Risks associated with red blood cell transfusions: potential benefits from application of pathogen inactivation

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BACKGROUND: Red blood cell (RBC) transfusion risks could be reduced if a robust technology for pathogen inactivation of RBC (PI-RBCs) were to be approved.

MATERIALS AND METHODS: Estimates of per-unit and per-patient aggregate infectious risks for conventional RBCs were calculated; the latter used patient diagnosis as a determinant of estimated lifetime exposure to RBC units. Existing in vitro data for the two technologies under development for producing PI-RBCs and the status of current clinical trials are reviewed.

RESULTS: Minimum and maximum per-unit risk were calculated as 0.0003% (1 in 323,000) and 0.12% (1 in 831), respectively. The minimum estimate is for known lower-risk pathogens while the maximal estimate also includes an emerging infectious agent (EIA) and endemic area *Babesia* risk. Minimum and maximum per-patient lifetime risks by diagnosis grouping were estimated as 1.5 and 3.3%, respectively, for stem cell transplantation (which includes additional risk for cytomegalovirus transmission); 1.2 and 3.7%, respectively, for myelodysplastic syndrome; and 0.2 and 44%, respectively, for hemoglobinopathy.

DISCUSSION: There is potential for PI technologies to reduce infectious RBC risk and to provide additional benefits (e.g., prevention of transfusion-associated graft-versus-host disease and possible reduction of alloimmunization) due to white blood cell inactivation. PI-RBCs should be viewed in the context of having a fully PI-treated blood supply, enabling a blood safety paradigm shift from reactive to proactive. Providing insurance against new EIAs. Further, when approved, the use of PI for all components may catalyze operational changes in blood donor screening, laboratory testing, and component manufacturing.

Although transfusion safety has increased greatly, risks are still associated with red blood cell (RBC) transfusion. These could be reduced if a robust technology was approved for pathogen inactivation of RBCs (PI-RBCs) by applying the PI technology either to the RBC component or to the parent whole blood (WB) unit. In a recent risk/benefit publication on PI platelets (PLTs) prepared using the Intercept system,[1] we emphasized that such an analysis should calculate benefits and risks on a per-patient rather than a per-unit basis. However, because this is a more difficult task for RBCs, we used a combined approach of calculating a risk per RBC unit and, when possible, a per-patient risk. Since the availability of PI-RBCs would result in the potential to transfuse a full complement of PI blood components (RBC, PLTs, plasma), we also

ABBREVIATIONS: EIA(s) = emerging infectious agent(s); GSH = glutathione; HSCT = hematopoietic stem cell transplantation; LR = leukoreduced; MDS = myelodysplastic syndrome; PI = pathogen inactivation; SCD = sickle cell disease; TA-GVHD = transfusion-associated graft-versus-host disease; TT-CMV = transfusion-transmitted cytomegalovirus; TTB = transfusion-transmitted babesiosis; WB = whole blood.

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discuss operational benefits that could be achieved under a full PI scenario.

CATEGORIZING RBC RECIPIENTS AND ESTIMATING NUMBER OF TRANSFUSED RBC UNITS

The 2011 HHS National Blood Collection and Utilization Survey estimated a mean per-patient RBC dose of 2.75 units annually,[2] and a 5-year retrospective study in a regional hospital system reported a mean of 2.9 (±2.7) RBC units per transfused inpatient.[3] However, there is substantial interpatient variability in RBC units transfused due to clinical diagnoses of the patient, the indication for transfusion, long established physician practice patterns, and the presence or absence of patient blood management programs.

Figure 1 provides a theoretical schema for understanding a recipient's risk of acquiring a transfusion-transmitted infection, which is dependent on two factors: the number of units transfused (e.g., a higher risk with more units) and whether transfusion occurs when an undetected emerging infectious agent (EIA) is in the blood supply. This latter time-related risk is higher for recipients whose transfusion exposure spans a longer time interval.[1,4] Factors relevant to clinical outcome of a transfusion-transmitted infection include the expected length of recipient survival due to underlying disease and the increased susceptibility of different patient populations (based on their degree of immunosuppression) to adverse clinical outcomes secondary to infectious disease transmission.[5] Thus, a logical way to categorize RBC recipients is both by number of units transfused and by the time interval over which transfusions occur. In Table 1, [6-19] which forms the basis of a per-patient risk analysis for selected patient groups, we synthesized existing RBC usage and transfusion practice data for illustrative diagnoses into a five-tiered classification scheme based on acute (single transfusion episode), intermittent (multiple episodes), or chronic (often lifetime) RBC transfusion therapy.

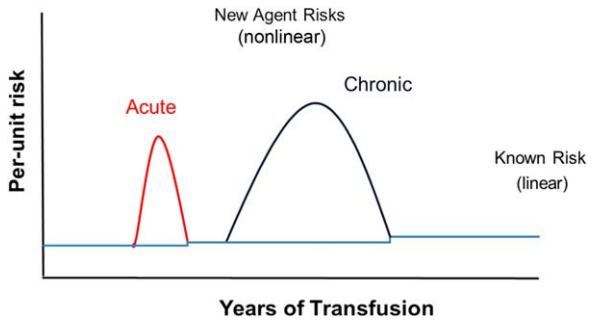

Fig. 1. Quantitating transfusion risk over time. In the absence of additional interventions, known per-unit infectious risks are consistent over time. These risks change when an EIA enters the blood supply. The figure indicates two types of EIAs: an acute agent and a chronic agent.* A past example of an acute EIA is West Nile virus and a past example of a chronic EIA is HIV. In contrast to known agents, EIA risks will vary over time. The intervals between an acute or chronic EIA entering the blood supply and the application of a successful intervention for that agent have been estimated as 1.5 and 5 years, respectively. After recognition of the EIA and development of a screening test, the risk from that agent will be decreased but a small residual risk will remain, thereby slightly increasing the overall per-unit risk above the previous level. This is indicated (though not to scale) by the stepwise increase in the horizontal line. *An acute agent is present only transiently (usually days to weeks) until the donor resolves the viremia or parasitemia. In contrast, the donor retains the chronic agent in their blood for many years (perhaps an entire lifetime) while remaining asymptomatic and capable of blood donation.

RISK REDUCTION BY PI

Infectious risks

Exclusively or predominantly from RBC transfusion

Babesiosis is a malaria-like illness transmitted by infected ticks and by transfusion. In healthy persons, infection is generally asymptomatic or mild and transient. However, clinically severe and even fatal disease has occurred in at-risk ill populations, especially patients who are immunosuppressed.[20,21]

Babesia spp. are intraerythrocytic protozoan parasites. *B. microti* is the primary agent of babesiosis in the United States and is highly endemic in the Northeast (Connecticut, Massachusetts, New Jersey, New York, Rhode Island) and the Upper Midwest (Minnesota and Wisconsin), areas that include 16% of the US population.[22] Recently, geographic expansion has been reported to neighboring states.[22,23] In the absence of surveillance in all 50 states, additional geographic areas where *Babesia* transmissions occur may go unrecognized.

A comprehensive CDC review reported 162 transfusion-transmitted babesiosis (TTB) cases (28 fatalities) in the United States between 1979 and 2009.[20] Since cases were compiled by passive surveillance, this is very likely an underestimate. *B. microti* was the agent in 159 cases while three involved *B. duncani*. All but four cases were from RBC units with transmission occurring throughout the storage period. Almost 80% were reported from 1999 to 2009; whether this represents increased transfusion transmission or lack of recognition of past cases or both is not known. Although nearly 90% of cases were in the seven highly endemic states, cases also occurred in nonendemic states.[20,22] These were attributed to shipment of RBC units from an endemic to a nonendemic area, a donor from an endemic area donating while visiting a nonendemic area, or a nonendemic area donor having acquired the infection while visiting an endemic area.

TABLE 1. Patients receiving RBC transfusions get exposed to different numbers of RBC units with different time frames of exposure*

RBC transfusion category	Diagnosis or procedure	Number of transfusion episodes	Total RBC unit exposure† (time)	Immune suppressed	Use of irradiated blood
Acute	Cardiac surgery[6,7]	Single	3‡	No	No
Acute	Trauma[8]	Single	5‡	Suppressed cell immunity	No
Intermittent	ICU[9]	Variable	3.5‡	No	No
Intermittent	Cardiovascular disease[10]	Variable	3‡	No	No
Sustained over limited time frame	HSCT[11,12]	Multiple	10-20 (3-6 months)	Yes	Yes
Chronic but time-limited	MDS[13]	Multiple	13/year (3 years)	Immunosuppressed in many cases	No§
Chronic, lifelong	SCD[14]	Multiple	24‡/year (30 years[15,16])	Asplenic	No§
	Thalassemia[17]		15/year (50 years[18,19])	No	

* These data are taken from representative publications for each RBC transfusion category and may not be fully reflective of all practice patterns. Depending on how the data were presented in the cited publication(s), they are expressed as a median, mean, or range thereof.
† The data include only the patients who received transfusions.
‡ Median.
§ Not routinely; may be irradiated if hospital-wide policies for hematology-oncology patients or for pediatric patients require.

There are no FDA-licensed blood donor screening assays. Recently, *B. microti* donor screening using serologic (automated immunofluorescence or enzyme-linked immunosorbent assay) and nucleic acid test (NAT) assays under an investigational new drug procedure has been ongoing on a portion of the inventory in several states.[24-27] Seroprevalence in these states ranged between 0.4 and 1.2% (17% being polymerase chain reaction [PCR] positive) and 1 in 10,000 donors showed PCR positivity without antibody.[27] Although PCR positivity increases the risk of transfusion transmission, PCR-negative but seropositive units may also transmit; furthermore low-level parasitemia or infectivity may be intermittent over a period of months to a year.[27-29] Whereas there have been no TTB cases from *Babesia*-negative RBC units, the TTB rate in these regions from unscreened units has ranged from 1 in 20,000 to 1 in 31,000 over the same time interval.[22]

These data suggest that a dual serology and NAT approach is needed to maximize risk reduction.[26,27] Given that *B. microti* is a mostly intracellular organism, this would require PCR testing of cellular material, which is logistically more challenging than the plasma NAT performed for other pathogens. Further, since *Babesia* infection is regional, a blood center's impetus to screen donations upon FDA licensure is likely to vary. In nonendemic areas, reluctance may be due to concerns about adding unnecessary cost for little safety gain and the loss of donors due to false-positive test results.[30] Finally, there is no legal or ethical precedent or model for regional screening of the US blood supply. These issues could be made moot by the use of PI-RBCs.

Transfusion-transmitted malaria is rare in the United States with an annual incidence of less than one case.[31] The major mechanism to reduce risk is the use of multiple predonation questions, including travel to a malaria-endemic area. Due to poor specificity for detecting malarial infections, large numbers of individuals who are not infected with malaria are deferred, thereby impacting blood availability.[32] Furthermore, errors in eliciting travel history lead to a large number of biologic deviation reports to FDA, which has a negative impact on blood operations and staff productivity. The introduction of a robust PI-RBC method that can inactivate all *Plasmodium* species in all their intra- and extracellular forms may allow elimination of the nonspecific and complex donor questioning used in the United States and eliminate malarial antibody testing that is used in many countries to shorten the deferral period.

Agents transmitted by RBCs or other components

Sepsis resulting from RBC bacterial contamination is rare but does occur. In France, this occurred at a rate of 1 per 2.6 million transfused RBC units from 2000 to 2008;[33] the rate in Germany was 1 in 1.9 million over a similar time frame.[34,35] In France, all seven septic cases were caused by Gram-negative bacteria, but in only a single case was the isolate (*Yersinia enterocolitica*) one that is commonly considered to be psychrophilic.[33] In Germany, multiple species of Gram-positive and Gram-negative organisms were reported.[34,35] In the United States, no RBC-mediated fatalities due to bacterial sepsis have been reported to the FDA in the past 5 years.[36]

Analogous to PLTs, it is likely that RBC bacterial contamination occurs more frequently than clinically detected sepsis.[37] As demonstrated in Table 2, 7% to 15% of RBC cocomponents associated with bacterially

TABLE 2. Bacterial contamination rates for WB-derived PC and associated results or disposition of RBC cocomponents

Period	Country (PC pool size)	Number of pools tested	Bacterial incidence*/10^6	PLT pools results	RBC cocomponent results or disposition
2000-2008[33]	France (4/5)	320,000	25	6 Gram-positive/2 Gram-negative (1 death)	NA
2008-2011[38]	United States (2-6)	70,867	99	7 (+) pools by POC test	1/7 (15%) RBCs (+) (CoNS); Culture-negative RBCs transfused
2003-2010[39]	Wales (4)	37,594	771	29 (+) pools (116 units)	7/105 (7%) RBCs; 7 Gram-positive
2005-2010[40]	Canada (4 [BC]; 5 [PRP])	228,142 (BC) (51,151 [PRP])	127 176	29 BC and 9 PRP culture (+)	NA
2008[41]	United States (5)	20,275	965	20 culture (+)	130 RBC units retrieved and discarded†

* This refers to bacterial contamination and is not a measure of clinical sepsis.
† The 130 RBC units were not cultured.
BC = buffy coat; NA = no data reported; PRP = platelet rich plasma.

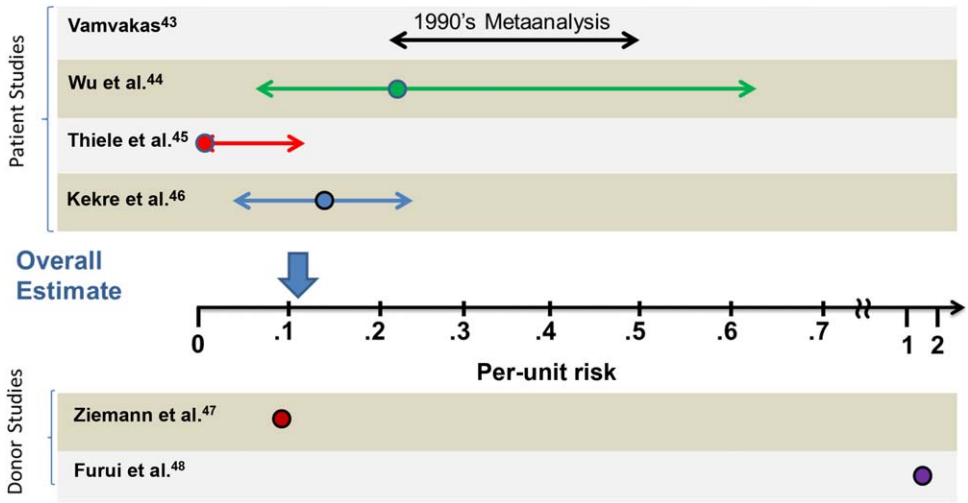

Fig. 2. CMV risk: historical data and recent studies. The graph depicts the per-unit risk as quantified in the different publications. The circles indicate the mean values. Patient studies are grouped above and donor studies are depicted under the x-axis. The length of the arrows corresponds to the 95% confidence intervals, when reported, or high and low estimates. The overall estimate is depicted with the vertical arrow above the x-axis and takes into account that only the approximately 50% of patients who are CMV seronegative are at risk for acquiring TT-CMV.

contaminated WB-derived PLTs contain bacteria; these data justify the policy of quarantining and either discarding or culturing RBC units associated with culture-positive PLT pools. PI-RBCs may prevent RBC-mediated Gram-negative sepsis as well as any potential deleterious effects from transfused Gram-positive bacteria.[33,38-41]

Transfusion-transmitted cytomegalovirus (TT-CMV) can be a serious medical complication in specific immunosuppressed populations such as CMV-seronegative hematopoietic stem cell transplantation (HSCT) recipients.[42,43] Strategies to reduce TT-CMV include the use of leukoreduced (LR) cellular products or CMV serology testing or both.[42,43] Despite these strategies, there is consensus that TT-CMV residual risk persists. As shown in Fig. 2, several recent transfusion transmission and/or donor-based PCR studies indicate that per-unit risk is approximately 0.1%.[42-48] A recent editorial indicated that, in the absence of PI, complex testing algorithms would be needed to reduce this residual CMV risk and would result in substantial loss of transfusable RBC units.[42]

Anaplasma phagocytophilum, the agent of human granulocytic anaplasmosis, is an intracellular, Gram-negative bacterium with neutrophil tropism.[49,50] Nine transfusion-transmitted cases have been reported; in seven cases, the implicated blood product was an RBC.[49,51] Transmission has occurred with both LR and non-LR RBC units; in one case the unit had been stored for 30 days. Eight of the cases were reported since 2007,

TABLE 3. Calculated and actual prevalence of EIAs

	Prevalence in blood donations (%)	
	Chronic agent	Acute agent
Model EIA[4] (range)	0.045 (0.01-0.08)	0.025 (0.007-0.075)
CHIKV		0.038-0.052 (Thailand) [52] 0.4 (Reunion)[53,54]
DENV		0.07 mean;[55] 0.45 max
HEV	0.01 (US);* 0.035 (UK)[56]	

* S.L. Stramer, personal communication, 2015.

indicating an incidence of about one case annually.[49,51] Similar to *B. microti*, these observations illustrate how increased scrutiny may uncover a pathogen prevalence and level of risk that had previously escaped the attention of public health and transfusion medicine specialists. Currently, there is no blood donor screening for this agent.

EIAs

Table 3 contains risk estimates for a theoretical EIA entering the US blood supply as well as recent data for EIAs detected in specific non-US and US locations.[4,52-56]The recent chikungunya epidemic in the Caribbean, including the US territory of Puerto Rico with some autochthonous cases in Florida,[57] validate the model and indicate that acute agents can rapidly materialize and not be limited to traditional geographical boundaries. Currently for dengue and chikungunya, risk is posed by donors with recent travel to "epidemic" regions, as illustrated by the very high estimated peak incidence of chikungunya in La Reunion (1500 per 10^5 donations)[53,58] and Thailand (38.2-52.3 per 10^5 donations).[52,59] This has prompted travel-related donor deferrals in some European and Asian countries, with similar policies under consideration elsewhere.[60] However, such deferrals have inherent nonspecificity and may require periodic revision to account for new epidemics. Extrapolating from data showing the effectiveness of PLT and plasma PI[61,62] against these agents, a reasonable assumption is that PI-RBC technology will also be effective. If so, application of PI to all components could obviate the need for EIA-related travel deferral.

Overall RBC infectious risks without PI

Per-unit risks for individual pathogens are summarized in Tables 4 and 5,[1,27,33,46,50,51,63-65] which also provide an aggregate per-unit risk, expressed as a minimum (0.00031%, based on lower risk pathogens) and a maximum (0.12031%, representing composite risk due to *Babesia* in an endemic area, an acute EIA, and lower risk pathogens). These risks are increased by 0.1% for HSCT patients due to their susceptibility to CMV transmission. Table 6 combines aggregate per-unit risk estimates from Table 5 with the number of transfused units from Table 1 to calculate a minimum and maximum per-recipient risk for different patient categories.[66] At the minimum risk levels, it is estimated that 1 in 67 HSCT recipients will acquire an infection during their period of intensive posttransplant transfusion support and that approximately 1 in 450 (0.22%) patients with hemoglobinopathies will do so over their entire course of transfusion therapy (approx. 30-50 years). At the maximum levels, infectious risk increases to 43% to 45% (1 in 2) for hemoglobinopathy patients. Risk is also high for patients with myelodysplastic syndrome (MDS; 1 in 27) and HSCT recipients (1 in 30) and is not insignificant (1 in 400 to 1 in 150) for other categories of patients who receive fewer units.

Noninfectious risks

Transfusion-associated graft-versus-host disease

Transfusion-associated graft-versus-host disease (TA-GVHD), an almost uniformly fatal condition, is prevented by completely inactivating T lymphocytes in RBCs or LR-RBCs.[67] This is currently accomplished by gamma irradiation, which although highly effective in preventing GVHD, has multiple limitations.[68] Rare TA-GVHD cases are still reported likely due to substandard treatment or failure to apply the procedure uniformly to all cellular units for patients at risk, due either to inappropriate institutional criteria or to incorrect patient diagnosis.[68-74]

Gamma irradiation is known to damage RBC membranes causing acute and delayed hemolysis and to damage the Na-K pump resulting in potassium leakage from the RBCs.[75] Consequently, storage of irradiated RBCs is limited to 28 days postirradiation.[76]

In the United States in 2011, an estimated 13.4% of transfused RBCs were irradiated.[2] It is likely that the criteria for irradiation varied among institutions. Similarly, the length of time that an irradiated RBC unit is stored before being transfused may also vary. In the scenario of batch mode irradiation with subsequent storage of units, there is the theoretical concern that these RBC units may function less optimally than nonirradiated RBCs and therefore should not be given to patients *not* at risk of TA-GVHD. The alternative scenario of irradiating units just before product issue poses logistic challenges and is only possible if the institution has its own blood irradiator. Finally,

TABLE 4. Per unit risk in transfused RBC under current donor testing protocols in the United States

Pathogen	Risk	Method of estimation
Higher-risk pathogens		
B. microti[27]	0.076% (1 in 1316)	Antibody and PCR data in endemic areas*under IND screening†
CMV[1,46]	0.1% (1 in 1000)*	Detection of infection in transfused recipients and PCR data in donors
EIA		
Acute-type agent[4]	0.025% (1 in 4000)	Mathematical modeling‡
Chronic-type agent[4]	0.045% (1 in 2222)	Mathematical modeling‡
Lower-risk pathogens		
Plasmodia—all species	Rare	Clinical case reporting (<1 TT case per year in United States)
Bacteria[33]	0.00005% (1 in 2 million) Clinical Sepsis	Based on French and German Data No documented clinical cases in the United States in past 5 years; May be more common for subclinical cases
A. phagocytophilum[50,51]	Rare	Clinical case reporting (<1 TT case per year in United States); May be more common for subclinical cases
HIV[63]	0.00007% (1 in 1.5 million)	Mathematical modeling§
HCV[63]	0.00009% (1 in 1.1 million)	Mathematical modeling§
HBV[64]	0.0001% (1 in 1 million)	Mathematical modeling§
WNV[65]	Rare	Clinical case reporting (<1 TT case per year in United States)

* Rare in nonendemic areas.
† Assumes that all PCR-positive donations, regardless of antibody status, would be infectious.
‡ Using data from previously detected EIAs.
§ Using NAT donor screening data and a window period model.
IND = investigational new drug.

TABLE 5. Aggregate single-unit risks in transfused RBC under current donor testing protocols in the United States

Aggregate risk category	Risk elements*	Risk
Minimum	• HIV + HCV + HBV • Bacteria • *Babesia*-nonendemic area	0.00031% (1 in 322,600)
Minimum + CMV†	• HIV + HCV + HBV • Bacteria • CMV risk for immunocompromised patients • *Babesia*-nonendemic area	0.10031% (1 in 996)
Maximum	• HIV + HCV + HBV • Bacteria • *Babesia*-endemic area • New chronic EIA	0.12031% (1 in 831)
Maximum CMV†	• HIV + HCV + HBV • Bacteria • CMV risk for immunocompromised patients • *Babesia*-endemic area • New chronic EIA	0.22031% (1 in 454)

* This column contains the components that are then summed together to provide the total risk (shown in the right-hand column), for each aggregate risk category. The numbers for each risk element are taken from Table 4.
† (HSCT patients).

facilities with irradiators have been subject to increasing regulatory scrutiny due to bioterrorism concerns, making the continued use of this equipment less desirable and more expensive.[76]

PI-RBCs and PI-WB procedures have been found effective in inactivating white blood cells (WBCs) and T cells and when applied routinely, could replace the use of gamma irradiation, and solve logistic challenges.[77,78] Data for one of these technologies indicate that PI-RBCs show lower hemolysis, lack of effect on the Na-K pump, and lower extracellular potassium and protein levels, resulting in better in vitro function than gamma-irradiated RBCs.[79]

RBC alloimmunization

Since PI is known to prevent donor WBCs from exerting their immunologic effects, PI could theoretically affect the

TABLE 6. Aggregate lifetime patient risks due to RBC transfusion for different patient categories under current testing algorithms in the United States

Diagnosis	RBC unit exposure	Aggregate risk per patient (%)	
		Minimum*[1]	Maximum†[2]
Cardiac surgery	3	0.0009 (1/107,000)	0.36 (1/277)
Trauma	5	0.0016 (1/65,000)	0.60 (1/167)
ICU	3.5	0.0011 (1/91,000)	0.42 (1/238)
Cardiovascular disease	3	0.0009 (1/107,000)	0.36 (1/277)
HSCT	15	1.49 (1/67)	3.25 (1/31)
MDS	39	0.012 (1/8,000)	3.76 (1/27)
SCD	720	0.22 (1/450)	43.17 (1/2)
Thalassemia	750	0.23 (1/430)	45.13 (1/2)

* The method of calculating risk when large numbers of units are transfused as described by Kleinman et al.[66]

† Lifetime risks, except for cardiovascular disease and ICU patient groups. In the latter groups, risk is for a single hospitalization or ICU stay. Lifetime risk would increase for patients transfused on multiple occasions.[1] Minimum per-unit risk is 0.00031% for all patient groups except for HSCT patients, where minimum risk is 0.10031% based on potential sequelae from TT-CMV infection.[2] Maximum per-unit risk is 0.12031% for the first four patient groups and 0.22031% for HSCT patients. For patients with MDS, SCD, and thalassemia, risk is 0.12031% for a 1.5-year period (when a new acute EIA is in the blood supply) and 0.07631% (due to *Babesia*) when transfused during other time intervals.

immune system's presentation of RBC antigens and thereby influence RBC alloimmunization rates; therefore, the impact of LR on RBC alloimmunization may help predict whether PI treatment of RBC would have a similar effect.[80-82]

The development of RBC alloantibodies has well-known potential deleterious consequences. The rate of RBC alloimmunization in transfused hospitalized patients (excluding patients with hemoglobinopathies) has been measured at 1.8% and 4% in two large studies.[83,84] The rate increases with the number of RBC units transfused; however, the majority of antibodies are formed early in the course of transfusion therapy. Specific primary diagnoses are associated with higher rates: 18% to 47% in sickle cell disease (SCD) patients in the absence of phenotypic matching,[85] 5% to 30% in thalassemia,[17,86-89] 15% in MDS,[90] and 9% in patients with malignant hematology diagnoses.[91]

A prospective small randomized control trial in 404 cardiac surgery patients examined the effect of universal LR on RBC alloimmunization.[92] Although the rate was lower in the LR group than in the non-LR group (3.4% vs. 7.1%), the difference was not significant. A smaller single-hospital study using a retrospective noncontemporaneous study design demonstrated a decreased alloimmunization rate in recipients of LR versus non-LR RBCs based on comparing data from two 1-year intervals separated by 14 years.[93] This same study showed that LR resulted in a decreased alloimmunization rate in acute myeloid leukemia patients whereas a different study showed no effect of LR in MDS patients.[90] Other smaller studies in thalassemia patients have suggested an association of LR with a decreased RBC alloimmunization rate;[94] however, these studies have had small sample sizes and have potential confounding factors. In summary, the available data do not allow for a firm conclusion. Unlike LR (which still leaves a small number of viable WBCs in the blood product),[80,81] PI renders WBCs nonviable and stops protein production and antigen expression, thus establishing a theoretical basis for why PI might reduce alloimmunization even if LR does not.[95,96]

Patients with leukemia usually receive both RBC and PLT transfusions. Current data suggest that PI treatment of PLTs may reduce the rate of HLA alloimmunization in this patient group.[96] It is possible that PI-RBCs may show the same benefit. If so, the application of PI to both components may protect against HLA alloimmunization and may improve the odds of finding a compatible HSCT donor for patients with leukemia as well as for patients with hemoglobinopathies who may become future HSCT candidates.[97,98] These possibilities need to be examined in the clinic.

It is important for PI clinical trial protocols to include an assessment of RBC alloimmunization rates. Since the overall RBC alloimmunization rate is low in the general transfused population,[83] it is unlikely that pivotal clinical trials will be powered to adequately evaluate this phenomenon and data from routine use will be required.

APPROACHES FOR PRODUCING A PI-RBC PRODUCT

There are two conceptual approaches to obtaining PI-RBCs (Fig. 3). WB can be separated into components and then PI can be applied to the RBCs, or PI treatment can be applied to the WB unit. The PI-WB unit can be transfused as WB or, alternatively, could subsequently undergo further processing to produce components;[99] the latter approach has the logistic advantage of producing multiple PI components from a single PI application. However, if the WB unit is stored before processing, this approach may require compromises since component storage

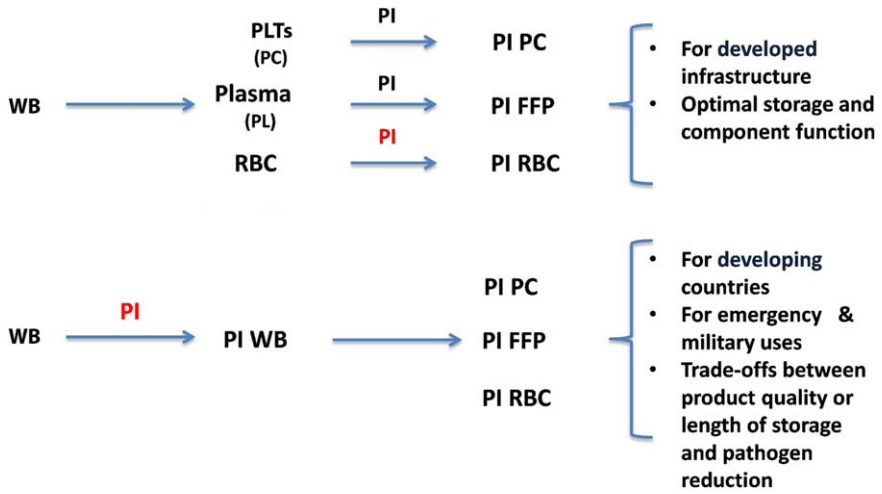

Fig. 3. Conceptual approaches for PI of blood products.

requirements are conflicting and will be difficult to satisfy simultaneously (e.g., RBCs and WB are stored refrigerated, PLTs at RT, and plasma frozen).

In developed countries, targeted blood component therapy for specific indications has been standard practice for many decades. Specialized storage containers have been tailored to each component and additive solutions developed to optimize quality and extend shelf life.[100] Furthermore, individual-component PI technology is compatible with apheresis component collection, which has become an important part of the blood supply chain.[101] In contrast, in countries with little infrastructure or in acute trauma situations (particularly in military conflicts), the need for WB transfusion is greater and suggests the value of the application of PI to WB units.[102,103]

Two methods are in commercial development for supplying PI-RBC products: WB photochemical inactivation using riboflavin and ultraviolet (UV) light (Mirasol System)[104] and RBC chemical inactivation using S-303 and glutathione (GSH; Intercept System).[105] In addition, use of the S-303 and GSH system to treat WB is being pursued for the developing world.[106] The basic characteristics of the two systems are summarized in Fig. 4. The riboflavin and UV WB system utilizes the same riboflavin dose and illuminator as for plasma and PLT PI, but uses a much higher UV dose, corresponding to a significantly longer illumination time (Fig. 4).[104]

The S-303 and GSH system, now in its second generation, utilizes a chemical system featuring a fast-acting compound (S-303) that reacts with nucleic acid bases to form stable adducts and cross-links with a mode of action similar to the Intercept systems for PLTs and plasma, but without the use of an illuminator.[105] To minimize nonspecific reactions with molecules in the extracellular domain, GSH is included in the process. Because of its size, GSH does not penetrate cell or viral membranes, so when added to the RBC unit, it remains exclusively in the extracellular space. This allows quenching of extracellular reactions without a significant impact on pathogen and WBC inactivation. The modifications present in the second-generation system were implemented to reduce the formation of immunogenic adducts on the surface of PI-RBCs.[107-109] The second-generation system uses the same dose of the active ingredient S-303, a buffered version of the quencher GSH at 10-fold higher concentration for improved quenching, and includes an exchange step after the overnight incubation that allows the effective removal of proteins and electrolytes from the RBC supernatant.[109,110]

DATA FOR PI-RBC AND PI-WB SYSTEMS

Licensure of PI-RBCs or PI-WB requires in vitro studies of RBC quality during storage, in vitro inactivation studies for representative transfusion-transmitted pathogens, in vivo recovery and survival studies of transfused RBCs in healthy volunteers to validate the maximum allowable length of product storage, and clinical trials of safety and efficacy in relevant patient populations. After licensure, postmarketing hemovigilance studies will allow the further characterization of PI-RBC safety and efficacy.

RBC quality during storage has been summarized in the literature.[104,105,110,111] PI studies are ongoing by both PI manufacturers; available data are summarized in Table 7.[77,78,99,104,105,112-121] These inactivation data still need to be validated by full-unit studies with multiple replicates, and hence care should be taken in their interpretation; nevertheless, a few points emerge. For the S-303 and GSH

TABLE 7. Compilation of published PI data in RBCs for S-303 and GSH and in WB by riboflavin and UV

	Mean log reduction		
Pathogen	S-303 and GSH		Riboflavin and UV (@ 80 J/mL$_{RBC}$)
Viruses			
HIV - cell free	>6.5[112]*		
HIV- cell associated	>5.9[105]		4.5[113]
BVDV (surrogate for HCV)	>4.8[105]		
DHBV (surrogate for HBV)	>5.1[121]	6.3[112]*	
CMV model viruses			
HSV	>6.0[112]*		
IBR			1.5[99]
VSV	5.7[112]		4.5[104]
Bluetongue	≥6.0[112]	>5[105]	1[99]
Adeno Type 5	>7.4[105]		
WNV	>6.0*[119]		
SARS	>6.5[120]*		
CPV			3.8[99]
HAV			1.5[99]
Parasites			
B. microti	>5.5[112]	>4.9[119]*	>4.73[114]
Plasmodium falciparum	>6.8[119]*		>6.4[118]
T. cruzi	>5.4[112]*	>5.3[119]*	>3.5[116]
Leishmania donovani			2.3[117]
Bacteria			
Y. enterocolitica	≥ 6.8[105]	7.4[112]*	2[99]†
Serratia marcescens	5.1[105]	4.1[112]*	
Serratia liquifaciens			2[99]†
Pseudomonas aeruginosa	4.5[112]*		
Escherichia coli	≥6.7[105]	7.4[112]*	
Staphylococcus aureus	5.1[105]	>5.1[112]*	
Staphylococcus epidermidis	>6.9[112]*		
Listeria monocytogenes	>7.1[112]*		
WBCs	>5[77]*		4.7[78]

* Inactivation achieved with first-generation system (0.2/2 mmol/L GSH).
† These data are from low-titer experiments and inactivation of higher bacterial titers was not evaluated.
CPV = canine parvovirus; DHBV = duck hepatitis virus; HSV = herpes simplex virus; IBR = infectious bovine rhinotracheitis virus; SARS = severe acute respiratory syndrome; VSV = vesicular stomatitis virus.

system, the extent of PI has remained comparable between the first- and second-generation approaches. For the riboflavin and UV system, the limited data indicate that inactivation of some pathogens is lower in the WB system than in the PLT and plasma systems, despite the higher dose of UV light used (80 J/mL$_{RBC}$ vs. 6.2 J/mL $_{PLASMA}$). This is consistent with the lower efficiency for UV light delivery in the presence of hemoglobin (Hb)-containing RBCs.[99]

Each technology has undergone recovery and survival studies independently performed by the same investigator (Table 8).[122,123] The S-303 and GSH system was tested in 27 healthy volunteers in two centers using a crossover design.[122] At 35 days of storage, the 24-hour recovery of autologous treated RBCs compared favorably with control RBCs (88% vs. 90%, $p = 0.31$), meeting FDA requirements. The survival of S-303 RBC (T_{50}) was lower than that of control RBCs, but within the normal range (32.7 days vs. 39.5 days; $p = 0.0001$; normal, 28-35 days).[122] In a different study,[123] RBCs stored for 42 days were manufactured from riboflavin and UV-treated autologous WB units prepared using variable UV doses (22, 33, and 44 J/mL$_{RBC}$). Only five of 11 subjects met the FDA requirement of more than 75% recovery at 24 hours; mean RBC survival was 24 ± 9 days. There was a trend toward lower recovery and lower survival for higher illumination doses. These data, along with recently published in vitro data at Storage Days 21 and 42, indicate that a storage time shorter than 42 days will be required for an 80 J/mL$_{RBC}$ dose.[111]

Table 8[122-131] also reports other Phase II and III studies, primarily using information from the ClinicalTrials.gov website.[124-131] For the first-generation S-303 and GSH system, a Phase III trial in SCD patients was terminated early when two patients developed apparent RBC antibodies, but no sequela.[108]

The companion Phase III first-generation PI-RBC study in cardiovascular surgery patients with acute anemia, conducted simultaneously with the SCD study, met its primary noninferiority composite endpoint despite its early termination due to the SCD study findings.[124] After S-303 reformulation, two Phase III clinical studies with the second-generation S-303 and GSH RBC system are in progress in Europe, targeting the indications of acute and chronic anemia in cardiovascular patients and patients with thalassemia, respectively.[125,127]

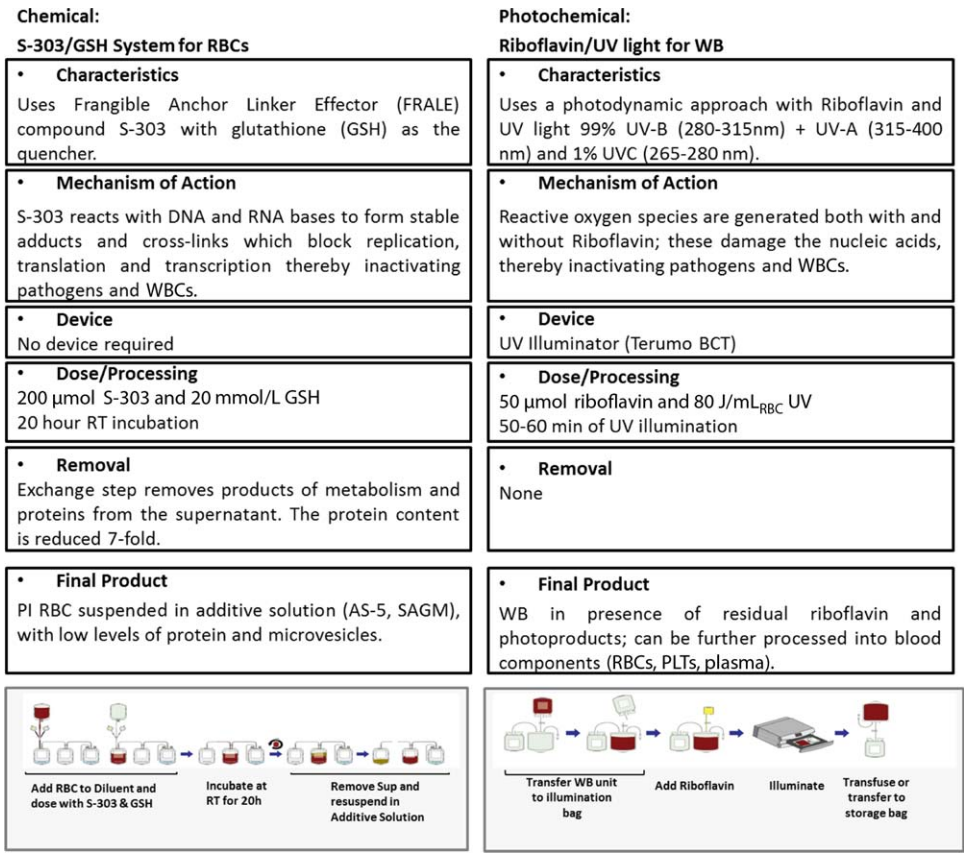

Fig. 4. Comparison of systems for PI of RBCs and WB.[104,105]

For riboflavin and UV, an additional Phase II study (presumably using an 80 J/mL$_{RBC}$ dose) has finished recruiting.[129] A Phase III study on the prevention of TT-malaria among recipients of PI-WB is under way in Africa.[130]

POTENTIAL ADVERSE EFFECTS OR RISKS OF PI-RBCs

Potential risks of transfusing PI-RBCs include toxicology-based adverse side effects, increased RBC alloimmunization, and reduced clinical benefit to patients (i.e., efficacy). Extensive toxicology data for both PI-RBC systems are available in the literature.[132,133] Such data have been reviewed by regulatory agencies and found robust enough to authorize Phase II and III clinical trial work. With regard to the primary chemical agents, S-303 completely decomposes during the 20-hour treatment process and, in addition, the chemically inert reaction by-products are significantly reduced through the exchange step. Riboflavin and its photodegradation products have a toxicology profile of "generally regarded as safe." Nevertheless, potential long-term toxicology risks can only be definitively assessed by collecting routine use data.

As mentioned, two SCD patients in a first-generation S-303 and GSH Phase III trial developed antibodies; these were found to be directed against adducts formed on the RBC surface during S-303 treatment. Further characterization of the antibodies showed they were low titer (2-8), were inhibited by acridine compounds (thereby pinpointing specificity for the anchor part of the S-303 molecule), and did not cause phagocytosis of S-303–treated RBCs in an in vitro model of RBC clearance.[134] In a rabbit mismatch transfusion model,[135] first-generation S-303 RBCs circulated normally in naive animals and did not cause the formation of antibodies. However the S-303 RBCs showed accelerated clearance when the animals were immunized with KLH-acridine compounds. These observations led to the technology modifications incorporated in the second-generation system. When second-generation S-303 RBCs were transfused to the same pre-immunized animals, they exhibited normal circulation. Finally, sera from the two antibody-positive patients were negative when cross-matched against second-generation S-303 RBCs.

Despite these encouraging observations, concerns still exist regarding alloantibody formation due to RBC alterations caused by second-generation S-303 treatment.

TABLE 8. Clinical experience

Study	Number	Description	Endpoints	Results
A. With the S-303 and GSH PI-RBC system				
First generation				
US Phase III chronic study	50	Transfusion-dependent SCD patients; two-arm double-blinded crossover design	Blood utilization	Terminated
US Phase III acute study[124]	148	CV surgery patients	Composite endpoint of MI, renal failure, and mortality[124]	Met primary endpoint, early termination
Second generation				
US Phase II study [122]	27	Healthy volunteers; crossover design	24-hour recovery: 88.0 ± 8.5 days (T) vs. 90.1 ± 6.9 (C)	Met primary endpoint, completed
EU Phase III acute study[125]	50	CV surgery patients; two-arm design	Primary efficacy: mean Hb content per RBC component Primary safety: adverse events over 90 days (related and unrelated to study RBC components) compared between the treatment groups	Met primary endpoint with similar AE profile between arms[126]
EU Phase III chronic study[127]	70	Transfusion-dependent thalassemia Major patients; crossover design	Primary efficacy: Hb consumption (g Hb/kg body weight/day). Primary safety: incidence of a treatment-emergent antibody with confirmed specificity to S-303 RBCs over 12 months	In progress
B. With the riboflavin and UV PI-WB system				
US Phase II Study–IMPROVE[123,128]	12 (4/4/3)	Feasibility trial to evaluate recovery and survival in RBCs obtained from WB units treated with the Mirasol system. Three study arms each using a different UV dose (22, 33, and 44 J/mL$_{RBC}$)	Primary: 24-hr posttransfusion RBC recovery Secondary: RBC survival; SAE	Terminated *Recovery ≥ 75%* 22-J dose: 1 of 4 33-J dose: 1 of 4 44-J dose: 1 of 3 *Survival ≥ 28 days* 22-J dose: 2 of 4 33-J dose: 2 of 4 44-J dose: 0 of 4
US Phase II study–IMPROVE II[129]	29	To evaluate, as per FDA criteria, the 24-hr posttransfusion RBC recovery in healthy adult subjects of LR-RBCs, derived from Mirasol-treated fresh WB units, and stored refrigerated for 21 days.	Primary: 24-hr posttransfusion RBC recovery Secondary: RBC survival, AUC; SAE; neoantigenicity	Completed Data not yet reported
Ghana Phase-III Study–AIMS[130]	250	Treatment of WB with the Mirasol system: prevention of Malaria caused by transfusion	Primary: TT malaria Secondary: TT bacterial infections	Completed

AUC = area under curve; CV = cardiovascular; MI = myocardial infarction; SAE = severe adverse event.

Thus, a primary aim of the second-generation S-303 thalassemia clinical trial is to monitor RBC immunogenicity. However, given the small size of any clinical trial, negative results will not be sufficient to resolve this issue. This will require an ongoing hemovigilance program to monitor routinely used product (as has been done for PI-PLTs and PI-plasma)[136-139] to achieve the numbers required to assess this potential transfusion complication. Similar clinical trial endpoints and hemovigilance monitoring will be required to determine if the riboflavin system affects RBC immunogenicity.

If an increased frequency of alloantibody formation were to be found, this should be viewed in the context of the known high alloimmunization rate in chronic RBC recipients;[80,82] that is, it may be that the benefits of PI-RBCs will exceed a small risk conferred by the development of additional antibodies, especially if these antibodies do not cause hemolysis and/or result in an increased difficulty in finding compatible RBC units.

The potential for decreased efficacy of PI-RBCs has not yet been fully assessed and will require Phase III clinical trial data as well as routine use data. For S-303 RBCs,

in vitro RBC quality assessment and in vivo volunteer studies indicate that at 35 days of storage, these cells would be expected to function as well as non–PI-RBCs when transfused. With regard to the riboflavin WB system, a full set of similar data is not yet available but preliminary results suggest that the shelf life of this product may be limited to shorter than 35 days.

DISCUSSION

RBC transfusion carries multiple risks, each of which (excepting some infections in highly endemic areas) is relatively small. Because of this, it appears unlikely that a laboratory screening intervention to minimize any one of these risks will be implemented. The chief deterrent is cost, but other factors such as lack of concern by clinicians (due to underrecognition of cases, underappreciation of the potential for severe outcomes, and the availability of treatment) and blood center concerns of unnecessary donor deferrals due to false-positive test results may also influence inaction. PI offers a solution to this dilemma in that multiple risks can be obviated by a single intervention. Since PI will come at additional cost, an economic analysis leading to a decision to implement PI may also need to factor in protection against an EIA that could result in a large number of transfusion transmissions and severe recipient outcomes.[4,5] In this worst-case scenario, failure to have implemented PI may create lack of trust in the entire blood system.

The potential specific benefits of PI-RBC technology include:

- Reducing risk to essentially zero for most current transfusion-transmitted pathogens (HIV, HCV, HBV, HTLV, WNV, syphilis, CMV, *Babesia*).
- Substantially decreasing or completely eliminating risk for pathogens with the potential for a very high genomic titer (e.g., dengue).[61,140] Depending upon the robustness of the PI technology and the infectious dose of a particular pathogen, full inactivation of such pathogens could be achieved for all units or a substantial proportion of cases depending on the pathogen load.
- A greater margin of protection against TA-GVHD than irradiation due to robust WBC inactivation.[67,69-74,141]
- The possible reduction of WBC alloimmunization would provide HSCT candidate recipients with a higher likelihood of successful HLA matching to potential HSCT donors.
- Operational benefits in blood manufacturing and inventory management. Dual RBC inventories for both CMV and irradiation status could be eliminated and recall of RBC units associated with bacterially contaminated PLTs could be discontinued.
- Eliminating a blind spot of the current testing paradigm, which requires that a pathogen be detectable by NAT in a plasma sample or that the donor has developed a robust serologic response to an intracellular organism (e.g., *Babesia*); PI would obviate the need to implement a more logistically complex cellular-based PCR platform.
- Eliminating donor screening questions including travel to malaria-endemic areas, a history of babesiosis or Chagas disease, and travel to WNV-endemic areas (asked ex-US). Since malaria travel deferrals are very common, their elimination could have a significant impact on the number of eligible donors.

With regard to cost containment, PI implementation for all components (RBCs/PLTs/plasma) should allow for the discontinuation of some donor screening tests and/or the modification of existing screening protocols.[142] Syphilis testing would no longer be needed as transfusion transmission risk is exceedingly low, serologic testing has limited ability to detect early infection, and PI methods have high efficacy in killing the organism in each component type (shown for PLTs and plasma and still to be verified for PI-RBCs).[143-145] CMV antibody, *Trypanosoma cruzi* antibody, and HBsAg testing (assuming that HBV NAT is implemented) could also be eliminated.

A robust PI technology that can inactivate 5 to 8 *infectious* log of most pathogens would allow for modification of NAT protocols. There would be no need to perform individual NAT for HIV, HCV, HBV, or WNV since minipool testing would be adequate to detect any units with high viral load that otherwise might theoretically escape the full effects of PI. Minipools could be made larger as is currently done in source plasma testing.[146] Consideration could also be given to removing some serologic assays that might be considered redundant; these include anti-HBc, anti-HCV, and even anti-HIV (although the latter might trigger public concern). Of course, modification of blood donor screening protocols would require regulatory authority approval and it would probably take some years of routine use with systematic hemovigilance efforts to accumulate the data required for such changes to be made.

A fully PI-treated blood supply would shape the response to threats from new EIAs, in that there would be less pressure to develop screening assays.[142] For years, this has been the case for fractionated plasma derivatives that routinely undergo PI. For example, recipients of these products were protected from WNV transmission at a time when transmission to recipients of blood components occurred.[146] Also, as has been seen with regard to agents posing a transfusion transmission risk that does not reach a crisis level and do not have compelling business cases due to factors such as geographic or seasonal

variations in incidence/prevalence (e.g., *Babesia* and dengue), new blood donor screening assay development cannot be relied upon to protect the blood supply.[30] The approach of PI-treated components may ultimately be less complex and less expensive than continued assay development.

Two PI-RBC and WB systems are in different levels of development and each may have its role with riboflavin and UV best suited for developing countries and S-303 and GSH for developed countries. Further studies with the actual system(s) used are still needed to demonstrate inactivation of CMV, spirochetes, selected EIAs, multiple bacterial species, and WBCs—the latter for replacement of gamma irradiation. The assessment of alloimmunization with PI-treated RBCs should be investigated with the realization that routine use is the only way to achieve the numbers required to assess this transfusion complication and other potential severe adverse events. Finally, PI-RBCs may still have its limitations since some pathogens (Parvovirus B19, HAV, HEV) are at least partially resistant to inactivation.

In summary, PI-RBCs should be viewed in the context of having a fully PI-treated blood supply, thereby shifting the blood safety paradigm from reactive to proactive[142] and as providing insurance against known and unknown pathogens that may enter the blood supply or are currently un(der)recognized.[4]

CONFLICT OF INTEREST

SK is a paid consultant to Cerus Corporation; AS is employed by Cerus Corporation.

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