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INVITED REVIEW

Progress towards an appropriate pathogen reduction technology for whole blood in Africa

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Background Interest in an effective pathogen reduction technology (PRT) for whole blood (WB) tailored to address specific needs of limited-resource settings is growing. The Swiss Red Cross Humanitarian Foundation (SRCHF) supports a clinical programme to promote PRT for WB in resource-limited settings where rates of WB use and transfusion-transmitted infections are high.

Pathogen reduction technology for whole blood Cerus Corporation (INTERCEPT® Blood System) and Terumo BCT (Mirasol®) are investing in clinical trials for red-blood-cell (RBC) and WB PRT. The SRCHF selected the INTERCEPT WB PRT for its clinical programme in Africa based on data related to the INTERCEPT system's pathogen reduction effectiveness and the company's goal of producing a processing kit requiring minimal electricity.

Adapting the INTERCEPT RBC PRT for WB The INTERCEPT PRT for RBC and WB uses amustaline (0·2 mM) to irreversibly cross-link nucleic acids and glutathione (GSH) (20 mM for RBC and 2 mM for WB) to quench unreacted amustaline. Laboratory and clinical trial results for the RBC PRT form the basis for the WB PRT. Preliminary studies have demonstrated preservation of RBC quality in amustaline/GSH-treated WB. A Phase 1 safety trial in Côte d'Ivoire and development of a WB processing kit requiring limited electricity are described.

Discussion Assessing the clinical safety of a WB PRT under local conditions is a positive step in the development of a suitable WB PRT for low-resource settings.

Key words: Africa, amustaline, pathogen reduction, whole blood.

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Background

Improvements in the selection of voluntary, non-remunerated blood donors and laboratory screening have reduced the risk of some transfusion-transmitted infections (TTI) in many low- and middle-income countries in sub-Saharan Africa [1]. However, these improvements remain focused on a small number of viral and bacterial markers, for example HIV, hepatitis B (HBV) and C (HCV), and syphilis, and are not evenly implemented across all 47 countries represented in the World Health Organization's Regional Office for Africa (WHO AFRO) (Fig. 1) [2–4].

Challenges facing blood transfusion services in sub-Saharan Africa include low rates of blood donation [1,5], quality assurance gaps in blood screening laboratories [6–10], high rates of inappropriate blood use [11,12] and insufficient funding from national and/or external sources [2,13]. Current blood donation rates are also insufficient to cover 100% of many countries' transfusion needs

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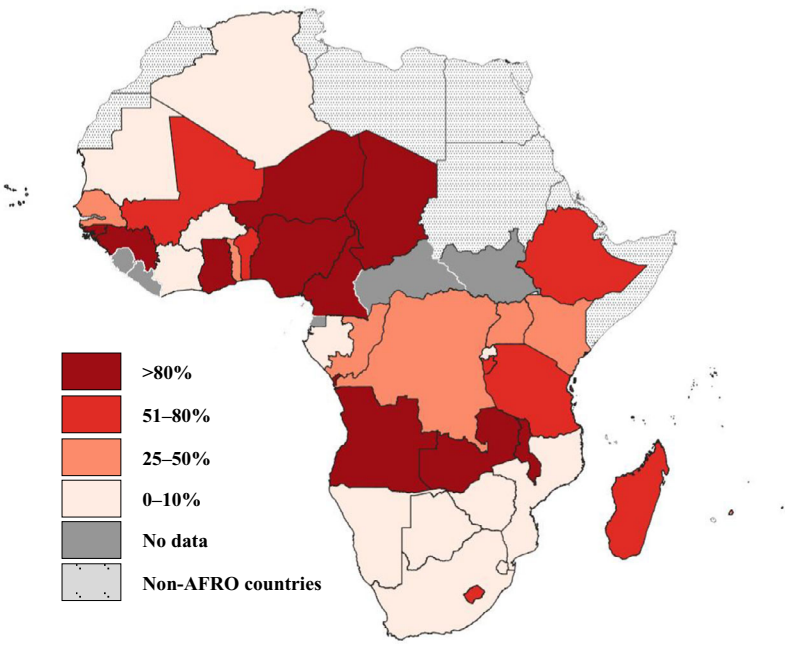

Fig. 1 Percentage of whole blood donations transfused as whole blood, AFRO Region, 2013. Source: WHO Global Status Report on Blood Safety and Availability, 2016. All data from the 2013 Global Database on Blood Safety data collection except Benin and Nigeria (2011).

[14,15] shortages that contribute to high rates of maternal and child mortality. An average of 26% (range 16–72%) of maternal and child deaths have been linked to deficits in the availability of blood to treat cases of maternal haemorrhage and severe childhood anaemia associated with malaria [16,17].

The prevalence of TTI is also notably higher in sub-Saharan Africa compared with other regions of the world [1,18,19]. In 2013, the WHO reported that in West and Central Africa, the median percentage of donated blood units reactive for HIV, HBV and HCV was 1·9–2·2% (range 0·08–6·7%), 6·3–7·7% (range 0·3–17·4%) and 1·2–1·8% (range 0·3–4·9%), respectively [20]. In 2016, 9 of 11 countries with the highest donor prevalence rates in a regional TTI prevalence study were located in West or Central Africa [15]. Other infectious agents are equally common, including the mosquito-borne parasites responsible for malaria and lymphatic filariasis, as well as human T-lymphotropic virus (HTLV). Studies in multiple African countries have estimated the prevalence of *Plasmodium* species infection in blood donors as high as 38%, with lymphatic filariasis and HTLV estimated at ~9% and 3%, respectively [21–25]. In addition to mosquito-borne parasitic infections, several transfusion-transmissible arboviruses, including dengue, West Nile, yellow fever and Zika viruses are endemic in many sub-Saharan African countries. The risk to transfusion recipients from these arboviral pathogens is not well documented in African blood donors since routine testing is not performed. However, surveillance studies in other regions, including the Caribbean, Latin America and the South Pacific, have estimated the prevalence of dengue and Zika viraemia in blood donors at up to 3% during outbreaks, with substantially higher proportions of donors remaining asymptomatic or reporting symptoms of these infections in the weeks following donation [26–29]. Yellow fever virus is another arboviral threat that has recently re-emerged in sub-Saharan Africa. Yellow Fever outbreaks and mass vaccination campaigns in Angola and the Democratic Republic of Congo have put additional pressure on limited blood stocks as potential blood donors must be deferred from donation for 4–6 weeks after vaccination with a live attenuated virus vaccine [30,31].

The high cost of infectious disease testing is a substantial barrier to improving blood safety in sub-Saharan Africa. While national blood transfusion services in several of the 47 countries in the WHO AFRO region have adopted some form of serological testing (either manual or automated), and two countries (Namibia and South Africa) have implemented nucleic acid testing (NAT) to screen for HIV, HCV and/or HBV, many blood centres and hospital blood banks still rely on inexpensive, but less sensitive antigen/antibody or antibody-only rapid test kits to screen donors and/or donated blood [15]. In countries where the use of rapid test kits is the standard, residual risks associated with viral and other infectious markers are higher due to higher rates of false-negative results. Likewise, increased false-positive rates associated

with rapid test kits lead to unnecessary blood wastage [6,7].

While the residual risk of HIV, HBV and HCV is relatively low in upper-income countries, the risks remain elevated in African countries that rely on less-specific screening tools. This risk can be further magnified – even in countries that use more advanced serology screening platforms – where quality control systems are not in place or are not routinely implemented [3,8,32,33]. As a result, while the cumulative risk for the three major viral markers is less than one per million units in upper-income countries, residual risk estimates in sub-Saharan Africa range from 1/4000 and 1/111 000 donations for HIV to 1/7000 and 1/15 000 for HBV and HCV, respectively [4,7,33–35]. Routine pre-donation testing for *Plasmodium falciparum*, the parasite responsible for the most severe form of malaria, is not widespread in the region due to concerns about donor deferrals. In endemic areas where up to a third of adult blood donors may have active parasitaemia at any given time, the loss of otherwise eligible donors to pre-donation screening would further reduce already scarce blood stocks.

Similarly, screening for bacterial contamination of blood components is not widespread in sub-Saharan Africa. The risk of bacterial contamination in red-blood-cells (RBCs) has been estimated at between one in 1·9 million and one in 2·8 million in two upper-income European countries [36]; however, estimates from sub-Saharan Africa are considerably higher. A study of bacterial contamination in whole blood (WB) units transfused over a 1 year period in a paediatric ward in a Kenyan hospital found 44 different bacterial species in 38/434 WB units (8·8%) [37]. A study of bacterial contamination in WB and RBC units in an Ethiopian hospital found contamination in 6·3% and 4·6% of units, respectively [38].

In West and Central Africa, the vast majority of blood transfusions are prescribed to correct severe anaemia associated with acute haemorrhage [39,40]. For many of the same structural and financial reasons that limit countries' access to more sensitive laboratory screening methods, most transfusions in the West and Central African subregion are performed with WB, not component therapy (Fig. 1). While the production and use of platelets and plasma has increased in some countries in sub-Saharan Africa [19], WB represented more than 80% of blood components used by 19 of 38 (50%) blood banks in a recent survey of blood use in 11 French-speaking countries in West and Central Africa [15].

Over the last 20 years, two large global health programmes – the U.S. President's Emergency Plan for AIDS Relief (PEPFAR) and the Global Fund – have invested heavily in blood safety projects. However, these projects were limited to a relatively small number of countries and, since 2010, have largely been phased out [1,13]. The sustainability of investments made by external donor-funded projects has also been questioned [41].

Pathogen reduction is an emerging approach that supplements some test- and donor deferral-based strategies to improve the safety and availability of donated blood [42]. Several pathogen reduction technologies (PRT) for plasma and platelets are in development or in routine clinical use in upper-income countries [42–45]. However, despite the progress with platelet and plasma PRT, options for pathogen-reduced red-blood-cells (RBC) and WB are limited. While no PRT is currently approved for RBC, both Cerus Corporation (INTERCEPT® Blood System) and Terumo BCT (Mirasol®) are investing in late-stage clinical trials for RBC and WB PRT. Given the growing interest in a WB PRT that meets low- and middle-income countries' unique challenges [4,46–48], the SRCHF considered both systems for use in its humanitarian programme in sub-Saharan Africa.

Mirasol® PRT (Riboflavin/UV)

The Mirasol® system uses riboflavin (vitamin B2) and an illumination device to inactivate pathogens in platelets, plasma and whole blood through the intercalation of riboflavin into nucleic acids and the release of reactive oxygen species under ultraviolet (UV) illumination. This process blocks replication by selectively damaging the guanine bases in nucleic acids [49]. A number of studies in platelets, plasma and/or WB treated with the Mirasol system have established the technology's effectiveness against a range of pathogens in different blood components [48,50]. A healthy subjects trial with RBCs derived from Mirasol-treated WB (n = 24) found acceptable cell quality and recovery in test arm patients compared to patients transfused with conventional RBCs [51]. A clinical trial in paediatric patients transfused with RBC derived from Mirasol-treated WB (n = 70) described similar clinical efficacy (measured by post-transfusion haemoglobin) and patient safety end-points between test and control RBCs. Beyond 14 days post-treatment, a statistically significant increase in haemolysis and free haemoglobin levels was observed [52]. An additional Phase 3 trial evaluating the efficacy and safety of RBCs derived from Mirasol-treated WB in patients requiring chronic transfusion (PRAISE trial [ClinicalTrials.gov Identifier: NCT03329404]) was suspended. To date, the only WB PRT clinical trial conducted in sub-Saharan Africa has utilized the Mirasol system. That trial documented the incidence of transfusion-transmitted malaria in non-parasitaemic blood recipients exposed to WB transfusions that were retrospectively found to carry infectious levels of *Plasmodium falciparum*. Sixty-five patients (28 treated and

37 untreated) were exposed to *P. falciparum*-infected WB. Transmission of transfusion-transmitted malaria (TTM) occurred but incidence of TTM was significantly lower in patients receiving Mirasol-treated WB (3/28, 10·1%, 95% CI: 2·3–28·3) than in the group of patients receiving untreated WB (13/37, 35·1%, 95%CI: 20·2–52·5; $P = 0·024$) [53].

The INTERCEPT PRT for RBC: The basis for an Amustaline/GSH-based PRT for WB

The research and clinical development programme for the amustaline/GSH-based RBC PRT is now at an advanced stage of development.

Findings from preclinical and clinical research related to the amustaline/GSH-based RBC PRT technology, coupled with a development programme that aims to minimize electricity use (vs. the Mirasol system's reliance on electricity for the illumination device), contributed to the Swiss Red Cross's decision to implement the amustaline/GSH WB PRT in a clinical trial in sub-Saharan Africa. Preclinical and clinical data from the RBC development programme are presented below to describe how the RBC PRT serves as a basis for the amustaline/GSH-based WB PRT.

Amustaline (S-303): structure and mechanism of action

Amustaline dihydrochloride, the active ingredient used for pathogen inactivation in the INTERCEPT Blood System for RBCs, is a highly soluble compound composed of three distinct components: (1) a planar acridine anchor, which targets nucleic acids and intercalates the molecule between target base pairs; (2) a bis-aklylator effector, which reacts with nucleic acids, specifically guanine bases creating covalent bonds; and (3) a linker, a frangible ester bond that connects the anchor and effector, and hydrolyses in neutral pH. Due to the amphiphilic nature of the acridine anchor, amustaline passes rapidly through cell membranes, bacterial cell walls and viral envelopes when added to blood or red cell suspensions. Inactivation of pathogens and leucocytes via covalent bonds formed between the effector and nucleic acids occurs within 2–3 h of treatment; the amustaline molecule then degrades into a non-reactive by-product (S-300) (Fig. 2). By the end of an 18- to 24-h treatment period, amustaline levels are below the level of quantitation [54].

The role of Glutathione

Because of its electrophilic nature, amustaline may react with other nucleophiles, including small molecules such as phosphate and water, and macromolecules such as plasma proteins. Side reactions between amustaline and proteins on RBC surface membranes have also been observed. While these side reactions become less common as amustaline degrades, the acridine component of amustaline bound to RBC surface membranes has been implicated in the generation of non-clinically significant antibodies [55]. To minimize non-specific side reactions with RBC surface membrane proteins, Glutathione (GSH) (γ-glutamylcysteinylglycine) is added with amustaline, as a quenching agent. GSH is a natural tripeptide found in the cytosol of cells [56]. In the amustaline/GSH system, GSH does not penetrate RBC but remains in the intercellular space where it binds to ('quenches') unreacted amustaline and reduces the immunogenic potential of RBC membrane-bound acridine moieties.

Pathogen and leucocyte inactivation in amustaline/GSH-treated RBC

Amustaline has demonstrated robust inactivation of pathogens in RBCs (Table 2), including viruses [57–64], bacteria [63,65–68] and protozoa [69,70] as well as residual donor leucocytes [71–74]. Due to its effectiveness against viral pathogens and donor leucocytes, the amustaline/GSH-based INTERCEPT system for RBCs has been

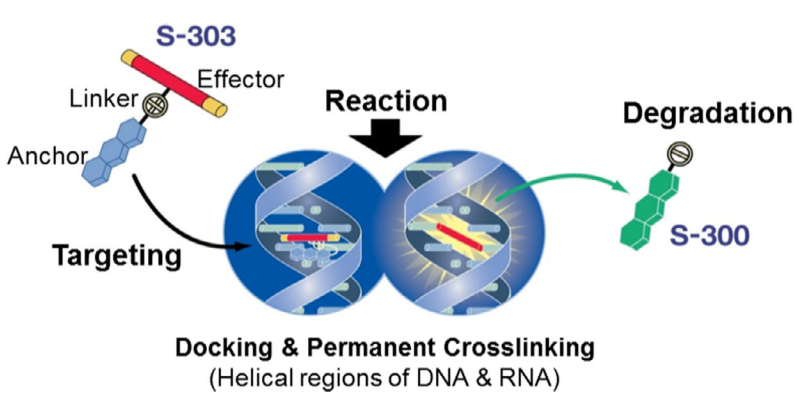

Fig. 2 INTERCEPT Blood System for RBC and WB mechanism of action.

proposed as an alternative to cytomegalovirus (CMV) serology and as a replacement for irradiation for the prevention of transfusion-associated graft-versus-host disease (TA-GVHD) [73].

RBC PRT toxicology studies

Preclinical evaluations of systemic toxicology and metabolism were conducted in rats and dogs exposed to single and multiple transfusions of the S-300 by-product and amustaline/GSH-treated RBCs. These studies found no impact on morbidity or mortality or any negative consequences linked to the animals' diet, body weight, clinical chemistry or urine analyses. In addition, plasma concentrations of S-300 after the first and last doses showed no accumulation after repeat transfusions [54,75]. Studies evaluating the genotoxic and carcinogenic potential of amustaline/GSH-treated RBC, treatment by-products and S-303 also demonstrated the safety of amustaline/GSH-treated RBC. No genotoxicity was identified using *in vitro* or *in vivo* bacterial reverse mutagenicity and chromosomal aberration assays, and no carcinogenicity was identified in *in vivo* clastogenicity and carcinogenic assays using validated murine models. These studies suggested that there is no measurable genotoxic hazard associated with transfusion of amustaline/GSH-treated RBCs.

Clinical trials with amustaline/GSH-treated RBC

Two generations of the amustaline/GSH PRT for RBCs have been tested in a total of 11 clinical trials, including six Phase 3 trials (Table 1). An original process, based on 0·2 mM amustaline and 2 mM GSH, successfully completed three Phase 1 survival, life span and tolerability studies. Two Phase 3 clinical trials with the original process – one in cardiac surgery patients and one in chronically transfused thalassaemia and sickle cell anaemia patients –were halted early due to the appearance of treatment-emergent antibodies in two of the chronic transfusion study patients. The antibodies were not clinically significant, low titre, transient and not associated with haemolysis. The treatment-emergent antibodies were determined to have been directed at the acridine moiety of amustaline bound to RBC surface membranes [76,77]. While the cardiac surgery study was halted before completing its enrolment target, a sufficient number of patients had been enrolled to demonstrate non-inferiority based on statistically similar rates of myocardial infarction, renal failure and death in the test and control arms. Comparable rates of adverse events were observed in both the test and control arms [55].

The RBC PR process was modified to further reduce extraneous amustaline binding to RBC surface membranes. The current RBC PRT uses 0·2 mM amustaline and 20 mM GSH. This modified process reduces the immunogenicity of treated RBC while maintaining their viability [78] as demonstrated in recovery and life span studies [79] and non-inferiority end-points in two Phase 3 trials in Europe. In cardiac surgery patients, the mean haemoglobin content of amustaline/GSH-treated RBCs was comparable to untreated controls [80]. In a larger study in Thalassaemia patients requiring chronic transfusion support, mean haemoglobin consumption (g/kg/day) in patients receiving test RBCs was not inferior to patients receiving control RBCs [81]. In both trials, no antibodies to amustaline were detected in any test arm patients [80–82]. Additional, larger and clinical studies are underway in the United States (Table 1).

Results from the two completed European Phase 3 clinical trials, as well as *in vitro* results showing robust efficacy against a broad spectrum of pathogens and leucocytes formed the basis of a CE Mark application for the amustaline/GSH-based RBC PRT in late 2018 [83]. That application remained under review at the time of this publication.

Adapting the amustaline/GSH-based RBC PRT for an Effective WB PRT

The amustaline/GSH-based WB PRT described below builds on the proven effectiveness of amustaline against a broad spectrum of transfusion-transmissible pathogens in RBCs, and the clinical efficacy and safety of RBCs treated with amustaline/GSH. The clinical development programme for the amustaline/GSH-based WB PRT includes preliminary studies on the impact of the amustaline/GSH process on selected RBC parameters over a standard 35-day life span and the immunogenicity of RBCs in treated WB. A Phase 1 clinical trial in Côte d'Ivoire is also described, as are modifications to the RBC PRT process to make the WB PRT more appropriate for austere settings where electricity may be less reliable.

The amustaline/GSH WB PRT Development Programme

The amustaline/GSH process for WB is based on the amustaline/GSH process for RBC described above, with 0·2 mM amustaline used in both processes. The objectives of the WB development programme may be considered in two parts: first, the performance of the amustaline/GSH WB PRT is being validated for the treatment of anaemia in settings where WB is frequently used instead of RBCs. Specific performance questions related to this development phase include pathogen inactivation and preservation of RBC parameters, for example haemolysis, ATP, glucose and lactate levels over a standard 35-day WB life span. The amustaline/GSH WB PRT's impact on coagulation factors and platelet function will be evaluated in

Table 1 Clinical development programme for amustaline/GSH RBCs

S-303/GSH treatment process	Clinical Trial Phase	Number of subjects receiving S-303/GSH RBCs	Primary endpoints and results
Original	1A	21	24 h recovery: 78·9% (test)/83·9% (control) [107]
	1B	28	24 h recovery: 81·1% (test) [107]
	1C	40	24 h recovery: 81·7% (test)/84·5% (control) Tolerability of full-unit transfusion: Passed [107]
	3	74	Incidence of myocardial infarction, renal failure, death: 21·6% (test)/20·5% (control) [55]
	3	17	Mean haemoglobin transfused (g Hb/kg/day): 1·3 (test)/0·9 (control) [76]
Interim modified	1	28	24 h recovery: 79·8% (test)/84·5% (control) [84]
Current[c]	1	26	24 h recovery: 88·0% (test)/90·1% (control) [85]
	3 STARS	25	Mean treatment difference (Hb content): −2·27 g/unit (−2·61, −1·92, within equivalence margin ± 5 g/unit).[a] [80]
	3 SPARC	81	Mean Hb consumption 9 g/kg/day): 0·113 ± 0·04 (test)/0·111 ± 0·04 (control) (P = 0·373).[a] [108]
	3 RedeS[b]	300 (target)[b]	Efficacy end-point: Adjusted haemoglobin increment averaged over multiple transfusions.[b]
	3 ReCePI[b]	300 (target)[b]	Efficacy end-point: Acute Kidney Injury within 48 hours of surgery.[b]

[a]STARS and SPARC study safety end-point results: No significant differences in RBC use or clinical outcomes; adverse events within expected range; no S-303 antibodies detected.

[b]The RedeS and ReCePI trials were both enrolling patients as of June 2019. As such, no results are currently available. For more information on both trials, please see the following clinicaltrials.gov summaries: https://clinicaltrials.gov/ct2/show/NCT03037164 (RedeS) and https://clinicaltrials.gov/ct2/show/NCT03459287 (ReCePI).

[c]An additional Phase 3 study in chronically transfused sickle cell anaemia patients is planned.

future studies in patients with clinical indications, requiring clotting and other coagulation functions, for example trauma and obstetric haemorrhage.

Adaptations to the RBC PRT

The same concentration of amustaline (0·2 mM) is used in both processes. However, while 20 mM of GSH is used in the amustaline/GSH-based RBC PRT, a lower concentration is used in the WB PRT. This adaptation was necessary due to the lack of an exchange step in the WB process to remove residual GSH (which produces RBC dehydration over storage) and the need to protect coagulation factors. Based on the results of studies on the impact of varying concentrations of GSH on the *in vitro* properties of RBCs, platelets and plasma in treated WB, a final concentration of 2 mM GSH was chosen for the amustaline/GSH WB PRT as this level had the least effect on coagulation factor activity [75,84,85].

Studies have been conducted to demonstrate pathogen reduction activity comparable to the amustaline/GSH process for RBC (Table 2) and assess the impact of the amustaline/GSH WB process on RBC viability and function in treated WB (Table 3).

The immunogenic potential of amustaline/GSH-treated RBCs was assessed in an animal model of chronic transfusion which evaluated the impact of reducing the level of RBC membrane-bound acridine on immunogenicity [86]. This study used rabbit RBCs processed to produce products with a range of different RBC-associated acridine which represented low (model for the current RBC process), intermediate (2× low) or high (4× low) acridine levels. There was a dose-response association between the level of RBC-bound acridine and the development of antibodies to amustaline/GSH-treated RBC. The absence of any immune responses in the low acridine group demonstrated that the immune potential of amustaline/GSH-treated RBCs was reduced by increasing the level of GSH (Fig. 3). Even in the high acridine group not all rabbits developed an immune response. This suggested that even at high exposures, RBC-associated acridine is poorly immunogenic. The presence of plasma in treated WB also provides additional quenching of the ability of amustaline

Table 2 Pathogen reduction with the amustaline/GSH system for RBCs

Type	Characteristic	Organism	Log reduction[a]
Bacterial	Gram-negative	*Yersinia enterocolitica* [63]	≥6·8 ± 0·2
		Serratia marcescens [63]	5·1 ± 0·1
		Escherichia coli [63]	≥6·7 ± 0·1
		Salmonella choleraesuis [65]	3·9 ± 0·1
		Pseudomonas fluorescens[65]	3·0 ± 0·1
		Klebsiella oxytoca [65]	≥6·3 ± 0·3
	Gram-positive	*Staphylococcus epidermidis* [63]	>7·1 ≥ ±0·1
		Staphylococcus aureus [36]	>5·1 ± 0·3
Viral	Enveloped, ssRNA (+)	Cell-associated human immunodeficiency virus [63]	>5·9 ± 0·1
		Bovine viral diarrhoea virus (BVDV; hepatitis C model) [63]	>4·8 ± 0·0
		Zika virus (ZIKV) [62]	>5·99 ± 0·2
		Chikungunya virus (CHIKV) [58]	>5·8 ± 0·2
		Dengue virus (DENV) [57]	>6·61 ± 0·2
		Vesicular stomatitis virus (VSV) [109]	5·7 ± 0·2
	Enveloped, dsDNA	Duck hepatitis B virus (DHBV; hepatitis B model) [59]	>5·1 ± 0·2
		Cytomegalovirus (HSV model for CMV) [36]	≥6·0
	Non-enveloped, sdRNA	Bluetongue virus (BTV) [63]	≥5·0 ± 0·1
	Non-enveloped, dsDNA	Human adenovirus 5 (AD5) [63]	>7·4 ± 0·2
	Non-enveloped, ssRNA (+)	Feline calicivirus (FCV) [64]	>6·8 ± 0·1
Protozoal	Intra-erythrocytic parasite	*Babesia microti* [70]	>5·0 ± 0·2
		Plasmodium falciparum [110]	>7·9 ± 2·3

[a]Based on input titre and post-treatment titre of viral plaque-forming units (pfu) or bacterial colony-forming units (cfu) in 1 ml.

to bind to cell surface proteins and reduces immunogenicity.

Given the strong evidence supporting the efficacy of amustaline to inactivate a wide range of pathogens in RBCs (Table 2), a similar level and scope of pathogen inactivation efficacy are expected for the amustaline/GSH-based process for WB. Investigations with 0·2 mM amustaline/2 mM GSH in WB have in fact shown that high levels of inactivation can be achieved with a number of pathogens: HIV (>6·5 $\log^{10}$), Bluetongue virus (>5·7 $\log^{10}$), *Yersinia enterocolitica* (>7·0 $\log^{10}$), *Serratia marcescens* (4·2 $\log^{10}$) and *Staphylococcus epidermidis* (7·5 $\log^{10}$) [87]. Additional bridging studies with the amustaline/GSH-based WB PRT demonstrated high levels of inactivation with two pathogens of particular importance to blood safety in sub-Saharan Africa: hepatitis B (represented by Duck hepatitis B virus, DHBV) and *Plasmodium falciparum*, the parasite that causes malaria. In each of these experiments, mean inactivation scores of >5·3 $\log^{10}$ and >7·5 $\log^{10}$ were achieved for DHBV and *P. falciparum*, respectively [87].

Phase 1 trial protocol

A protocol for a Phase 1 clinical trial titled 'A randomized, controlled Phase 1 clinical trial to assess the safety of whole blood treated with amustaline (S-303) and glutathione (GSH), a pathogen inactivation system, in anaemic patients in an African hospital' has been developed and will be implemented in Abidjan, Cote d'Ivoire by the

Table 3 Red-blood-cell parameters for INTERCEPT-treated and conventional whole blood at days 2 and 7

		Day 2		Day 7	
Parameter[a]	Method	Test (*n* = 12)	Control (*n* = 12)	Test (*n* = 12)	Control (*n* = 12)
pH at 37°C	ABL analyser	6·67 ± 0·03	6·66 ± 0·04	6·51 ± 0·03	6·50 ± 0·06
Plasmatic glucose (mmol/l)	Colorimetric	12·57 ± 1·08	13·24 ± 1·57	9·39 ± 1·64	10·34 ± 2·04
Plasmatic lactate (mmol/l)	Colorimetric	15·50 ± 1·63	17·37 ± 2·76	22·87 ± 2·81	22·73 ± 3·49
ATP (μmol ATP/g Hb)	Bioluminescence	3·65 ± 0·32	3·32 ± 0·57	3·37 ± 0·52	3·17 ± 0·55
% Haemolysis[b]	Calculated	0·02 ± 0·01	0·08 ± 0·03	0·02 ± 0·01	0·07 ± 0·05

[a]Plasmatic Sodium, Potassium and Albumin showed the same consistency over storage (data not shown).
[b]Plasmatic free haemoglobin was measured by infrared spectrophotometry.

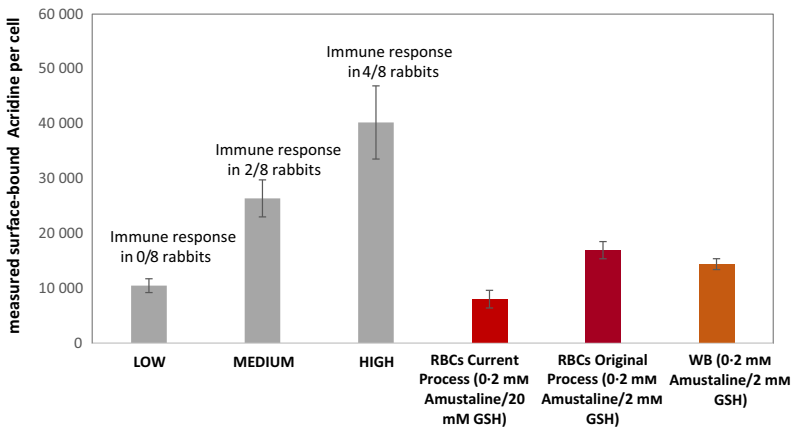

Fig. 3 Immunogenic potential of RBC surface-bound acridine in amustaline/GSH-treated RBCs and WB in a rabbit model of chronic transfusion.

Swiss Red Cross, with support from Cerus Corporation, the Centre National de Transfusion Sanguine (CNTS) and the Centre Hospitalier Universitaire (CHU) de Yopougon. This open-label clinical trial will enrol 20 adult patients (age > 18 years) with haemoglobin levels ≥5 g/dl into test (n = 10) and control arms (n = 10). The sample size has been deliberately limited in this first-in-humans study in a real-life clinical environment in sub-Saharan Africa. Anaemia will be diagnosed by local clinical criteria. Immunohaematology tests, including an indirect antiglobulin test (IAT), will be performed to exclude any patients with a pre-existing antibody to amustaline. Subjects randomized into the Test arm will receive one unit of amustaline/GSH-treated WB (non-leucoreduced). Control subjects will receive one unit of conventional (non-treated) WB or one unit of non-treated RBCs. The selection of the type of control unit will be made by the investigator based on actual blood product availability. All study components will be collected locally in compliance with local donor selection and donation screening policies. Subjects will be hospitalized for 24 h after the study transfusion for initial adverse event (AE) and transfusion reaction (TR) monitoring. Patients may consent to provide an additional blood sample for future TTI research. Subjects will receive clinical care based on local standards.

The trial's primary safety end-point is the proportion of patients with possible, probable or certain transfusion reactions ≥Grade 2 (Swissmedic criteria). Secondary safety and efficacy outcomes will include AEs and serious AEs. Post-transfusion haemoglobin increments adjusted by the haemoglobin content transfused will also be compared in Test and Control subjects.

If successful, results from this trial will inform the development of larger Phase 2 and 3 clinical trials for the amustaline/GSH WB PRT. In anticipation of these future trials, laboratory assessments of the impact of amustaline/GSH on platelet function (e.g. activation and aggregation), coagulation factor activity (via PT and aPTT assays), plasma protein parameters and the kinetics and strength of clot formation (e.g. described by viscoelastometry) are underway.

Finally, the amustaline/GSH RBC PRT processing kit has been adapted to reduce the WB technology's reliance on the need for a stable electrical supply. Unlike the INTERCEPT technology for RBC, which requires a centrifuge to perform the washing step and the Mirasol WB PRT that requires an illumination device, the commercial version of the amustaline/GSH WB PRT will only require a low-voltage tube sealing device which may be battery-operated.

Discussion

The risk of TTI remains unacceptably high in lower-income countries. The problem is particularly severe in sub-Saharan Africa, where high burdens of infectious diseases including HIV/AIDS, hepatitis B and C, and malaria in the blood donor pool increase the risk of undetected pathogens in donated blood components. Challenges to improving the safety of blood supplies in sub-Saharan Africa are compounded by weak healthcare infrastructure, especially unreliable electricity [88], limited access to quality control systems in transfusion centres and testing laboratories, human resource challenges, and an absence of haemovigilance systems to monitor the supply, safety and use of blood components [32,40,89].

In addition to the challenges described above, a pressing need remains for adequate supplies of safe blood in sub-Saharan Africa. By including WB on the List of Essential Medicines and incorporating blood transfusions as part of the Comprehensive Emergency Obstetric Care package, the WHO has highlighted the importance of safe

blood to address high levels of anaemia due to malaria and maternal haemorrhage and for other indications, including the treatment of patients with sickle cell anaemia.

Pathogen reduction technologies, including the amustaline/GSH PRT, present a clear opportunity to improve the safety and availability of blood transfusion in sub-Saharan Africa, either as a comprehensive safety intervention (i.e. every WB collection is tested and treated) or as an intervention used in special situations (e.g. remote/rural areas, humanitarian emergencies). Indeed, examples of successful emergency use of PRT in remote areas already exist. The INTERCEPT Blood System for Platelets was successfully implemented as an emergency countermeasure during chikungunya virus outbreaks on the French Indian Ocean territory of La Reunion [90] and in Puerto Rico [91]. Likewise, the investigational amustaline/GSH PRT for RBCs was approved for emergency clinical trial use in Puerto Rico during the recent Zika outbreak [92] [Rodriquez, 2017].

Countries will need to evaluate their own needs and resources before selecting a WB PRT implementation strategy. Indeed, given the costs associated with introducing PRT – purchase of processing sets, staff training, maintenance and need for more or less electricity depending on technology – economic considerations may dictate whether a national or a targeted implementation strategy is selected. Studies to quantify and compare PRT investment costs against potential savings – for example due to the removal of certain infectious disease tests – and other benefits, including an expanded donor pool due to the lifting of certain donor deferral criteria, are needed. Some of this work is already underway. Estimates from a recent model-based study in Uganda suggested that testing costs could be reduced if current testing algorithms were replaced with a combination of a cost-effective PRT and a rapid test [93]. While cost increases are likely to accompany the initial introduction of a WB PRT, evidence from the introduction of platelet PRT in upper-income countries suggests cost savings and better patient outcomes are achievable over time [94–96]. The benefits derived from improved health outcomes may be substantial in African countries where the direct and indirect costs and humanitarian burdens associated with maternal and child mortality are high [97,98] and the burden of clinical syndromes such as sepsis and TA-GVHD are poorly defined [99]. Indeed, PRT has been shown to reduce the incidence of costly sepsis in platelet recipients in Switzerland and other countries [100], and regulators are increasingly aware of these opportunities. Recently, the U.S. FDA described the potential for PRT to 'measurably increase the safety of the blood supply while reducing cost' [101].

In the short term, the introduction of a WB PRT in low-resource settings should reinforce and not necessarily replace current strategies to improve blood safety. Pre-donation behavioural screening, laboratory testing for major viral markers, education for clinicians on the appropriate use of blood and the introduction of haemovigilance systems to monitor patient safety will continue to play a role in protecting blood supplies in Africa and elsewhere. However, as clinical experience with RBC and WB PRT grows, some strategies may be adapted – for example combining PRT with rapid tests or with pooled NAT assays – or relaxed – for example allowing recruitment of family/replacement donors [4,48].

While WB remains the most frequently transfused labile blood product in much of sub-Saharan Africa, countries that have expanded the production and use of blood components, including platelets collected by aphaeresis [19], may also weigh the benefits associated with the use of currently available platelet and plasma PRT. Until a comprehensive PRT is available that allows for the separation of components from a pathogen-reduced WB collection, approved platelet and plasma PRTs may have a role in countries (or hospitals) where component therapy is increasing.

Emerging health technologies including mobile devices, solar power and drones are playing an increasingly important role in the search for sustainable responses to the chronic logistical, geographic and power challenges in lower and middle-income countries' healthcare systems [102–104]. Recent studies have shown the potential for investments in transportation, information technology, adequate staffing and appropriate health technologies to make an enormous difference in the lives of patients in lower-income countries, especially pregnant women and their babies [105,106]. The time has come for an accelerated pace of clinical development to add a safe, effective, technologically appropriate and affordable PRT for WB to the list of life-saving technologies available to clinicians and patients in sub-Saharan Africa.

Conflict of interests

Marion C. Lanteri, Anne North, Nina Mufti and John Pitman are employees of Cerus Corporation.

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