Improving the safety of whole blood-derived transfusion products with a riboflavin-based pathogen reduction technology

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Abstract

Worldwide safety of blood has been positively impacted by technological, economic and social improvements; nevertheless, growing socio-political changes of contemporary society together with environmental changes challenge the practice of blood transfusion with a continuous source of unforeseeable threats with the emergence and re-emergence of blood-borne pathogens. Pathogen reduction (PR) is a proactive strategy to mitigate the risk of transfusion-transmitted infections. PR technologies for the treatment of single plasma units and platelet concentrates are commercially available and have been successfully implemented in more than 2 dozen countries worldwide. Ideally, all labile blood components should be PR treated to ensure a safe and sustainable blood supply in accordance with regional transfusion best practices. Recently, a device (Mirasol® Pathogen Reduction Technology System) for PR treatment of whole blood using riboflavin and UV light has received CE marking, a significant step forward in realising blood safety where WB transfusion is the norm, such as in sub-Saharan Africa and in far-forward combat situations. There is also keen interest in the ability to derive components from Mirasol®-treated whole blood, as it is seen as a more efficient and economical means to implement universal PR in the blood centre environment than treatment of components with different PR systems.

Keywords: transfusion medicine, blood safety, bloodborne pathogens, emerging infectious diseases.

Introduction

Blood transfusion safety is considered by the World Health Organization an integral part of each country's national health care policy and infrastructure[1]. Worldwide safety of blood has been positively impacted by technological, economic and social improvements[2] although in some regions availability, safety and quality of blood is still an issue[3-5].

In most countries, measures to reduce the risk of transmission of diseases to recipients through blood have been continuously implemented and improved[6]. In the past four decades, regulatory agencies, blood providers and medical professionals in a joint effort have implemented risk reduction measures and practices that resulted in a prodigious increase in blood transfusion safety[2,4,6]. Nevertheless, growing socio-political changes of contemporary society, together with environmental changes, challenge the practice of blood transfusion with a continuous source of unforeseeable threats with the emergence and re-emergence of blood-borne pathogens[7,8]. This realisation triggered a discussion among experts about a possible paradigm shift from a reactive to a proactive approach to blood safety, since the most common reactive measures, like donor deferrals and/or screening donated blood, can potentially lead to blood shortages if risks accumulate[3,9].

History of pathogen reduction for blood products

Pathogen reduction (PR) is one such proactive strategy to mitigate the risk of transfusion-transmitted infections (TTI). The majority of available PR methods involve physicochemical disruption of pathogen structural elements or photochemical modification of nucleic acids to prevent replication[10]; as such, these technologies act upon a broad spectrum of infectious agents as well as white blood cells (WBCs) that can cause immunological complications. The plasma fractionation industry has the longest and most successful experience with PR, resulting in more than three decades with no reported cases of hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV) transmission with fractionated plasma-derived therapeutics[11]. However, the earliest PR methods used to treat plasma (solvent-detergent [SD] and methylene blue [MB] treatment) are not effective for cellular blood components. SD is ineffective because the surfactant activity also disrupts the plasma membrane of the desired blood cells and MB exhibits poor penetration through plasma membranes, making it ineffective against intracellular pathogens and WBCs[12].

Two ultraviolet (UV) light-based photochemical PR methods have been developed for use with both plasma and platelets. One system uses amotosalen

hydrochloric acid (also known as S-59) activated by UV-A light to crosslink nucleic acids. It requires a post-PR processing step of up to 24 hours to remove residual S-59 and its photoproducts due to toxicity concerns[13,14]. The second system utilises riboflavin and UV light for the inactivation of pathogens and WBC. Riboflavin photoproducts and catabolites are found endogenously in normal blood[15], so no compound removal steps are required after treatment.

Pathogen reduction treatment of red blood cells (RBC) has been more challenging due to the absorption of light by haemoglobin[15], which limits the use of photochemical technologies. Two RBC PR technologies based upon alkylating agents that do not require photoactivation have been tested in clinical trials in patients. One of these technologies used amustaline (also known as S-303)[16]. The second lightless method utilised the inactine PEN110 as its active agent. Both technologies were shown to be effective against selected enveloped and non-enveloped viruses, bacteria, protozoa, and WBCs[17-25], but clinical trials were brought to an early end due to the formation of antibodies to treated RBCs[26-28]. Development of the PEN110-based PR system was discontinued in 2005, while an improved version of the S-303 system has recently been evaluated in a small clinical trial[29].

Neither of the RBC PR methods is suitable for treatment of whole blood (WB). As a class, alkylating agents are frequently genotoxins with varying potency in inducing chromosomal damage and mutagenesis[30]. As such, the RBC PR treatments utilising these agents rely upon cell washing and other methods, such as compound adsorption and quenching, to control the toxicity of the infused RBC product. Furthermore, alkylating agents are nucleophiles that readily form covalent bonds with other organic molecules; while they are intended to target nucleotides and inhibit DNA replication, there remains the potential for adduct formation with cell membrane components and the induction of neoantigenicity[26-28].

Recently, a device for PR treatment of WB using riboflavin and UV light has received CE marking. This system, the Mirasol® Pathogen Reduction Technology System (Terumo BCT, Lakewood, CO, USA), utilises the same core technology as was developed for the treatment of platelets and plasma. The spectral output of the Mirasol® UV lamps is centred at 313 nm, with more than 99% of the total energy reaching the product falling within the UVB (280-315 nm) and UVA (315-400 nm) spectra. This is an optimal range for riboflavin-mediated photosensitisation of nucleic acids (Figure 1)[31]. Mixing by linear agitation during illumination further promotes exposure of the full WB/riboflavin suspension to UV light.

Development of a universal pathogen reduction method for whole blood and components

The Mirasol® Pathogen Reduction Technology System uses riboflavin (vitamin B2) plus UV light to induce damage in nucleic acid-containing agents.

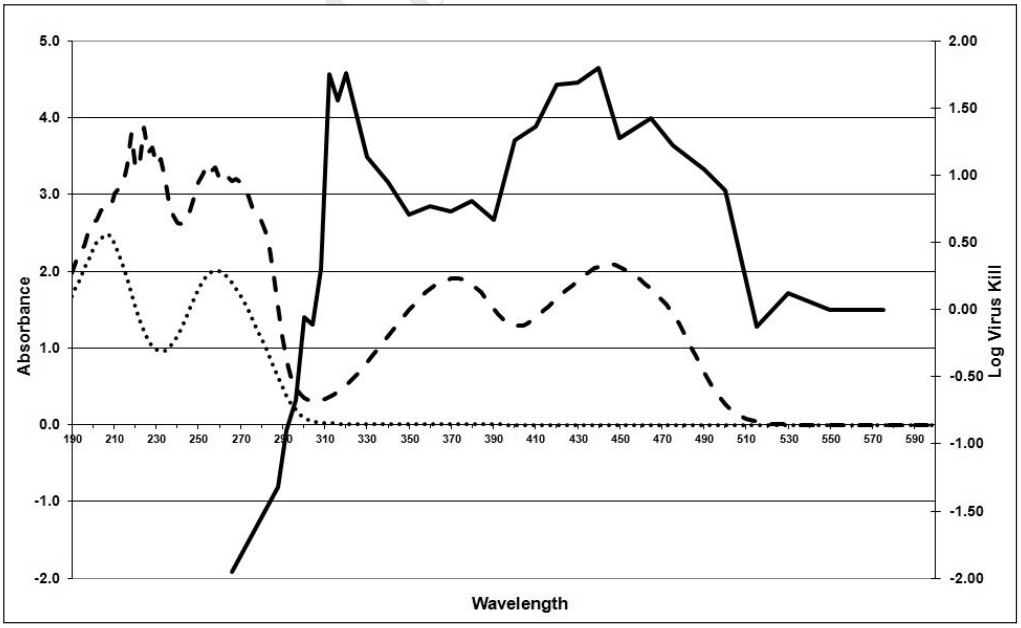

Figure 1 - Light absorption and virus inactivation in solution as a function of wavelength of light. Solid line: action spectrum (virus inactivation *vs* wavelength); dotted line: absorption of 1 mg/mL DNA in saline; dashed line: absorption of 200 μM riboflavin in saline. *Reproduced with permission*[31].

The system has been shown to be effective against clinically relevant pathogens and inactivates leucocytes without significantly compromising the efficacy of the product or resulting in product loss[10,15]. Riboflavin is a naturally occurring vitamin with a well-known and well-characterised safety profile[32]. The system is currently in use in many parts of the world for the treatment of platelets and plasma. Haemovigilance data on over 190,000 transfusions has confirmed consistently low adverse reaction (AR) rates during six years of surveillance at multiple sites and implies that these components are safe for patients[33]. However, the ultimate goal of pathogen reduction technologies should be to universally safeguard the blood supply and will have to include a solution for all blood products: WB, RBCs, platelets and plasma.

Pathogen reduction treatment of WB brings the benefit of improved blood safety to areas and situations where WB transfusion is the norm, as in sub-Saharan Africa and in far-forward combat zones, but just as important is the ability to derive components from PR-treated WB. The initial development efforts for a component derived from Mirasol®-treated WB have focused upon RBCs.

Pre-clinical testing of Mirasol®-treated whole blood and red blood cells

Pre-clinical testing of Mirasol®-treated WB and RBCs derived thereof has been aligned around three major priorities: 1) validation of PR and donor WBC inactivation capability; 2) evaluation of the effects of PR-treatment on transfusion blood product quality; and 3) the toxicology of riboflavin and its photoproducts. Experiments have included quantitative *in vitro* and *in vivo* animal model studies of PR efficacy against specific pathogens and WBCs, *in vitro* cell quality assays, and additional toxicology tests to supplement the extensive work performed in support of Mirasol®-treated platelets and plasma.

Pathogen reduction and white blood cell inactivation

The effectiveness of Mirasol® treatment for WB has been evaluated with tests of parasitic, viral, and bacterial pathogen reduction. Generally, PR performance has been assessed according to Committee for Proprietary Medicinal Products (CPMP) guidelines[34], whereby the calculation of reduction factors is based upon a statistical evaluation of pathogen titres that includes the determination of the probability of detecting an infectious agent at low concentration. A variety of pathogens have been tested using *in vivo*[35] and *in vitro* cell culture[36-39] models. The generalised methodology for PR testing involves the inoculation of WB units with the pathogen of interest followed by treatment with the Mirasol® System. The appropriate *in vivo* or *in vitro* test system was then challenged with serial dilutions of post-treatment samples in order to statistically determine the infectious dose at which 50% of the test system replicates were infected (ID_{50}) and reduction factors were calculated based upon the pre- and post-treatment ID_{50} values. PR results are summarised in Table I.

White blood cell inactivation is a significant potential benefit of the Mirasol® technology. Transfusion of viable leucocytes has been shown to lead to a number of donor anti-recipient responses such as transfusion associated graft-*versus*-host disease (TA-GvHD)[40]. The current standard of care to inhibit such responses includes leucoreduction followed by exposure of blood components to 25 Gray (Gy) of gamma-irradiation. The effectiveness of WBC inactivation by Mirasol® treatment of WB in comparison to gamma irradiation has been evaluated with both *in vitro* studies and with an *in vivo* mouse model[41]. Overall, PR-treatment and treatment by gamma-irradiation were similarly effective in reducing viable T cells in treated WB, PR treatment was more effective in suppressing antigen presentation, cellular activation, and cytokine secretion, and TA-GvHD was prevented in animals receiving PR-treated and gamma-irradiated cells,

Table I - Log reduction results for tested pathogens. Parasite and virus assessed in whole blood; bacteria assessed in leucoreduced red blood cells in AS-3.

Parasite	Log reduction	Virus	Log reduction	Bacteria+	Log reduction
Babesia microti[35]	≥5.0	HCV	2.7	*Bacillus cereus*	5.3
Babesia divergens	7.3*			*Escherichia coli*	4.9
Leishmania donovani[37]	2.3			*Klebsiella pneumoniae*	5.0
Plasmodium falciparum[36]	≥6.4			*Staphylococcus aureus*	4.1
Trypanosoma cruzi[38]	≥3.5			*Staphylococcus epidermidis*	3.2
				Serratia liquefaciens	5.9
				Streptococcus pyogenes	≥5.1
				Yersinia enterocolitica	4.4

HIV: Human Immunodeficiency Virus. *Unpublished data, assessed in whole blood. +Unpublished data, assessed in leucoreduced red blood cells.

whereas clinical signs of TA-GvHD were evident in all animals receiving untreated cells (Figure 2).

Red blood cell quality

In vitro testing forms the foundation for the evaluation of new blood processing methodologies. By assessing the physical and biochemical changes in investigative blood products, a risk-based assessment can be made to determine the extent of further testing necessary to assure safety and efficacy for eventual approval of the device.

For the core cell quality verification study for RBCs derived from Mirasol®-treated WB, a total of 61 paired units of leucoreduced RBCs were evaluated (Dimberg *et al., manuscript to be submitted*). The main finding from the *in vitro* measurements was that all acceptance criteria based upon AABB standards were met: haemolysis remained below 1%, pH was greater than or equal to 6.2, post-leucoreduction RBC recovery was greater than 85%, and post-leucoreduction residual WBCs were below 5×10^6. Crossmatch compatibility to pre-treatment autologous plasma was also designated an acceptance criterion as a screen for treatment-induced non-specific protein binding and potential neoantigen formation. All units remained crossmatch compatible. The most prominent difference between RBCs derived from Mirasol®-treated *vs* untreated WB after 21 days of storage is that the concentration of extracellular potassium is higher in RBCs derived from Mirasol®-treated WB than in conventional RBCs; the difference in potassium concentration is similar to that seen with storage of gamma-irradiated RBCs.

Toxicology

From a toxicological perspective, the safety profile for the Mirasol® technology is well-understood based upon the extensive literature for riboflavin and its metabolites[42-54]. Furthermore, since the same mechanism of action is used for pathogen reduction treatment of WB as is used for platelets and plasma, the specific toxicology work previously performed to support treatment of platelets and plasma is applicable to PR-treatment of WB as well. The Mirasol® toxicology program for platelets and plasma was developed in accordance with US Food and Drug Administration (FDA) Good Laboratory Practices (GLP) guidelines and aligning to International Organization for Standards (ISO)-10993: Biological Evaluation of Medical Devices. As per this guideline, toxicology testing for platelets and plasma has included assessments of cytotoxicity, haemocompatibility, genotoxicity, acute and sub-chronic systemic toxicity, reproductive toxicity, and leachables/extractables. Additionally, neoantigenicity testing was performed to address concerns driven from the experience with other PR technologies[55]. No evidence of toxic effect was observed in any of these studies[32].

In addition to the support provided by the toxicology programme for the platelets and plasma system, several toxicology studies have been performed using Mirasol®-treated WB or RBCs derived from Mirasol®-treated WB. *In vitro* testing included evaluations of cytotoxicity and complement activation, and *in vivo* models have been used to screen for potential neoantigenicity and to assess systemic toxicity upon acute exposure to Mirasol®-treated WB and WB-derived RBCs. The *in vitro* tests were negative for

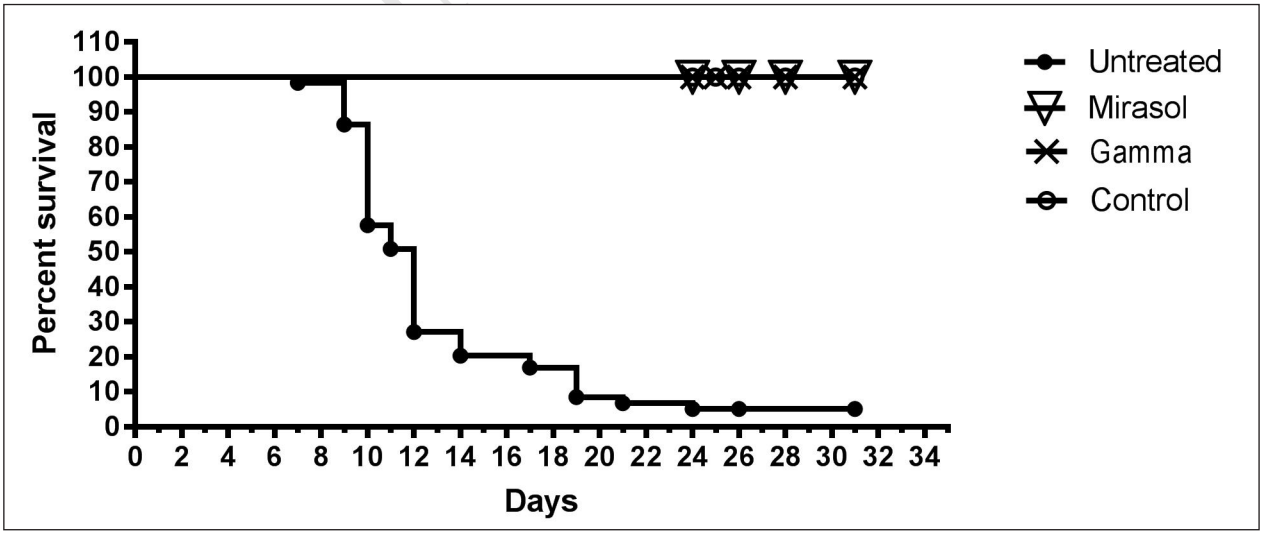

Figure 2 - Survival of mice injected with untreated, Mirasol®-treated or gamma-irradiated donor cells (n=60 per group). Control mice were injected with PBS (n=18)[41].
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toxic effects; mouse L929 fibroblasts showed no reactivity when exposed to Mirasol®-treated WB, and no inappropriate activation of the complement system was observed.

Red blood cells treated with the Mirasol® technology have been tested for potential neoantigenicity in a baboon model and in a rabbit model. The baboon study was performed with an earlier generation Mirasol® System in which RBC were treated in riboflavin solution rather than in WB[55]; this study showed that RBC treated with riboflavin and UV light do not induce antibody formation in recipient animals. This is in contrast to the strong antibody response and clearance of cells induced by treatment with quinacrine mustard, a compound related to S-303 that was used as the positive control in the experiment. RBCs from PR-treated WB were also assessed for potential neoantigenicity in a rabbit model[32]. Human PR-treated WB was used to immunise rabbits in one study arm; the other study arm was immunised with untreated control whole blood. PR-treated WB was also separated to provide RBCs after treatment, and the RBCs were stored for 42 days and used to immunise rabbits in another study arm, with untreated control RBC in a fourth study arm. No treatment-related neoantigenicity was observed.

Two acute toxicity studies were performed specifically to support the Mirasol® System for WB. These studies were aimed at evaluating not only the safety, but also the efficacy of transfusion. In one study, RBCs derived from Mirasol®-treated WB were administered to dogs through 1 total blood volume (TBV) manual RBC exchange[56]. One TBV was taken to be the maximum feasible dose. The Mirasol®-treated RBCs were well tolerated; there were no histopathological differences, nor was there any evidence of severe transfusion reactions, morbidity, or mortality. In the other study, liver injury was induced in pigs and Mirasol®-treated WB was used to resuscitate the animals[57]. There were no significant differences between animals transfused with untreated WB and Mirasol®-treated WB in terms of survival, blood loss, tissue oxygen delivery, or coagulation parameters *in vivo*.

Clinical evaluation of Mirasol®-treated whole blood products

The pre-clinical testing revealed no significant safety signals and suggested sufficient patient benefit to justify moving into clinical trials. To date, clinical evaluation of the Mirasol® WB System has included three feasibility clinical trials as well as a clinical trial in patients assessing the incidence of transfusion-transmitted *Plasmodium spp.* infection that was conducted in Kumasi, Ghana.

Radiolabelled recovery and survival feasibility clinical trials

The "Inactivation of Whole Blood with Mirasol: Performance in Red Blood Cells in Healthy Volunteers" (IMPROVE) feasibility clinical trial compared the 24-hour recovery and survival of RBCs derived from WB treated with a prototype Mirasol® System at varying UV illumination energy levels (22, 33, or 44 joules per millilitre of RBCs [J/mL_{RBC}])[58], while IMPROVE II evaluated RBCs produced with the final configuration as CE marked[59]. In the IMPROVE study, *in vitro* RBC quality parameters were compared to the *in vivo* recovery and survival of transfused autologous RBCs derived from Mirasol®-treated WB illuminated at the 3 different levels of UV light energy, allowing for *in vitro* parameters correlating with *in vivo* recovery and survival to be identified. These correlations were then used to fine tune the UV energy dose for the final configuration of the device. The results from the subsequent, larger IMPROVE II study indicated that RBCs derived from WB treated by the Mirasol® System with an 80 J/mL_{RBC} UV energy dose maintained acceptable cell quality and met the FDA criterion for recovery as compared with untreated RBCs. Furthermore, subjects re-infused with leucoreduced RBCs derived from Mirasol®-treated WB demonstrated no evidence of neoantigenicity or other major safety concerns.

The recovery and survival of platelets derived from Mirasol®-treated WB has also been evaluated[60]. In the MEDIC clinical trial, WB that was Mirasol®-treated using the final device configuration was stored at room temperature for 24±1 hour, after which a 50 mL sample was removed to produce a platelet pellet for radiolabelling. The mean recovery for Mirasol®-treated, stored platelets was 82.9% of the untreated, fresh control and mean survival was 82% of the untreated, fresh control. These results were well above the FDA acceptance criteria for recovery (66.7% of fresh control) and survival (58% of fresh control).

Clinical trial in patients

The AIMS study was a randomised, double-blind, parallel-group clinical trial that was conducted in Kumasi, Ghana, an area endemic for malaria where over 90% of transfusions are performed with WB[61]. The primary end point was the incidence of transfusion-transmitted malaria (TTM) in non-parasitemic recipients exposed to parasitemic whole blood. Of 226 randomised patients, 65 (28 Mirasol, 37 Control) were non-parasitemic patients who were exposed to parasitemic blood. The incidence of TTM was significantly lower in the Mirasol® group (1 [4%] of 28 patients *vs* 8 [22%] of 37 patients; p=0.039). Moreover, there was no difference in the incidence of adverse reactions or in the haemoglobin increment seen in patients who received

Mirasol®-treated WB *vs* those receiving untreated WB. This was the first clinical study for any PR technology to demonstrate a reduction in transfusion transmission of any type of infection.

The Mirasol® System has recently been approved by the Ghanaian Food and Drugs Authority, and plans are underway to introduce Mirasol®-treated WB into routine use at two sites in Ghana, initially focusing upon patient populations that are particularly susceptible to complications from transfusion-transmitted disease.

Components derived from Mirasol®-treated whole blood

The Mirasol® System has been CE marked for transfusion of WB, a significant step forward in ensuring blood safety where WB transfusion is the norm, such as in sub-Saharan Africa and in far-forward combat situations. There is also keen interest in the ability to derive components from Mirasol®-treated WB, as it is seen as a more efficient and economical means to implement universal PR in the blood centre environment than treatment of components with different PR systems.

Although processes for separation of Mirasol®-treated WB into components have not yet been optimised, there has been considerable interest in evaluating such components. Herzig *et al*. and Schubert *et al.* have recently reported on components separated from Mirasol®-treated WB *via* the platelet rich plasma (PRP)[62] and buffy coat (BC)[63] methods, respectively, and Terumo BCT has performed a preliminary study of automated componentisation using the REVEOS device. Reduced platelet recoveries were observed, whether separated by manual or automated methods. However, platelets produced from Mirasol®-treated WB demonstrated more moderate treatment effects when compared to a Mirasol®-treated platelet product[63]. Declines in plasma protein quality were also observed, though for key plasma factors such as fibrinogen and factor VIII the Council of Europe (CoE) guidelines were met for frozen plasma produced by the BC method and the automated method with the REVEOS device. RBCs demonstrated acceptable cell quality through 14 days of storage when stored in saline-adenine-glucose-mannitol (SAG-M) additive solution and up to 21 days in additive solution 3 (AS-3).

The quality of WB-derived components is influenced by numerous factors, including hold times, processing methods (PRP, BC, automated), and choice of storage solutions. Work performed to date suggests that two viable components, plasma and RBCs, can be obtained without significant alterations to current component separation processes. Reduced platelet recoveries suggest that more individual platelet units will need to be pooled to achieve clinical platelet doses.

Conclusions

Blood safety has been a major focus of concern in the last three decades; hence considerable efforts and resources have been invested in this cause by governmental and non-governmental agencies as well as commercial companies. In accordance with the precautionary principle, PR technologies have been developed to reduce the risk of TTI from single-donor or fractionated blood components. Nowadays, blood transfusion stakeholders expect that the transfusion of labile blood components should reach similar safety levels as seen with fractionated plasma-derived therapeutics. Currently, PR technologies for the treatment of single plasma units and platelet concentrates are commercially available and have been successfully implemented in more than 2 dozen countries worldwide. Ideally, all labile blood components should be PR treated to ensure a safe and sustainable blood supply in accordance with regional transfusion best practices, and PR treatment of WB represents the most efficient implementation path to achieve this goal. It has been recently demonstrated through a clinical trial in a malaria-endemic country that a WB PR technology based upon riboflavin and UV light does reduce the risk of transfusion-transmitted malaria. RBCs derived from PR-treated WB will soon be evaluated in a pivotal clinical trial, and further development work to optimise separation and to evaluate plasma and platelet components is warranted.

Disclosure of conflicts of interest

SY, SD, SK, HP and MC are employees of Terumo BCT. RG is a former employee of Terumo BCT.

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