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*Original article*

# ELP protocol: an original approach for the mitigation of anti-CD38 interference

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**Background** - Transfusion medicine is facing new challenges from therapies which interfere with pre-transfusional tests, such as monoclonal antibodies targeting blood-cell antigens. Anti-CD38 monoclonal antibodies, widely used to treat multiple myeloma, cause panreactivity of indirect antiglobulin test; this can be resolved by treating cells with dithiothreitol to disrupt the CD38 disulphide bonds expressed on red blood cell surfaces. Interference mitigation strategy with dithiothreitol, however, has some drawbacks: it entails losing the traceability of results and the denaturation of blood group systems sensitive to reducing agents; it takes time to perform and quality controls are lost.

**Materials and methods** - Panels were treated with 0.2 mol/L dithiothreitol and stored for 30 days with a commercial preservative solution. On day 30, we measured the hemolysis indices and ability to eliminate daratumumab and isatuximab interference in the treated cells using indirect antiglobulin test. We also tested the stability of erythrocyte antigenic structure by screening 42 samples with known antibodies; tests were repeated on day 1, 7, 15 and 30. All indirect antiglobulin testing was performed on gel card.

**Results** - After 30 days from treatment, panels preserved in preservative solution showed hemolysis indices comparable to untreated panels: all cases of interference by anti-CD38 in pre-transfusional tests were successfully mitigated. All antibodies were detected after 30 days, except for KEL system antibodies, as expected, although there was a detectability of anti-Kell antibodies in high titer samples (the first detection in dithiothreitol-treated cells since 1983).

**Discussion** - We propose the Extended Lifetime Protocol; a simple card-based method which is cheap and traceable, that combines the strengths of anti-CD38 mitigation strategies. It makes it possible to treat and store, at the same time, a sufficient volume of red blood cells, that can be used for the following 30 days, to avoid any delay in transfusional requests.

**Keywords:** *interference, anti-CD38, dithiothreitol, turnaround time, anti-Kell.*

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## INTRODUCTION

Since the introduction of anti-CD38 monoclonal antibodies (MoAbs) for the treatment of oncohematological disorders such as multiple myeloma (MM), the problem has arisen

of how to mitigate interferences in the pre-transfusional tests. The most common method currently involves using dithiothreitol (DTT), but its advantages, such as easy availability and low cost are counterbalanced by obvious disadvantages, including the denaturation of certain erythrocyte antigens, the long lead times for the execution of the procedure, the tendency of in vitro hemolysis of the treated erythrocytes and the lack of traceability of the results mainly linked to the use of test-tube methods.

The aim of this paper is to suggest a method for performing pre-transfusional tests that allows safe recognition of the presence of clinically significant irregular antibodies, an appropriate timeframe for the execution of the interference mitigation protocol and the necessary traceability of the results.

## MATERIALS AND METHODS

Performing serological or molecular extended erythrocyte typing prior to treatment with anti-CD38 MoAbs, as well as correctly assigning the packed red blood cells (pRBCs) that respect the patient's blood group systems sensitive to reducing agents and involved in the development of clinically significant antibodies, has made it possible to develop the extended lifetime protocol (ELP), using 0.2 mol/L DTT in accordance with both the internationally validated protocol for resolving daratumumab (DARA) interference[1,2] and with AABB Technical Manual[3].

Following the treatment of erythrocytes in tubes, the tests were performed in microcolumn, a technology able to guarantee, through digital readers, the traceability of the results. The treated erythrocytes were finally resuspended in preservative solution and stabilized up to 30 days after DTT treatment and/or until expiry, as suggested by Sigle and Lorenzen[4,5].

Erythrocytes treated with 0.2 mol/L DTT and stabilized in preservative solution were evaluated for:

1. reduction/absence of development of *in vitro* hemolysis;
2. stability of erythrocyte antigenic set of screening and identification panels;
3. ability to mitigate serological interference from anti-CD38 MoAbs.

Both screening and antibody identification tests were carried out on EDTA anticoagulated plasma samples, aliquoted and stored at -80°C in the laboratory serum library.

### Preparation of 0,2 mol/L Dithiothreitol

For the preparation of 0.2 mol/L DTT, 1 g of DTT (DL-Dithiothreitol, Sigma-Aldrich [St. Louis, MI, USA]) was diluted in 32 mL of saline solution (0.9% NaCl). 1,5 mL aliquots were stored at –20°C for a maximum of twelve months until use[6].

### Erythrocyte DTT treatment and stabilization in preservative solution

800 µL of erythrocytes of screening and/or identification panels (red blood cell [RBC] Screen-Cyte 3% Medion Grifols Diagnostics AG [Düdingen, Switzerland]/Data-Cyte Plus Reagent Red Blood Cells 3% Medion Grifols Diagnostics AG), positive control cells (RBC pool from group O pRBCs E+e+, E+e-) and negative control cells (RBC pool from group O pRBCs K+k+, K+k–), were washed 3 times in NaCl (3,500 rpm for 5 minutes) with a cell washing centrifuge (Cell Washer Centrifuge Hettich Rotolavit II [Hettich AG, Bäch, Switzerland]), as suggested by the Osaka method[7], in order to reduce the carrying out time of erythrocyte washing procedure.

50 µL of washed packed erythrocytes were incubated for 40 minutes at 37°C in water bath with 200 µL of 0.2 mol/L DTT, previously thawed at room temperature. During incubation, the RBC-DTT suspension was mixed 3 times. At the end, 4 washes were carried out with automated washing machine at 3,500 rpm for 5 minutes.

To verify the effectiveness of the DTT treatment the positive and negative control erythrocytes were typed; an aliquot of the positive Kell and E pools were suspended at 0.8 % in DG Gel Sol (Diagnostic Grifols S.A. [Barcelona, Spain]) and dispensed in the dedicated wells (E and Kell) of the Rh and Kell phenotype determination card (DG Gel Rh Pheno+Kell Diagnostic Grifols S.A).

After centrifugation in dedicated devices, the procedure highlighted phenotype E+ Kell- erythrocytes. If typing did not show the expected results, the entire protocol needed to be repeated. Typing for E and Kell antigens on positive and negative control pools must be repeated before any irregular antibody screening (IAT) is performed.

While the suspended erythrocyte stability at 0.8% in DG Gel Sol (Diagnostic Grifols S.A.) is seven days after storage at +4°C, the residual packed erythrocytes are stabilized with the addition of 4 mL of Red Blood Cell (RBC) Storage Solution (Immucor Inc. [Norcross, GA, USA]) and stored at +4°C until 30 days from the date of DTT treatment

and/or until the expiry date of the test cells (the validity of the untreated screening and identification panels in use at our Transfusion Service is 30 days).

To prevent false positive reactions related to the components of the preservative solution, before each use, the preservative solution is removed and the erythrocytes manually washed in NaCl (3,500 rpm × 3 minutes).

After each use of the test cells, the preservative solution is replaced, and this replacement takes place weekly (days 7, 14 and 21 from the treatment date) even if panels are not used. In case of cross-match assignment of blood components, the same procedure was also used in the treatment of the pRBCs to be tested.

In this protocol, the choice was made to perform the auto-control with DTT-treated erythrocytes, in case there was a new positive finding to the indirect Coombs test performed with treated cell tests.

## RESULTS

### Hemolysis

Six cell test sets (Screen-Cyte 3% Medion Grifols Diagnostics AG) were treated with 0.2 mol/L DTT; in 3 sets (AutomaticW), a Hettich Rotolavit II cell washer centrifuge (Hettich AG) was used to wash the erythrocytes before and after DTT incubation, while in the other 3 sets (ManualW) the erythrocyte washing was performed manually (in NaCl at 3,500 rpm for 5 minutes).

For each of the AutomaticW and ManualW sets, three different storage methods were adopted:

1. RBC SS 30 day: resuspension for 30 days in RBC Storage Solution preserving medium (Immucor Inc.).
2. RBC SS 30 day/1week: 30-day resuspension in RBC Storage Solution (Immucor Inc.) with replacement of the preserving medium at 7, 14 and 21 days from the date of treatment with DTT.
3. NaCl 30 day: resuspension in NaCl for 30 days. This method was used for the sole purpose of comparing (within the same time-frame) the stabilization provided by our protocol with the standard conservation of erythrocytes in NaCl/Phosphate-Buffered-Saline (PBS).

The development of in vitro hemolysis was calculated on the thirtieth day from the date of treatment, comparing the values obtained then to those obtained from the same batch of untreated erythrocytes with 0.2 mol/L DTT (UT).

In all 6 sets, hemoglobin concentrations were measured using both the Plasma/Low Hb quantitative system (HemoCueR AB, Ängelholm, Sweden) (**Figure 1 A**, **B**) and a semi-quantitative determination of the hemolytic index (H) using the Cobas c 311 system (Roche Diagnostics [Rotkreuz, Switzerland]) (**Figure 1 C**, **D**).

The photometric determinations of both systems showed comparable results for all the conditions analyzed.

As expected, the highest hemolysis values were found in the determinations carried out on NaCl 30-day erythrocytes. Regardless of the storage method, the AutomaticW sets showed lower hemolysis values than those found in the ManualW sets. Irrespective of the type of washing protocol followed, RBC SS 30 day/1 week showed a lower hemoglobin concentration and hemolytic index than RBC SS 30 day; the values obtained using the Plasma/Low Hb test were below the lower limit of the linearity range, and were even lower than those found in the UT.

### Stability of the erythrocyte antigenic set of screening panels

The stability of the erythrocyte antigen set, together with the antibody detection capacity of the screening and identification panels were verified on 40 patient samples. To these, 3 samples were added relating to internal quality control (IQC) (Essential II Control Medion Grifols Diagnostics AG). The characterization of the antibody specificities is reported in **Table I**.

An IAT was carried out on all the samples using microcolumn DG Gel Coombs Diagnostic Grifols S.A. (AHG) with screening panels (Screen-Cyte 3% Medion Grifols Diagnostics AG) resuspended at 0.8% in DG Gel Sol (Diagnostic Grifols S.A.) treated and untreated (T0) with 0.2 mol/L DTT. The IATs performed with treated and stabilized RBCs were repeated at a distance of one (T1), seven (T7), fifteen (T15) and thirty (T30) days after treatment with DTT. For 36/45 specificity (80%), the results obtained in IAT between T1 and T30, were comparable, also in terms of score, with those obtained with the untreated RBC test. The negative quality control showed no deviation between T0 and T30. In 9/45 specificity (20%, 4 anti-Kell and 5 anti-Kpa), on the other hand, the IAT performed with treated RBC tests failed to detect the antibody detected with untreated erythrocytes.

As expected, of the 7 antibodies with anti-Kell specificity in 4 (57%), in which the antibody titer was ≤64, it was

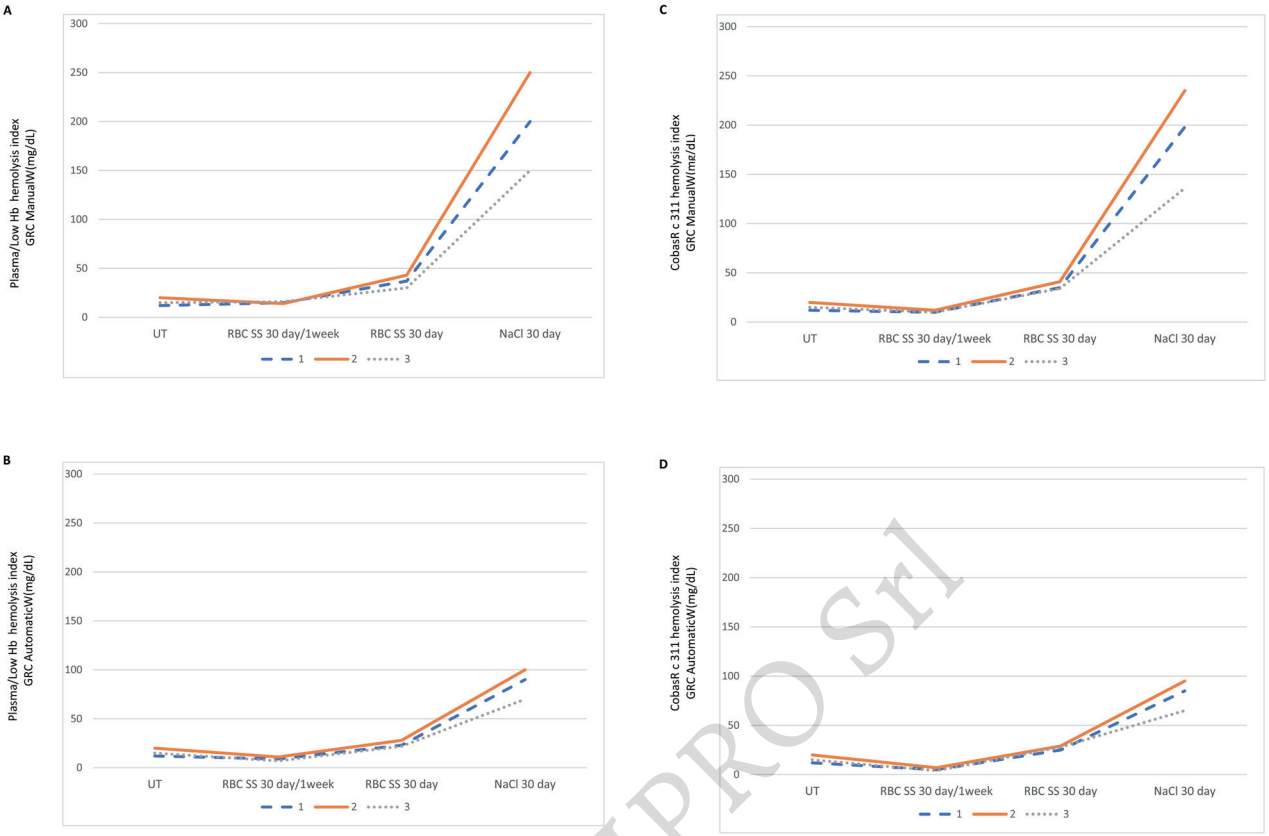


**Figure 1 - Determinations of the hemoglobin concentration with the Plasma/Low Hb quantitative system (A, B) and semi-quantitative determinations of the hemolytic index (H) on the Cobas c 311 system (C, D) of RBCs test treated with 0.2 mol/L DTT from 30 days, according to the AutomaticW (b, d) and ManualW (a, c) protocols, under the conditions: UT, RBC SS 30 day/1week, RBC SS 30 day, NaCl 30 day**



**Table I** - *Antibody screening test performed with untreated panel cells and with DTT-treated panel cells on day 1, 7, 15, 30*

| Antibody | Cases | Percentage (%) of detection | | | | |
|---|---|---|---|---|---|---|
| | | Untreated | DTT-treated T1 | DTT-treated T7 | DTT-treated T15 | DTT-treated T30 |
| **Anti-D** | 11 | 100 | 100 | 100 | 100 | 100 |
| **Anti-D (QC)** | 1 | 100 | 100 | 100 | 100 | 100 |
| **Anti-C** | 2 | 100 | 100 | 100 | 100 | 100 |
| **Anti-c** | 2 | 100 | 100 | 100 | 100 | 100 |
| **Anti-E** | 9 | 100 | 100 | 100 | 100 | 100 |
| **Anti-e** | 1 | 100 | 100 | 100 | 100 | 100 |
| **Anti-Cw** | 1 | 100 | 100 | 100 | 100 | 100 |
| **Anti-K** | 7 | 100 | 43 | 43 | 43 | 43 |
| **Anti-Kpa** | 5 | 100 | 0 | 0 | 0 | 0 |
| **Anti-Fya** | 1 | 100 | 100 | 100 | 100 | 100 |
| **Anti-Fya (QC)** | 1 | 100 | 100 | 100 | 100 | 100 |
| **Anti-M** | 3 | 100 | 100 | 100 | 100 | 100 |
| **Anti-S** | 1 | 100 | 100 | 100 | 100 | 100 |

impossible to detect the antibody due to the denaturation of the antigen following treatment with DTT, while in the other 3 cases (256≤ titers ≤2,048) the antibody search was positive, although the score was lower than that obtained with the untreated RBC test. In none of these 3 cases were changes in score and positivity with treated erythrocytes found in monitoring between T1 and T30.

The antibody tests performed with treated erythrocyte tests produced negative results in all 5 cases of immunization by anti-Kpa which, as expected, were no longer detectable, regardless of the titer.

To assess the appropriateness of treatment with DTT, a closer look was taken at the expression of Kell antigen on the K+ erythrocyte treated pool, used for the negative control of the procedure. For the new typing, performed in microcolumn (DG Gel Neutral Diagnostic, Grifols S.A.) and from the same result as the previous one, a different clone of anti-Kell was used (human monoclonal IgM clone AEK-4, Anti-K Mono-Type 2 KEL1 Medion Grifols Diagnostics AG) from the one used in the first determination (anti-K IgM human monoclonal clone MS-56 DG Gel Rh Pheno+Kell Diagnostic Grifols S.A). The antiserum (Anti-K Mono-Type 2 KEL1 Medion Grifols Diagnostics AG), certified for a titer ≥16 in liquid phase, was titrated with DTT untreated erythrocyte tests K+k+, (Screen-Cyte 0.8% Medion, Grifols Diagnostics AG) and presented in card (DG Gel Neutral Diagnostic, Grifols S.A.) a titration of 64. To confirm the antibody identification of samples positive for anti-Kell with titers 256, 1024 and 2048, compatibility tests were carried out in AHG card, by testing the three samples with the K+ pool DTT-treated; the cross-match resulted incompatible.

### Stability of erythrocyte antigenic set of identification panels

Identification panels were performed on the samples and quality controls reported in **Table II**. All antibody identification tests were carried out with AHG testing, using column agglutination technology. The same panel (Data-Cyte Plus Reagent Red Blood Cells 3% Medion Grifols Diagnostics AG), resuspended at 0.8% in DG Gel Sol (Diagnostic Grifols S.A.), was performed both at 30 days from the DTT treatment of the erythrocyte tests and their stabilization in preservative solution, and with untreated cells. The identification of the negative and positive IQC for anti-D, performed with treated RBCs, showed no change compared to the panel performed with untreated RBCs. Neither the quality control or the sample



**Table II** - *Antibody identification performed on samples with untreated panel cells and with DTT-treated panel cells on day 30*

| Antibody | Titer | Panel | RBC1 | RBC2 | RBC3 | RBC4 | RBC5 | RBC6 | RBC7 | RBC8 | RBC9 | RBC10 | RBC11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IQC NEG | | Untreated | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | DTT-treated T30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anti-D (IQC) | 8 | Untreated | 0 | 0 | 0 | 0 | 0 | 2+ | 3+ | 2+ | 3+ | 3+ | 2+ |
| | | DTT-treated T30 | 0 | 0 | 0 | 0 | 0 | 2+ | 3+ | 2+ | 3+ | 3+ | 2+ |
| Anti-Fya (IQC) | 8 | Untreated | 0 | 3+ | 3+ | 3+ | 3+ | 0 | 0 | 0 | 3+ | 3+ | 3+ |
| | | DTT-treated T30 | 0 | 3+ | 3+ | 3+ | 3+ | 0 | 0 | 0 | 3+ | 3+ | 3+ |
| Anti-Fya | 32 | Untreated | 0 | 1+ | 2+ | 1+ | 2+ | 0 | 0 | 0 | 2+ | 2+ | 1+ |
| | | DTT-treated T30 | 0 | 1+ | 2+ | 1+ | 2+ | 0 | 0 | 0 | 2+ | 2+ | 1+ |
| Anti-D+Anti-C | 512 - 64 | Untreated | 0 | 0 | 4+ | 0 | 0 | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| | | DTT-treated T30 | 0 | 0 | 4+ | 0 | 0 | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ |
| Anti-K+Anti-Kpa | 1,024-8 | Untreated | 0 | 3+ | 0 | 0 | 0 | 0 | 2+ | 3+ | 0 | 0 | 0 |
| | | DTT-treated T30 | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | 1+ | 0 | 0 | 0 |
| Anti-Kell | 256 | Untreated | 0 | 3+ | 0 | 0 | 0 | 0 | 0 | 3+ | 0 | 0 | 0 |
| | | DTT-treated T30 | 0 | 2+ | 0 | 0 | 0 | 0 | 0 | 2+ | 0 | 0 | 0 |
| Anti-Kell | 2,048 | Untreated | 0 | 3+ | 0 | 0 | 0 | 0 | 0 | 3+ | 0 | 0 | 0 |
| | | DTT-treated T30 | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | 1+ | 0 | 0 | 0 |

positive for anti-Fya showed any decrease in score or loss of positivity.

In the case of anti-D + anti-C, for both antibodies, no changes in score were detected following the execution of the treated panel. Otherwise, in the case of anti-Kell + anti-Kpa, treatment of the test RBCs with DTT did not allow identification of the anti-Kpa, as has already been highlighted in the screening performed between T1 and T30. All the anti-Kell tested were also identified with the panel treated with DTT, showing scores in agreement with the screening performed between T1 and T30; both identification and screening panel erythrocytes were characterized by expression of Kell antigen in heterozygous cells.

In order to reduce the variability of the results related to the phenotypic expression of the test RBCs, all the screening tests and identification panels were performed with the same batch of erythrocytes. Furthermore, to limit operator-dependent variability in the attribution of the scores (from 0 to 4+), all the readings of the tests performed manually manually were carried out using a digital reader (DG Reader Net, Grifols S.A.).

## Detection of Kell antigen

In contrast to what has been described in the literature since 1983[8], these results show that treatment of erythrocytes with 0.2 mol/L DTT does not lead to a complete denaturation of the Kell antigen, which was still detectable by serums containing anti-Kell antibodies with titers ≥256.

The lack of positive results in all the IATs performed between T1 and T30, with anti-Kell titers between 8 and 64, led us to consider the titer of 128 as a possible cut-off point for detection in cases of anti-Kell immunization in IATs performed with RBC tests treated with 0.2 mol/L DTT. To obtain an antibody titration of 128, samples positive for anti-Kell at titers 256 and 1024 were diluted in NaCl (1: 2 for titer 256, 1: 8 for titer 1024) and titrated with Erytra (Grifols S.A.) in AHG with K+k+ RBCs test (Screen-Cyte 0.8% Medion Grifols Diagnostics AG) untreated with DTT. Since both samples showed the expected titration, IATs were performed in AHG card with 30-day DTT-treated RBCs test and stabilized in preservative solution as required by the protocol. In both cases the IAT gave a positive result.

After analyzing a range of titrations between 8 and 2,048, we were able to establish that treatment of erythrocytes with 0.2 mol/L DTT does not lead to complete denaturation of the Kell antigen, which continues to be detectable by patient sera containing anti-Kell antibodies of titer ≥128 (**Table III**).

## Interference mitigation capability

The validation of the protocol was conducted on samples of 28 patients with MM, 25/28 (89%) of whom were being treated with daratumumab and 3/28 (11%) with



**Table III** - *Antibody screening test performed on anti-Kell and anti-Kpa with untreated panel cells and with DTT-treated panel cells on day 1, 7, 15, 30*

| Antibody | Titer | Untreated | | | DTT-treated T1 | | | DTT-treated T7 | | | DTT-treated T15 | | | DTT-treated T30 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | RBC1 | RBC2 | RBC3 | RBC1 | RBC2 | RBC3 | RBC1 | RBC2 | RBC3 | RBC1 | RBC2 | RBC3 | RBC1 | RBC2 | RBC3 |
| **Anti-Kell** | 8 | 0 | 0 | 3+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Anti-Kell** | 16 | 0 | 0 | 3+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Anti-Kell+Anti-Kpa** | 32-256 | 3+ | 0 | 3+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Anti-Kell** | 64 | 0 | 0 | 3+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Anti-Kell**[*1] | 128 | 0 | 0 | 2+ | 0 | 0 | 1+ | 0 | 0 | 1+ | 0 | 0 | 1+ | 0 | 0 | 1+ |
| **Anti-Kell**[*2] | 128 | 0 | 0 | 3+ | 0 | 0 | 2+ | 0 | 0 | 2+ | 0 | 0 | 2+ | 0 | 0 | 2+ |
| **Anti-Kell** | 256 | 0 | 0 | 3+ | 0 | 0 | 2+ | 0 | 0 | 2+ | 0 | 0 | 2+ | 0 | 0 | 2+ |
| **Anti-Kell+Anti-Kpa** | 1,024-8 | 3+ | 0 | 3+ | 0 | 0 | 1+ | 0 | 0 | 1+ | 0 | 0 | 1+ | 0 | 0 | 1+ |
| **Anti-Kell** | 2,048 | 0 | 0 | 3+ | 0 | 0 | 1+ | 0 | 0 | 1+ | 0 | 0 | 1+ | 0 | 0 | 1+ |
| **Anti-Kpa** | 4 | 2+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Anti-Kpa** | 16 | 2+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Anti-Kpa** | 16 | 2+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[*1]starting titer 256, diluted 1: 2; [*2]starting titer 1024, diluted 1: 8

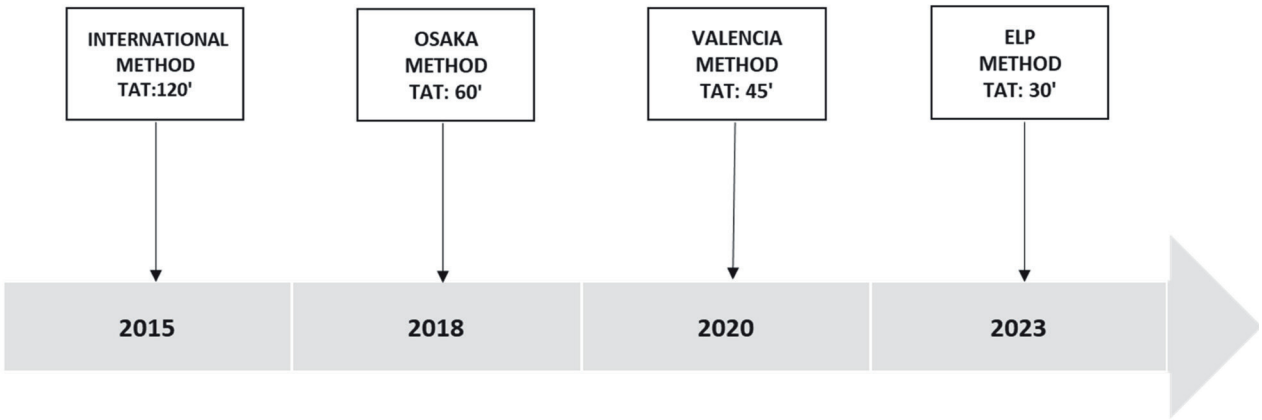


**Figure 2 - TAT of methods: internationally validated by Chapuy *et al.*[1,2], Osaka[7], Valencia[10], ELP to mitigate serological interference from anti-CD38 MoAbs. TAT of the ELP method is subject to the availability of erythrocytes pre-treated with DTT, following an IAT performed in card and with a negative result**
TAT: turn around time.

isatuximab. IATs were performed, both with untreated screening panels (T0), with RBC test at 1 (T1) and 30 days (T30) from treatment with DTT in accordance with the ELP protocol. All screening tests were performed using the same batch of erythrocytes (Screen-Cyte 3% Medion Grifols Diagnostics AG). Both the screening tests and the identification panels (Data-Cyte Plus Reagent Red Blood Cells 3% Medion Grifols Diagnostics AG) were resuspended at 0.8% DG Gel Sol (Diagnostic Grifols S.A.) and the tests were performed in AHG card. Although it is known that serological interference from anti-CD38 MoAbs is found in 100% of patients treated with DARA, but is detectable in only 63-68% of patients treated with isatuximab[9], antibody studies performed at T0 showed a panreactivity reaction, regardless of the type of anti-CD38 given.

Once drug-induced panagglutination was removed, antibody search was negative in 27/28 cases (96%), while in 1/28 (4%) cases it was possible to detect an anti-Fya immunization already known from the basic immunohematological study. In this case, both the screening panel and the identification panel performed at T30 showed no change in terms of score and positivity compared to those performed at T1. None of the cases analyzed showed false positive results at T30. The results obtained with the ELP protocol showed no deviations from those obtained by running in parallel the internationally validated method at T1 and T30[1,2].

### Turn Around Time (TAT)

Thanks to the use of the automated washer, the ELP method provides the DTT-erythrocyte treatment in 80 minutes, regardless of the number of samples being treated. It is therefore possible therefore, within 30 days of test cell treatment, to mitigate the interference from MoAbs anti-CD38 in 30 minutes and ensure a T&S management of the transfusion request (**Figure 2**). If erythrocytes pretreated with DTT are available, the test execution times of IAT are similar to those obtained by performing the test (in card) manually.

Similarly, in presence of irregular antibodies with known specificity, the availability of the identification panel and treated and stabilized pRBCs, allows the assignment of blood components with similar times to those required for performing tests (antibody identification and serological cross-match) manually.

## DISCUSSION

Unifying the state of the art as regards anti-CD38 mitigation strategies, the Transfusion Department of San Bortolo Hospital in Vicenza drew up an easy, standardized protocol designed to ensure the resolution of panreactivity in pre-transfusion tests requiring the use of antiglobulin reagents.

In all protocols where the RBC washing is performed manually, the timing of the procedure is generally related to the assignment method of blood components

and to the outcome of the antibody screening carried out with a treated RBC test. The Valencia[10] method, too, characterized by the absence of erythrocyte washings, is influenced by these variables, as well as requiring great manual skill on the part of the operator responsible for resuspending the erythrocyte-DTT-plasma mixture directly into the card incubation chamber.

Our ELP method not only allows the blood components to be assigned within 30 minutes, even in T&S, but has also proved extremely advantageous from the economic point of view, given the low cost of the DTT (which most laboratories are accustomed to using), and the reduced consumption of the erythrocyte panels which, once stabilized, are available for 30 days from treatment.

Chapuy *et al.*[1] associated the denaturation of CD38 with the denaturing capacity of Kell antigen by 0.2 mol/L DTT. The undetectability of Kell antigen on the erythrocyte surface was therefore considered an indicator of DTT treatment effectiveness (negative control). This can only be considered appropriate if, as a result of the application of the procedure, the selected erythrocytes for the negative control (K+) tested with anti-Kell antisera, show a K- typing.

Both the Osaka[7] and the Valencia[10] methods showed how treating erythrocytes with DTT in concentrations below 0.2 mol/L could mitigate anti-CD38 interference while preserving the erythrocyte antigen structure.

However, the need to keep the erythrocyte antigenic structure unchanged makes it impossible to use the denaturation of Kell antigen as an means of controlling the efficiency of the procedure; therefore, all protocols using DTT in concentrations between 0.01 mol/L (Osaka method)[7] and 0.04 mol/L (Valencia method)[10] are characterized by the absence of a negative control.

Our laboratory was able to find new immunizations directed towards antigens resistant to treatment with reducing agents, and showed that the presence of positive and negative controls, in presence of a positive IAT after procedure application, makes it possible to distinguish between a DTT treatment of the erythrocytes not properly performed, and the presence of auto/alloantibodies masked by a drug-induced panagglutination.

Therefore, the protocols subsequent to the publication of the internationally validated method[1,2], by intervening on the problems related to the denaturation of the blood group systems sensitive to treatment with reducing agents (KEL, DO, YT, KN, LU, LW, IN, JMH)[2,3,11], are aimed at ensuring the preservation of the erythrocyte's antigenic structure, and neglect the importance of a patient's phenotypic and/or genotypic characterization before starting anti-CD38 MoAb treatment.

According to the experience of our laboratory, a patient treated with anti-CD38 rarely arrives at a transfusion event without an immunohematological study involving extensive erythrocyte typing: similarly, these patients are rarely assigned RBCs that do not respect the blood group systems involved in the development of clinically significant antibodies, especially if they are known to be sensitive to treatment with reducing agents.

We used 0.2 mol/L DTT for the ELP method because in our opinion, once the patient's serological and/or molecular typing has been correctly performed prior to starting anti-CD38 treatment, the denaturation of the Kell antigen is not a limit, but is actually an indicator of effectiveness as regards the correct execution of the procedure.

Although treatment with 0.2 mol/L DTT resulted in changes in the erythrocyte antigens analyzed (Kell and Kpa) which are known to be sensitive, despite what has been reported in the literature, we were able to show that the destruction of the Kell antigen by the 0.2 mol/L DTT is actually only partial; in fact, the antigenic structure was still detectable by anti-Kell obtained from patients with titers ≥128.

In order to evaluate the correct denaturation of the CD38 for typing of the erythrocytes selected as negative control, we suggest using commercial anti-Kell with titer <128. Typing erythrocytes with anti-Kell with titer ≥128 may lead to DTT treatment being considered inappropriate, whereas the typing results are only attributable to partial preservation of Kell antigen and the high antibody concentration that determines its detection.

No further investigations have been carried out on blood-group systems sensitive to reducing agents,

since this aspect was not included in the development of the protocol, although particular attention was paid to the FY system; despite what has been reported by Sigle *et al.*[4], Fya and Fyb antigens don't have shown lack of expression on the erythrocytes treated.

## CONCLUSIONS

This original ELP method was developed as a pathway for the transfusion management of patients undergoing treatment with anti-CD38 MoAbs, from the technical protocol for the mitigation of serological interference, to the assignment of blood components. The stabilization of DTT-treated erythrocyte panels for antibody screening and identification using a commercial stabilizing solution appears to be an effective, exportable solution, which by reducing the amount of laboratory work involved makes it possible to offer the most appropriate transfusion support also in situations of emergency.

### Ethical consideration

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.

Written informed consent was obtained from each participant/patient for study participation and data publication.

## AUTHORS' CONTRIBUTIONS

FF, EM, MS were involved in the design of the study; EM, SC, CO, EP, KS, TT and GV were involved in the technical execution of the study; MB, AS, GL and FF were involved in the collection and analysis of patient data; FF e MS were involved in reviewing the results of the protocol and the manuscript.

*The Authors declare no conflicts of interest.*

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