Transfusion Medicine and Hemotherapy

Research Article

Transfus Med Hemother 2024;51:187–194
DOI: 10.1159/000534273

Received: May 8, 2023
Accepted: September 22, 2023
Published online: October 25, 2023

Evorpacept-Induced Interference and Application of a Novel Mitigation Agent, Evo-NR, in Pretransfusion Testing

Eungjun Yoon[a] Tae Yeul Kim[a] Hyungsuk Kim[b] Duck Cho[a, c]

[a]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea; [b]Department of Laboratory Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea; [c]Department of Health Sciences and Technology, Samsung Advanced Institute for Health Sciences and Technology, Sungkyunkwan University, Seoul, Republic of Korea

Keywords

CD47 antigen · Immune checkpoint inhibitors · Blood safety · Erythrocyte transfusion · Clinical laboratory technique

Abstract

Introduction: Evorpacept is a CD47-blocking agent currently being developed for the treatment of various cancers. Interference by evorpacept in pretransfusion compatibility testing has been reported at limited plasma concentrations. Although various mitigation strategies have been proposed, none are practical. This in vitro study assessed evorpacept-induced interference at extended concentrations and investigated the capability of a novel mitigation agent, Evo-NR. ***Methods:*** Antibody screening tests were performed on evorpacept-spiked plasma with (anti-E and anti-Jk^a) or without alloantibodies at evorpacept concentrations up to 2,000 μg/mL using manual gel cards and automated analyzers. Evorpacept-coated red blood cells (RBCs) (rr [ce/ce], Fy[a+b−], S−s+) were tested by direct antiglobulin testing (DAT) and antigen typing using anti-Fy^b and anti-S reagents at indirect antiglobulin testing (IAT) phase. Evo-NR was used to resolve the interference in plasma and RBC samples. Flow cytometry was used to assess the mitigation effects. ***Results:*** Evorpacept-spiked plasma showed panreactive interference in antibody screening tests using manual gel cards (2+ to 3+) and automated analyzers (4+). A carryover effect was also observed in the automated analyzers. The use of a 3- to 6-fold molar excess of Evo-NR effectively resolved the interference in the plasma and enabled accurate alloantibody identification. Although the reduction in evorpacept binding to RBCs was identified via flow cytometry, Evo-NR was incapable of resolving the serologic interference observed in DAT and antigen typing at IAT phase. ***Discussion:*** Evorpacept showed constant panreactivity and a carryover effect at high concentrations. Evo-NR successfully resolved the interference in the plasma samples and could be considered a practical and efficient mitigation solution. Implementation of Evo-NR has the potential to support RBC transfusion for patients undergoing evorpacept treatment.

Introduction

Advances in antitumor biological agents have remarkably improved clinical outcomes in patients with advanced cancer. However, because some target molecules are not specific to tumor cells, unexpected consequences have been reported. These issues are not limited to clinical manifestations; they include interference in laboratory tests, particularly pretransfusion compatibility

Eungjun Yoon and Tae Yeul Kim contributed equally to this work.

karger@karger.com
www.karger.com/tmh

Correspondence to:
Hyungsuk Kim, hyungsuk.kim79@gmail.com
Duck Cho, duck.cho@skku.edu

testing. A well-known interference caused by a biological agent is the in vitro panreactivity observed during pretransfusion compatibility testing induced by unintended binding of anti-CD38 monoclonal antibodies to red blood cells (RBCs) [1–3]. Currently, these agents are widely used for treating patients with multiple myeloma, and many blood banks have implemented practical mitigation strategies, including dithiothreitol (DTT)-based methods.

CD47, first identified in RBCs as a "marker for self," is a glycoprotein that is ubiquitously expressed in a broad range of human cells and is associated with innate immune responses [4–8]. Signal regulatory protein alpha (SIRPα), an inhibitory receptor expressed on myeloid cells, recognizes CD47 and inhibits macrophage phagocytic activity. The upregulation of CD47 in tumor cells is a key component in evading myeloid cell-mediated elimination. Therefore, the CD47-SIRPα pathway, which functions as an immune checkpoint, has become a promising therapeutic target for cancer treatment [4, 7–9]. In addition to tumor cells, RBCs highly express CD47 [10–13]. CD47 is a component of the Rh complex, and CD47 expression in RBCs is affected by the Rh phenotype. CD47 is most abundantly expressed in D– RBCs, followed by D+, D––, and Rh_{null} RBCs [10–12, 14]. CD47 expression also decreases with RBC age, which leads to the clearance of aged RBCs by macrophages [14, 15]. Direct binding of CD47 targeting agents with an active Fc to RBCs, resulting in the direct activation of FcG receptors and myeloid clearance, is considered as the cause of anemia, which is a commonly reported adverse effect observed in patients treated with this class of agents [5, 7, 8]. In addition, binding of CD47 interferes with pretransfusion test causing panreactive results. Interference in pretransfusion compatibility testing has the potential to delay blood transfusion and/or result in hemolytic transfusion reaction by masked unexpected alloantibodies. Such interference has been consistently reported for several CD47-blocking agents [16–25]. Magrolimab (Hu5F9-G4), an anti-CD47 IgG4 monoclonal antibody, was the first CD47-blocking agent reported to interfere with pretransfusion compatibility testing [21]. Magrolimab caused strong false-positive results in antibody testing, including the immediate spin (IS), room temperature (RT), and IAT phases; however, false positivity is weak (microscopic) or absent in DAT and antigen testing [20–24]. The use of non-IgG4 reactive anti-human globulin and multiple rounds of allogeneic adsorption were effective in resolving the interference. SRF231, another anti-CD47 IgG4 monoclonal antibody, also interfered with pretransfusion compatibility testing; no spontaneous agglutination was observed in vitro; however, strong interference was detected in the IAT phase [25]. In contrast, other CD47-blocking agents, such as ontorpacept and AO-176, have been reported to cause minimal erythrocyte binding [26, 27]. However, whether such agents are interference-free in pretransfusion compatibility testing remains unclear because hemagglutination reactivity can be augmented in the antiglobulin phase.

Evorpacept, also known as ALX148, is a CD47-blocking fusion protein comprised a high-affinity engineered D1 domain of SIRPα and an inactive human IgG1 Fc domain [28], designed to minimize dose-dependent cytopenias. Currently, evorpacept is being studied in numerous clinical trials in combination with conventional chemotherapy or immune checkpoint inhibitors for patients with various advanced cancers (NCT03013218, NCT04417517, NCT04675294, NCT04675333, NCT04755244, NCT05002127, NCT05025800, NCT05027139, NCT05167409, NCT05467670, and NCT05524545). Similar to other CD47-blocking agents, interference in pretransfusion compatibility testing has been reported in patients using evorpacept, which may impact interpretation of on-treatment unexpected RBC antibody identification [16–19]. Previously reported pattern of interference by evorpacept was considerably different from that of magrolimab. In contrast to magrolimab, evorpacept did not cause spontaneous agglutination, DAT was positive, and strong interference was primarily observed in the IAT phase and not in the IS or RT phases during antibody testing [18, 19]. For patients receiving evorpacept, blood type and baseline unexpected antibody status are determined before starting treatment, often with extended RBC antigen genotyping and/or phenotyping. This information is used to guide RBC transfusion while receiving evorpacept treatment. Previous in vitro studies have identified interference caused by evorpacept at plasma concentrations up to 500 μg/mL. Several mitigation strategies, including allogeneic adsorption, soluble CD47, and high-affinity SIRPα monomers, have been suggested for resolving the interference. Although some have been shown effective, routine use of these methods in clinical laboratories does not seem practical due to efficacy, material availability, and high cost. Furthermore, in a phase 1 dose-escalation study, the maximum administered dose of evorpacept was 30 mg/kg once every other week, which was consistent with a maximum plasma concentration of 701 ± 169 μg/mL [29].

Despite previous studies, continued investigation of evorpacept interference and possible strategies for mitigation is warranted, as a robust and practical solution would optimize the pretransfusion testing process. It is necessary to further assess the interference of evorpacept in pretransfusion compatibility testing and investigate potential mitigation strategies under high plasma concentrations of evorpacept that can be practically implemented for routine use in blood banks. In this in vitro study, the interference of evorpacept in pretransfusion compatibility testing was evaluated beyond the maximum plasma concentration previously reported. In addition,

Evo-NR, a newly developed potential mitigation agent for proof-of-concept testing, which is a high-affinity chimeric anti-SIRPα monoclonal antibody containing a mouse Fc region, was evaluated to resolve the effect of evorpacept in pretransfusion compatibility testing.

Materials and Methods

Samples

Residual blood samples from routine pretransfusion compatibility testing were collected: group AB, D+ plasma with no unexpected antibody, anti-E or anti-Jk^a antibody, and group O, D−− RBCs. Samples were carefully selected after confirming the results of ABO typing, RhD typing, RhCE typing, antibody screening, and antibody identification.

Serologic Testing of Evorpacept-Spiked Plasma

To assess the interference induced by evorpacept in indirect antiglobulin testing (IAT), evorpacept (ALX Oncology, South San Francisco, CA, USA) was spiked into residual plasma without unexpected antibodies. Briefly, group AB, D+ plasma without unexpected antibodies were pooled, and evorpacept was added to the pooled plasma at final plasma evorpacept concentrations of 0.1, 1.0, 10.0, 100.0, 1,000.0, and 2,000 μg/mL. The highest concentration of 2,000 μg/mL was expected to be above that reported in patients participating in recent dose-escalation clinical trials [29]. Manual antibody screening tests were performed using gel IAT (ID-Card LISS/Coombs, Bio-Rad, Cressier, Switzerland) with four RBC reagents: R1R1 (DCe/DCe) and R2R2 (DcE/DcE) RBCs from ID-DiaCell I-II (Bio-Rad), rr (ce/ce) RBCs from ID-DiaPanel (Bio-Rad), and an in-house manufactured 0.8% suspension of group O+, D−− RBCs. Automated antibody screening tests were also performed with the same set of evorpacept-spiked samples using two automated immunohematologic analyzers: a solid-phase red cell adherence (SPRCA) testing platform Galileo Neo (Immucor, Norcross, GA, USA) with Capture-R Ready-Screen cells (Immucor) and the erythrocyte-magnetized technology (EMT)-based QWALYS 3 (Diagast, Loos, France) with HEMASCREEN cells (Diagast). To assess the carryover effect in automated analyzers, evorpacept-spiked samples were first analyzed, and blank plasma was subsequently tested three times consecutively in each device.

We intended to prevent the binding of evorpacept to RBCs by neutralizing evorpacept in the plasma with Evo-NR. To assess the mitigation capacity of Evo-NR, 1-, 2-, 3-, 4-, 5-, and 6-fold molar excess of Evo-NR was added to evorpacept-spiked plasma samples at concentrations of 0.1, 1, 10, 100, 1,000, and 2,000 μg/mL and incubated for 30 min at RT. Manual antibody screening tests were performed using gel IAT (ID-Card LISS/Coombs, Bio-Rad) with three RBC reagents: R1R1 (DCe/DCe) and R2R2 (DcE/DcE) RBCs from ID-DiaCell I-II (Bio-Rad) and rr (ce/ce) RBCs from ID-DiaPanel (Bio-Rad). In addition, we demonstrated the resolution of evorpacept interference using Evo-NR in plasma samples containing alloantibodies. Anti-E and anti-Jk^a were chosen because both antibodies cause clinically significant hemolytic transfusion reaction, and anti-E is most frequently observed antibody against Rh complex, and anti-Jk^a generally requires IAT phases due to its low reactivity [30]. Group AB, D+ plasma samples with anti-E or anti-Jk^a antibodies were tested by manual antibody screening using the same gel IAT method described above to obtain the initial baseline results. After being spiked with evorpacept to a plasma concentration of 2,000 μg/mL to induce interference, the samples were subsequently tested before and after being spiked with a 6-fold molar excess of Evo-NR, followed by a 30-min incubation at RT. The E, Jk^a, and Jk^b phenotypes of R1R1 (DCe/DCe), R2R2 (DcE/DcE), and rr (ce/ce) cells used were E−Jk(a−b+), E+Jk(a+b−), and E−Jk(a+b−), respectively.

Serologic Testing of Evorpacept-Coated RBCs

To assess evorpacept interference in direct antiglobulin testing (DAT) and extended blood group antigen typing at IAT phase, rr (ce/ce) RBCs (Panoscreen, Immucor) of Fy(a+b−), S−s+ phenotype were selected as CD47 is the most abundant in rr (ce/ce) RBCs [10–12, 14]. Fy(a+b-) and S-s+ phenotype was selected to investigate the mitigation capability of Evo-NR in antigen typing at the IAT phases. In vivo binding of evorpacept to the RBCs of patients was mimicked by in vitro incubation of one volume of RBCs, with two volumes of 10 μg/mL evorpacept-spiked plasma at 37°C for 30 min, followed by two washes with normal saline. DAT for evorpacept-coated RBCs was performed via the tube method with Gamma-clone anti-IgG (Immucor). To dissociate evorpacept from the RBCs, 500-, 1,000-, 1,500-, and 2,000-fold molar excess of Evo-NR was added to one volume of evorpacept-coated RBCs, incubated for 2 min at RT, and washed twice with normal saline. For comparison, 10 volumes of ethylenediaminetetraacetic acid/glycine hydrochloric acid (EGA) elution solution and phosphate-buffered saline (PBS) were separately added to one volume of evorpacept-coated RBCs and incubated. After incubation, another round of DAT was performed. Additional antigen typing was performed with pre- and post-Evo-NR-treated RBCs using tube IAT with ORTHO Sera anti-Fy^b and anti-S murine monoclonal antibodies (Ortho-Clinical Diagnostics, Raritan, NJ, USA) and Gamma-clone anti-IgG (Immucor).

Flow Cytometry

Flow cytometry was performed to evaluate the attachment of evorpacept to RBCs and the neutralizing or dissociation capabilities of Evo-NR. The following three mixtures were prepared: 25 μL evorpacept-spiked plasma (0.1, 1, 10, 100, 1,000, and 2,000 μg/mL) was mixed with 50 μL 0.8% rr (ce/ce) RBCs (ID-DiaCell III, Bio-Rad); 25 μL evorpacept-spiked plasma (2,000 μg/mL) preincubated with 1-, 3-, 6-, and 9-fold molar excess of Evo-NR was mixed with 50 μL 0.8% rr (ce/ce) RBCs (ID-DiaCell III); and one volume of 3% rr (ce/ce) RBCs (Panoscreen, Immucor), preincubated with two volumes of 10 μg/mL evorpacept-spiked plasma followed by two washes with normal saline, was mixed with 10 volumes of EGA or PBS, or 500-, 1,000-, 1,500-, and 2,000-fold molar excess of Evo-NR. FITC-conjugated F(ab')2 goat anti-human IgG (Jackson ImmunoResearch, West Grove, PA, USA) was used for staining, as described in a previous study [18]. The test results were compared to those obtained using a blank plasma sample as a negative control. Approximately 20,000 events were acquired on a flow cytometer (FACSVerse, BD Biosciences, San Jose, CA, USA) for each mixture, and RBCs were gated using forward and side scatter parameters.

Results

Interference of Evorpacept in Plasma Samples and the Mitigation Capability of Evo-NR

The summary of serologic test findings on evorpacept interference and mitigation using Evo-NR is described in Table 1. Panreactive interference was observed in both manual and automated antibody screening tests at plasma

Table 1. Summary of serologic testing on evorpacept interference and mitigation using Evo-NR

Test	Results
Antibody screening/identification (IAT)	
Interference test (evorpacept-spiked plasma)	
Manual gel method	
Plasma without unexpected antibody	Panreactive (2+ to 3+)
Plasma with anti-E or anti-Jk[a]	Panreactive (3+)
Automated analyzer	
SPRCA (Galileo Neo, Immucor)	Panreactive (4+), carryover at evorpacept 10.0 μg/mL or higher
EMT (QWALYS 3, Diagast)	Panreactive (4+), carryover at evorpacept 1,000.0 μg/mL or higher
Mitigation test (evorpacept-spiked plasma, Evo-NR incubation)	
Manual gel method	
Plasma without unexpected antibody	Not reactive at 3- to 6-fold molar excess Evo-NR
Plasma with anti-E or anti-Jk[a]	Anti-E and anti-Jk[a] identified at 6-fold molar excess Evo-NR
RBC antigen typing (rr [ce/ce], Fy[a−b+], S−s+ RBCs)	
Interference test (evorpacept-coated RBCs)	
DAT	Reactive (2+)
Fy[b] and S antigen (IAT)	Reactive (2+)
Mitigation test (evorpacept-coated RBCs, Evo-NR incubation)	
DAT	Reactive (2+) at 2,000-fold molar excess Evo-NR
Fy[b] and S antigen (IAT)	Reactive (2+) at 2,000-fold molar excess Evo-NR

SPRCA, solid-phase red cell adherence; EMT, erythrocyte magnetization technology; RBC, red blood cells; DAT, direct antiglobulin test; IAT, indirect antiglobulin test.

evorpacept concentrations as low as 0.1 μg/mL (online suppl. Table S1; for all online suppl. material, see https://doi.org/10.1159/000534273). The strength of hemagglutination was 2+ to 3+ in the manual gel card method, with the D−− cell showing weaker reactivity than the cells of other phenotypes. Strong reactivity (4+) was observed at all evorpacept concentrations in both automated analyzers (online suppl. Table S1). Flow cytometric analysis confirmed evorpacept binding to RBCs, showing saturation at an evorpacept concentration of 1 μg/mL (Fig. 1a). Noticeable carryover was observed in plasma evorpacept concentrations starting from 10.0 μg/mL and 1,000.0 μg/mL for SPRCA and EMT methods, respectively, affecting subsequent blank samples in automated analyzers (online suppl. Table S1). For alloantibody-positive samples, the presence of anti-E and anti-Jk[a] was masked by panreactivity in evorpacept-spiked plasma (online suppl. Table S2).

To resolve evorpacept-induced interference in IAT, 6-fold molar excess Evo-NR-treated plasma showed complete resolution of the interference caused by the highest plasma evorpacept concentration of 2,000 μg/mL (online suppl. Table S2). At lower concentrations, a smaller amount of Evo-NR could clear the effect of evorpacept; however, at least a 3-fold molar excess of Evo-NR was required. The addition of Evo-NR to evorpacept-spiked plasma samples containing anti-E or anti-Jk[a] successfully resolved the interference, revealing the corresponding alloantibody patterns (online suppl. Table S2). In flow cytometric analysis, the addition of Evo-NR remarkably reduced the binding of evorpacept to RBCs, with the mean fluorescence intensity reaching a plateau at an approximately 3-fold molar excess of Evo-NR (Fig. 1b). Although a 6-fold molar excess of Evo-NR was sufficient to resolve the interference in serological testing, a minimal degree of evorpacept binding was still detected in flow cytometry, even when treated with a larger amount of 9-fold molar excess. Overall, evorpacept interference in plasma samples was identified in multiple platforms, and Evo-NR effectively resolved the interference.

Interference of Evorpacept in RBC Samples and the Mitigation Capability of Evo-NR

The initial baseline DAT result for evorpacept-coated RBCs was 2+ (Table 1). The DAT results were the same after EGA or Evo-NR treatment. Similar to PBS, neither EGA nor Evo-NR could successfully reduce the reactivity of DAT. The Fy[b] and S antigen typing results (IAT) for evorpacept-coated RBCs before and after Evo-NR treatment also showed 2+ reactivity, resulting in false-positive results. Flow cytometry revealed partial dissociation of evorpacept from RBCs after EGA and Evo-NR treatment (Fig. 1c). Although a ≥500-fold molar excess of Evo-NR showed higher dissociating power than EGA, the mean fluorescence intensity of Evo-NR-treated RBCs were still considerably greater than the negative control

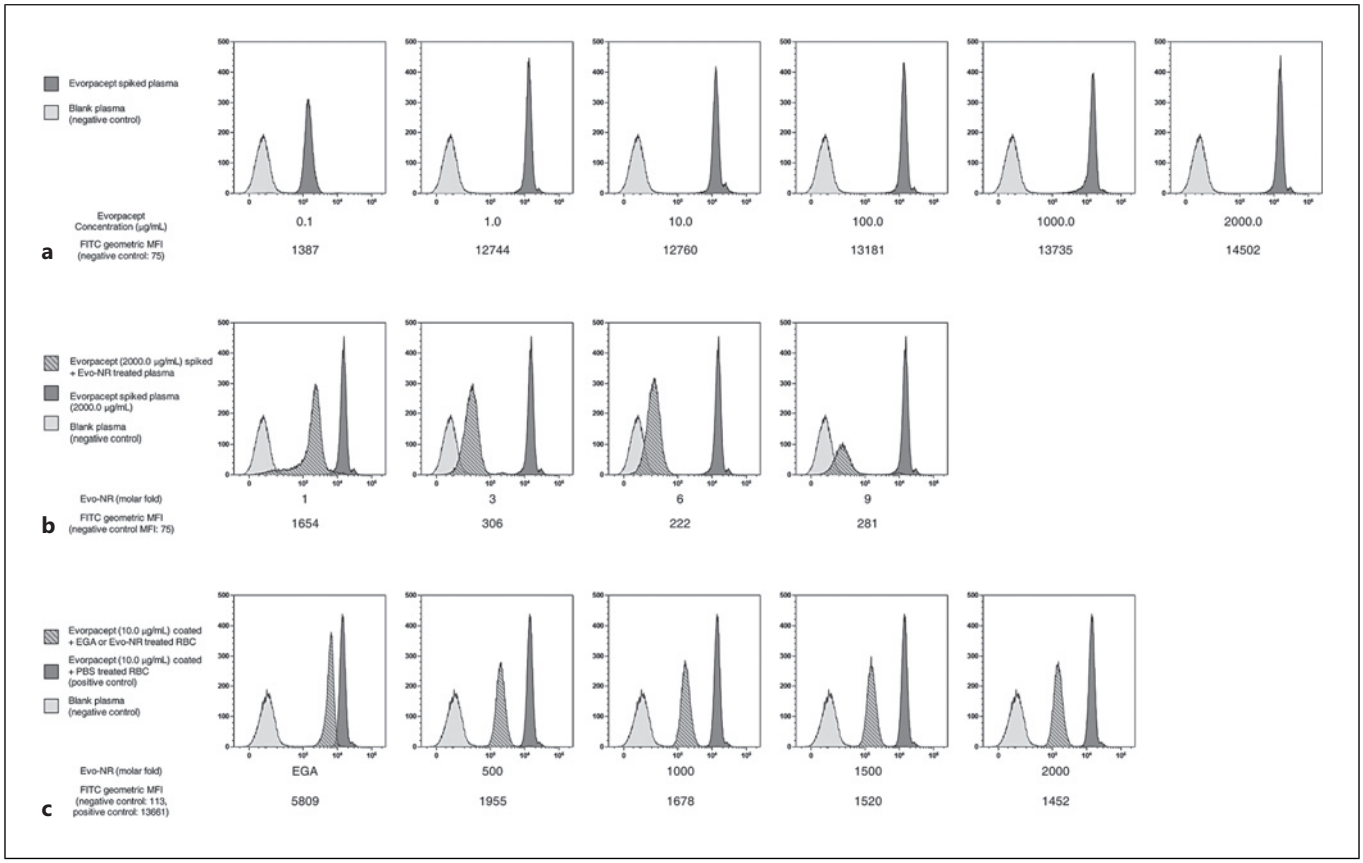

Fig. 1. Flow cytometric analysis of evorpacept binding and the mitigation effect of Evo-NR. **a** Evorpacept-spiked plasma. **b** Evorpacept plasma with Evo-NR treatment. **c** Evorpacept-coated RBCs with Evo-NR treatment. The horizontal axis shows FITC fluorescence intensity, and the vertical shows the number of cells. EGA, ethylenediaminetetraacetic acid/glycine hydrochloric acid; MFI, mean fluorescence intensity; RBC, red blood cell.

and were similar to the evorpacept-spiked plasma of 0.1 μg/mL (which showed a 2+ reactivity in serology). Overall, in the platforms evaluated, evorpacept interference in RBC samples was identified, but Evo-NR and EGA were unable to fully resolve the interference.

Discussion

To the best of our knowledge, this is the first study demonstrating the interference of evorpacept in pretransfusion compatibility testing at high plasma concentrations of up to 2,000 μg/mL, with the inclusion of the carryover effect in automated analyzers and assessment of the capability of a new mitigation agent, Evo-NR. In this study, evorpacept-spiked plasma was panreactive with all reagent RBCs in IAT, as previously reported [18]. Differences in reactivity were minimal among the cells of various Rh phenotypes, with the lowest reactivity being 2+ in D−− RBCs up to a concentration of 100 μg/mL. Unlike daratumumab, which only produced weak positive panreactivity to panel RBCs [1], evorpacept produced moderate to strong reactions, even at a low concentration of 0.1 μg/mL. This may be because CD47 is more highly expressed in RBCs than CD38 [24] and/or the picomolar binding affinity of evorpacept to CD47 [28] versus nanomolar binding of daratumumab to CD38 [31]. Additional speculations can be made based on the greater reactivity observed in plasma containing evorpacept compared with daratumumab. Evorpacept may have a greater carryover effect than daratumumab does. The carryover of daratumumab during antibody screening test was described in a solid-phase instrument, TANGO optimo (Bio-Rad) [32]. However, in our institution, daratumumab carryover in automated analyzers using SPRCA (Galileo Neo) and EMT (QWALYS 3) methods has been considered insignificant because carryover problems have not been identified until now. In contrast, evorpacept showed considerable carryover effects on both platforms, particularly in Galileo Neo. Although we cannot speak to the occurrence of this issue on other platforms yet, it would be advisable for blood banks to be informed about the history of evorpacept administration prior to pretransfusion compatibility testing. Blood banks should either evaluate the specific automated platforms used for antibody testing to avoid potential false-positive

test results or consider the use of manual testing methods. Additionally, similar to daratumumab, which can cause pretransfusion serological testing interference for up to 6 months after administration [29, 33], it may take several months for evorpacept-induced interference to resolve after the cessation of treatment.

While the use of DTT has been generally adopted as a feasible method to resolve the interference by anti-CD38 in pretransfusion compatibility testing [1–3], a reliable and practical method to eliminate evorpacept-induced interference has not yet been established. Although multiple rounds of allogeneic adsorption and high volumes of soluble CD47 (50–100-fold molar excess) or high-affinity SIRPα monomers (300-fold molar excess) have been reported to affect the elimination of evorpacept-induced interference in IAT [16–19], these methods are either laborious or cost-ineffective for daily use in the blood bank. In this study, Evo-NR successfully negated evorpacept-induced interference in antibody screening tests and enabled accurate identification of alloantibodies using only a small amount (3–6-fold molar excess) compared with soluble CD47 and high-affinity SIRPα monomers. Evo-NR neutralizes free evorpacept in the plasma by binding to the SIRPα domain, which is not expressed in RBCs. Therefore, unlike DTT treatment, which leads to the denaturation of RBC antigens containing disulfide bonds, including those from the clinically important Kell blood group [34], Evo-NR likely has no or minimal effect on the antigens of RBCs. Hence, it is expected that performing pretransfusion compatibility test using Evo-NR would enable safe transfusion without additional RBC antigen typing.

Although it did show some effect in flow cytometry, Evo-NR was incapable of sufficiently kicking off RBC-bound evorpacept, as shown in serologic testing. The possible reason for this is that Evo-NR and CD47 may target overlapping epitopes on evorpacept. Anti-SIRPα monoclonal antibodies targeting different epitopes on evorpacept may be able to bind to the evorpacept/CD47 complex and displace evorpacept from RBCs sufficiently to resolve the interference in DAT and RBC antigen typing. For antigens not requiring IAT for typing, this may be of less significance because a previous study revealed that antigen phenotyping can be conducted without interference at the IS and RT phases even after the administration of evorpacept [18]. However, antisera reagents that require IAT for testing differs according to manufacturer and supply. If the patient requires transfusion of antigen-matched RBCs to avoid alloimmunization, extended RBC phenotyping or genotyping prior to use of evorpacept would be recommended as Evo-NR cannot solve interference in antigen typing (such as Fy^b and S in this study) which requires the IAT phase. Another limitation of Evo-NR is that because it specifically targets the SIRPα domain, it is expected to be ineffective in resolving the interference by anti-CD47 monoclonal antibody-based drugs. However, it has the potential to be useful in mitigating the interference by CD47-blocking agents that are similarly structured on a SIRPα fusion protein, such as TTI-621.

For the transfusion of patients receiving CD47-blocking agents, alternative mitigation strategies can be considered. A universally applicable method is to transfuse phenotypically and/or genotypically matched RBCs. This approach can be helpful for preventing alloimmunization, leading to potential hemolytic transfusion reactions, which is well demonstrated in cases of safe transfusion in patients on evorpacept using prior genotypic blood group data via next-generation sequencing [35]. Although there can be debate on the appropriate extent of the type of antigens that need to be matched before RBC transfusion, this approach is particularly useful when serologic methods have limitations in resolving the problem. Although blood group phenotyping and genotyping are still useful for chronically transfused patients requiring the transfusion of antigen-matched RBC to significantly reduce the risk of alloimmunization, the routine use of Evo-NR may be able to diminish the needs for routine blood group phenotyping and genotyping prior to evorpacept use by enabling antibody screening and IAT crossmatching. This would be particularly helpful for patients who require urgent transfusion because finding antigen-matched RBC may take significant amount of time and resources. Patients referred from an outside hospital with unknown RBC antigen profile could also be benefitted. In addition, transfusion of antigen-matched RBCs still has the risk of hemolysis due to antibodies that are missed out depending on the extent of antigen matching. Evo-NR would have the potential to allow IAT crossmatching to cover this problem.

Our study had some limitations. First, because this was an in vitro study, the in vivo interaction between RBCs and evorpacept was not investigated. No apparent downregulation or deterioration of CD47 has been reported in patients using evorpacept; however, long-term in vivo exposure to evorpacept may have unforeseen effects on pretransfusion compatibility testing. Second, all serologic tests on the plasma were conducted in the IAT phase. It would be best if we evaluated IS and RT phases in extended concentration; however, in this study, we focused on IAT because IAT is capable of detecting most clinically significant antibodies, and evorpacept-induced interference was not observed in the IS and RT phases at concentrations ≤500.0 μg/mL in the previous study [18]. However, interference at an extended concentration (1,000–2,000 μg/mL) in the IS and RT phases has not been investigated, and therefore, currently remains unclear. Third, as identification of antibodies other than anti-E and anti-Jk^a under Evo-NR was not tested, the possibility that unexpected interaction between Evo-NR and the RBC antigen or antibody may hinder the accurate

identification of other clinically significant antibodies was not evaluated. Future studies on the validation of Evo-NR's ability to mitigate evorpacept interference using patient samples are anticipated.

Conclusions

In summary, we demonstrated the effect of evorpacept in pretransfusion compatibility testing at high plasma concentrations up to 2,000 μg/mL in IAT, which was in line with the findings from previous studies, and successfully resolved the interference with the use of Evo-NR. Evorpacept showed constant panreactivity and a carryover effect at high concentrations. Although the ability of Evo-NR to kick off RBC-bound evorpacept was limited, its ability to effectively mitigate evorpacept-induced interference in antibody screening has the potential to be considered a practical and efficient solution than previously suggested methods and support RBC transfusion for patients undergoing evorpacept treatment. CD47-blocking agents have shown promising results as immunotherapy candidates for the treatment of cancer. The fast track designation by the United States Food and Drug Administration was granted for the development of magrolimab and evorpacept. As these agents are already being used in hospitals conducting clinical trials and are expected to potentially be available in the market in the coming years, it is necessary for clinicians and blood banks to recognize and prepare for the interference caused by evorpacept and other CD47-blocking agents and their mitigation strategies.

Acknowledgments

The authors thank Soo Hyun Hong for her technical assistance in performing the laboratory experiments. We also thank ALX Oncology for their support in this study and their efforts to develop a reagent for distribution to blood banks and use in neutralizing evorpacept-induced interference in pretransfusion testing.

Statement of Ethics

This in vitro study was approved by the Institutional Review Board of Samsung Medical Center (SMC 2022-04-112-001), and informed consent was waived because clinical information was not collected for research purposes.

Conflict of Interest Statement

All authors declare no potential conflicts of interest.

Funding Sources

Evorpacept and Evo-NR were provided by ALX Oncology. The sponsor has collaborated in the study design but has not been specifically involved in data acquisition and interpretation.

Author Contributions

T.Y.K. designed the study and performed the laboratory experiments. E.Y. and T.Y.K. summarized the data and drafted the first version of the manuscript. H.K. and D.C. supervised the study, edited the manuscript, and contributed equally to this work as co-corresponding authors. All authors have reviewed and approved the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further inquiries can be directed to the corresponding author.

References

1 Chapuy CI, Nicholson RT, Aguad MD, Chapuy B, Laubach JP, Richardson PG, et al. Resolving the daratumumab interference with blood compatibility testing. Transfusion. 2015 Jun;55(6 Pt 2):1545–54.
2 Jones AD, Moayeri M, Nambiar A. Impact of new myeloma agents on the transfusion laboratory. Pathology. 2021 Apr;53(3):427–37.
3 Murphy MF, Dumont LJ, Greinacher A; BEST Collaborative. Interference of new drugs with compatibility testing for blood transfusion. N Engl J Med. 2016 Jul 21;375(3):295–6.
4 Logtenberg MEW, Scheeren FA, Schumacher TN. The CD47-SIRPα immune checkpoint. Immunity. 2020 May 19;52(5):742–52.
5 Matlung HL, Szilagyi K, Barclay NA, van den Berg TK. The CD47-SIRPα signaling axis as an innate immune checkpoint in cancer. Immunol Rev. 2017 Mar;276(1):145–64.
6 Matozaki T, Murata Y, Okazawa H, Ohnishi H. Functions and molecular mechanisms of the CD47-SIRPalpha signalling pathway. Trends Cell Biol. 2009 Feb;19(2):72–80.
7 Qu T, Li B, Wang Y. Targeting CD47/SIRPα as a therapeutic strategy, where we are and where we are headed. Biomark Res. 2022 Apr 13;10(1):20.
8 Veillette A, Chen J. SIRPα-CD47 immune checkpoint blockade in anticancer therapy. Trends Immunol. 2018 Mar;39(3):173–84.
9 Zhang W, Huang Q, Xiao W, Zhao Y, Pi J, Xu H, et al. Advances in anti-tumor treatments targeting the CD47/SIRPα axis. Front Immunol. 2020:1118.
10 Cartron JP. RH blood group system and molecular basis of Rh-deficiency. Baillieres Best Pract Res Clin Haematol. 1999 Dec;12(4):655–89.
11 Lindberg FP, Lublin DM, Telen MJ, Veile RA, Miller YE, Donis-Keller H, et al. Rh-related antigen CD47 is the signal-transducer integrin-associated protein. J Biol Chem. 1994 Jan 21;269(3):1567–70.
12 Mouro-Chanteloup I, Delaunay J, Gane P, Nicolas V, Johansen M, Brown EJ, et al. Evidence that the red cell skeleton protein 4.2 interacts with the Rh membrane complex member CD47. Blood. 2003 Jan 1;101(1):338–44.

13 Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. 3rd ed. London: Elsevier; 2012. p. 147–204.
14 Oldenborg PA, Zheleznyak A, Fang YF, Lagenaur CF, Gresham HD, Lindberg FP. Role of CD47 as a marker of self on red blood cells. Science. 2000 Jun 16;288(5473):2051–4.
15 Olsson M, Oldenborg PA. CD47 on experimentally senescent murine RBCs inhibits phagocytosis following Fcγ receptor–mediated but not scavenger receptor–mediated recognition by macrophages. Blood. 2008 Nov 15;112(10):4259–67.
16 Aye T, Er LS, Armour R, Teramura G, Wu Y. Use of novel recombinant protein to overcome serologic interference by ALX148 CD47 blocker [abstract P-NI-22]. Transfusion. 2020;60(S5):191A.
17 Er LS, Aye T, Armour R, Teramura G, Wu Y, Haile A. First experience with CD47 blocker ALX148 interference in pretransfusion testing [abstract P-IM-56]. Transfusion. 2019;59(S3):136A.
18 Kim TY, Yoon MS, Hustinx H, Sim J, Wan HI, Kim H. Assessing and mitigating the interference of ALX148, a novel CD47 blocking agent, in pretransfusion compatibility testing. Transfusion. 2020 Oct;60(10):2399–407.
19 Velliquette RW, Smeland-Wagman R, Shakarian G, Lomas-Francis C, Kaufman RM, Westhoff CM. Overcoming serological interference in a patient receiving CD47 antagonist ALX148 immunotherapy [abstract P-IM-55]. Transfusion. 2019 2019;59(S3):136A.
20 Brierley CK, Staves J, Roberts C, Johnson H, Vyas P, Goodnough LT, et al. The effects of monoclonal anti-CD47 on RBCs, compatibility testing, and transfusion requirements in refractory acute myeloid leukemia. Transfusion. 2019 Jul;59(7):2248–54.
21 Degtyaryova D, Hong H, Lomas-Francis C, Shakarian G, Westhoff CM. Serological observations in patients receiving Hu5F9-G4 monoclonal anti-CD47 therapy [abstract CP246]. Transfusion. 2017;57(S3):159A.
22 Sikic BI, Lakhani N, Patnaik A, Shah SA, Chandana SR, Rasco D, et al. First-in-human, first-in-class phase I trial of the anti-CD47 antibody Hu5F9-G4 in patients with advanced cancers. J Clin Oncol. 2019 Apr 20;37(12):946–53.
23 Troughton MC, Simmons LW, Langley C, Youssef M, Marques MB. A novel use of human platelet concentrate to resolve interference of anti-CD47 in serologic testing [abstract IGT46]. Transfusion. 2018;58(S2):179A.
24 Velliquette RW, Aeschlimann J, Kirkegaard J, Shakarian G, Lomas-Francis C, Westhoff CM. Monoclonal anti-CD47 interference in red cell and platelet testing. Transfusion. 2019 Feb;59(2):730–7.
25 Velliquette RW, Cai C, Shakarian G, Burgos A, Lomas-Francis C, Westhoff CM. Serological interference in patients receiving SRF231 anti-CD47 immunotherapy [abstract P-IG-37]. Transfusion. 2020;60(S5):159A–60A.
26 Petrova PS, Viller NN, Wong M, Pang X, Lin GH, Dodge K, et al. TTI-621 (SIRPαFc): a CD47-blocking innate immune checkpoint inhibitor with broad antitumor activity and minimal erythrocyte binding. Clin Cancer Res. 2017 Feb 15;23(4):1068–79.
27 Puro RJ, Bouchlaka MN, Hiebsch RR, Capoccia BJ, Donio MJ, Manning PT, et al. Development of AO-176, a next-generation humanized anti-CD47 antibody with novel anticancer properties and negligible red blood cell binding. Mol Cancer Ther. 2020 Mar;19(3):835–46.
28 Kauder SE, Kuo TC, Harrabi O, Chen A, Sangalang E, Doyle L, et al. ALX148 blocks CD47 and enhances innate and adaptive antitumor immunity with a favorable safety profile. PLoS One. 2018;13(8):e0201832.
29 Lakhani NJ, Chow LQM, Gainor JF, LoRusso P, Lee KW, Chung HC, et al. Evorpacept alone and in combination with pembrolizumab or trastuzumab in patients with advanced solid tumours (ASPEN-01): a first-in-human, open-label, multicentre, phase 1 dose-escalation and dose-expansion study. Lancet Oncol. 2021 Dec;22(12):1740–51.
30 Fung MK, Eder AF, Spitalnik SL, Westhoff CM. Technical manual. 19th ed. Bethesda: AABB; 2017.
31 Chami B, Okuda M, Moayeri M, Pirenne F, Hidaka Y, Nambiar A, et al. Anti-CD38 monoclonal antibody interference with blood compatibility testing: differentiating isatuximab and daratumumab via functional epitope mapping. Transfusion. 2022 Nov;62(11):2334–48.
32 Anani WQ, Duffer K, Kaufman RM, Denomme GA. How do I work up pretransfusion samples containing anti-CD38? Transfusion. 2017 Jun;57(6):1337–42.
33 Janssen Biotech, Inc. Horsham (PA): Darzalex package insert [cited 2022 Aug 10]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/761036s004lbl.pdf.
34 Wagner FF. Antibody testing in patients treated with anti-CD38: there is still room for improvement. Blood Transfus. 2020 Jul;18(4):244–6.
35 Kim TY, Yu H, Pahn MT, Jang J, Cho D. Application of blood group genotyping by next-generation sequencing in various immunohaematology case. Transfus Med Hemother. 2022:4988–96.