk allele, whereas 438 patients (87%) represented the wild type (G/G) with no amino acid exchange in position 91. This distribution matched Hardy-Weinberg equilibrium expectations ($P = .952$). Compared to the general European population, there was a slight overrepresentation of heterozygous (G/A) and homozygous (A/A) carriers for the *PRF1* A91V variant. Baseline clinical characteristics did not differ significantly between carriers and wild type (supplemental Tables 2 and 3).

In patients treated with CHOP only, *PRF1* A91V carriers showed a favorable prognostic trend in event-free survival (EFS; $P = .051$), progression-free survival (PFS; $P = .053$), and a significantly improved overall survival (OS; $P = .033$), with 36-month EFS at 61% vs 48%, PFS at 65% vs 52%, and OS at 81% vs 64% for carriers vs wild type (Figure 1). These effects were confirmed in multivariate analyses, with significant differences for all outcomes (EFS hazard ratio [HR], 0.6 [$P = .031$]; PFS HR, 0.5 [$P = .031$]; OS HR, 0.5 [$P = .033$]; supplemental Table 4).

In contrast, *PRF1* A91V carriers did not show additional benefit from rituximab, which was however strongly evident in patients with *PRF1* wild type. In *PRF1* A91V carriers, the 36-month EFS was 61% with CHOP vs 63% with R-CHOP, PFS was 65% vs 66%, and OS was 81% vs 73% (Figure 1; supplemental Table 5). Multivariate interaction analyses confirmed a significant interaction between *PRF1* A91V status and rituximab for PFS (HR, 2.3; $P = .038$) and OS (HR, 3.1; $P = .013$), supporting a negative predictive impact of *PRF1* A91V on rituximab efficacy (supplemental Table 6). Patients with *PRF1* wild type, representing the majority (87%), however, benefited highly significant from addition of rituximab. Here, the 36-month EFS rose from 48% to 71%, PFS from 52% to 76%, and OS from 64% to 79% (all $P < .001$). This benefit was consistent regardless of the international prognostic index, sex, or age (EFS HR, 0.5; PFS HR, 0.5; OS HR, 0.6; all $P < .001$; supplemental Table 5).

In the NHL-B2 validation cohort of 300 patients with diffuse large B-cell lymphoma, 22 (7%) were heterozygous and 1 (0.3%) homozygous for variants resulting in *PRF1* A91V. No clinical baseline differences were noted, and genotype frequencies aligned with Hardy-Weinberg expectations (supplemental Table 7). A favorable trend for *PRF1* A91V carriers treated with CHOP or cyclophosphamide, doxorubicin, vincristine, etoposide and prednisone or prednisolone 14/21 was observed, with a 36-month EFS of 68% vs 50% ($P = .058$) and significantly higher OS at 87% vs 60% ($P = .030$; Figure 2). Multivariate analysis supported the findings from the CHOP-only arm of the RICOVER-60 trial (supplemental Table 4).

To further validate *PRF1* A91V as a potential predictor for the benefit due to rituximab in patients with aggressive B-NHL, interim data from the OPTIMAL >60 trial (all treated with rituximab) were analyzed, including 411 patients with available blood samples. Among those, 45 (11%) were *PRF1* A91V carriers. Patient characteristics were balanced, except for a poorer performance status among carriers (Eastern Cooperative Oncology Group performance status >1, 13% vs 5%; supplemental Table 9). No significant differences were seen in PFS (HR, 0.8; $P = .635$) or OS (HR, 0.7; $P = .526$) for carriers of *PRF1* A91V or wild type, replicating the observation of RICOVER-60 that carriers of *PRF1* A91V treated with R-CHOP have not a better outcome than those with wild-type *PRF1* (Figure 2; supplemental Table 4).

In the CLL-8 trial, the predictive role of *PRF1* A91V for rituximab was not validated in multivariate interaction analysis, although trends similar to those in aggressive B-NHL were observed (supplemental Figures 2 and 3; supplemental Tables 10-11). The allele frequency of *PRF1* A91V (rs35947132) is ~4% in the White population (~8% heterozygosity), with lower frequencies in Asians. Interestingly, CLL-8 had a higher proportion of heterozygous carriers (119/589 [20%]) than aggressive lymphoma cohorts or the general population, indicating a potential contribution in CLL pathogenesis (supplemental Table 12).

In summary, this retrospective analysis showed that carriers of the *PRF1* A91V variant in the context of aggressive B-NHL had favorable outcomes with sole CHOP chemotherapy but did not benefit from the addition of rituximab, suggesting *PRF1* A91V as a possible predictive biomarker for impaired ADCC, which may relate to impaired cytotoxic immune function. Despite impaired perforin-mediated cytotoxicity, compensatory mechanisms such as FASL- or TRAIL-mediated killing or complement activity could however suffice in these patients.[19] Alternatively, our findings may be explained by *PRF1* A91V carriers presenting with inherently less aggressive disease, consistent with findings in murine models of lymphomagenesis showing perforin to be more critical in controlling low-grade lymphomas.[20] Although OS was significantly improved and EFS and PFS trends were favorable in CHOP-treated *PRF1* A91V carriers, the absence of a rituximab-related benefit in this group introduces a predictive dimension not yet fully explained, particularly given its limited replication in CLL, which is, however, more dependent on complement-dependent cytotoxicity compared to ADCC.

Collectively, our study is limited by the targeted nature of variant selection. Future studies should broaden the genetic scope to assess other known cytotoxic pathway components such as MUNC13-4, syntaxin 11, and MUNC18-2, which are also implicated in fHLH and might also influence response to rituximab.[21-23] Considering the predictive role of *PRF1* A91V alongside other

Figure 2. Survival analysis validating results from the RICOVER-60 trial with NHL-B2 and OPTIMAL >60 cohorts. Kaplan-Meier curves for patients treated with CHOP alone in the RICOVER-60 trial (A) and the NHL-B2 trial (B) according to *PRF1* wild-type (blue) or *A91V* carrier status (yellow) showing EFS (left) and OS (right). Kaplan-Meier curves for patients with CHOP and rituximab in the RICOVER-60 trial (C) and of the interim analysis of OPTIMAL >60 trial (D) according to PRF1 wild-type (blue) or A91V carrier status (yellow) with PFS (left) and OS (right).

known modulators of rituximab efficacy, such as low FcγRIIIa expression, vitamin D deficiency, and KIR2DS1-HLA-C2 interactions, might refine patient stratification.[9-11] Exploring the relevance of *PRF1* variants across lymphoma entities and immunotherapeutic settings appears to be worthwhile.

The study was approved by the Ethikkommission der Ärztekammer des Saarlandes (approval number N147/17).

The trials were registered at multiple clinical trials registries as follows: NCT0052936/EU-20243; NCT01478542 NHL-B2 (Pfreundschuh et al); and NCT00281918.

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References

1. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med*. 2002;346(4):235-242.
2. Pfreundschuh M, Trümper L, Osterborg A, et al. CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. *Lancet Oncol*. 2006;7(5):379-391.
3. Weng WK, Levy R. Expression of complement inhibitors CD46, CD55, and CD59 on tumor cells does not predict clinical outcome after rituximab treatment in follicular non-Hodgkin lymphoma. *Blood*. 2001;98(5):1352-1357.
4. Reff ME, Carner K, Chambers KS, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood*. 1994;83(2):435-445.
5. Dall'Ozzo S, Tartas S, Paintaud G, et al. Rituximab-dependent cytotoxicity by natural killer cells: influence of FCGR3A polymorphism on the concentration-effect relationship. *Cancer Res*. 2004;64(13):4664-4669.
6. Lefebvre ML, Krause SW, Salcedo M, Nardin A. Ex vivo-activated human macrophages kill chronic lymphocytic leukemia cells in the presence of rituximab: mechanism of antibody-dependent cellular cytotoxicity and impact of human serum. *J Immunother*. 2006;29(4):388-397.
7. Bowles JA, Wang SY, Link BK, et al. Anti-CD20 monoclonal antibody with enhanced affinity for CD16 activates NK cells at lower concentrations and more effectively than rituximab. *Blood*. 2006;108(8):2648-2654.
8. Zöphel S, Küchler N, Jansky J, et al. CD16+ as predictive marker for early relapse in aggressive B-NHL/DLBCL patients. *Mol Cancer*. 2024;23(1):210.
9. Ahlgrimm M, Pfreundschuh M, Kreuz M, Regitz E, Preuss KD, Bittenbring J. The impact of Fc-γ receptor polymorphisms in elderly patients with diffuse large B-cell lymphoma treated with CHOP with or without rituximab. *Blood*. 2011;118(17):4657-4662.
10. Kaddu-Mulindwa D, Altmann B, Robrecht S, et al. KIR2DS1–HLA-C status as a predictive marker for benefit from rituximab: a post-hoc analysis of the RICOVER-60 and CLL8 trials. *Lancet Haematol*. 2022;9(2):e133-e142.
11. Bittenbring JT, Neumann F, Altmann B, et al. Vitamin D deficiency impairs rituximab-mediated cellular cytotoxicity and outcome of patients with diffuse large B-cell lymphoma treated with but not without rituximab. *J Clin Oncol*. 2014;32(29):3242-3248.
12. Neumann F, Acker F, Schormann C, Pfreundschuh M, Bittenbring JT. Determination of optimum vitamin D3 levels for NK cell-mediated rituximab- and obinutuzumab-dependent cellular cytotoxicity. *Cancer Immunol Immunother*. 2018;67(11):1709-1718.
13. De Saint Basile G, Ménasché G, Fischer A. Molecular mechanisms of biogenesis and exocytosis of cytotoxic granules. *Nat Rev Immunol*. 2010;10(8):568-579.
14. Clementi R, Locatelli F, Dupré L, et al. A proportion of patients with lymphoma may harbor mutations of the perform gene. *Blood*. 2005;105(11):4424-4428.
15. Chia J, Yeo KP, Whisstock JC, Dunstone MA, Trapani JA, Voskoboinik I. Temperature sensitivity of human perforin mutants unmasks subtotal loss of cytotoxicity, delayed FHL, and a predisposition to cancer. *Proc Natl Acad Sci U S A*. 2009;106(24):9809-9814.
16. Pfreundschuh M, Schubert J, Ziepert M, et al. Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20+ B-cell lymphomas: a randomised controlled trial (RICOVER-60). *Lancet Oncol*. 2008;9(2):105-116.
17. Pfreundschuh M, Trümper L, Kloess M, et al. Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of

elderly patients with aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. *Blood*. 2004;104(3):634-641.

18. Pfreundschuh M, Christofyllakis K, Altmann B, et al. Radiotherapy to bulky disease PET-negative after immunochemotherapy in elderly DLBCL patients: results of a planned interim analysis of the first 187 patients with bulky disease treated in the OPTIMAL>60 study of the DSHNHL. *J Clin Oncol*. 2017;35(suppl 15):7506, 7506.
19. Knörck A, Schäfer G, Alansary D, et al. Cytotoxic efficiency of human CD8+ T cell memory subtypes. *Front Immunol*. 2022;13:838484.
20. Bolitho P, Street SEA, Westwood JA, et al. Perforin-mediated suppression of B-cell lymphoma. *Proc Natl Acad Sci USA*. 2009; 106(8):2723-2728.
21. Feldmann J, Callebaut I, Raposo G, et al. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). *Cell*. 2003;115(4): 461-473.
22. zur Stadt U, Rohr J, Seifert W, et al. Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired binding to syntaxin 11. *Am J Hum Genet*. 2009;85(4):482-492.
23. zur Stadt U, Schmidt S, Kasper B, et al. Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. *Hum Mol Genet*. 2005;14(6):827-834.