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Inactivation of infectious pathogens in labile blood components: meeting the challenge

L.Corash*

Cerus Corporation and Department of Laboratory Medicine, University of California, San Francisco 2411 Stanwell Drive, Concord, CA 94520, United States

Summary – Substantial improvement in the safety of blood transfusion has been achieved through the addition of new tests, such as nucleic acid tests, yet residual risk associated with transfusion of blood components persists. Transfusion of blood components has been implicated in the transmission of viruses, bacteria, and protozoa. While it is commonly recognized that hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), and the retroviruses, such as human immunodeficiency virus (HIV) and the human lymphotrophic viruses (HTLV) can be transmitted through cellular components, other pathogens are emerging as potentially significant transfusion-associated infectious agents. For example, transmission of protozoan infections due to trypanosomes and babesia have been reported. In addition to viral and protozoal infectious agents, bacterial contamination of platelet and red cell concentrates continues to be reported; and may be an under-reported transfusion complication. More importantly, new infectious agents may periodically enter the donor population before they can be definitively identified and tested for to maintain consistent safety of the blood supply. The paradigm for this possibility is the HIV pandemic, which erupted in 1979. During the past decade a number of methods to inactivate infectious pathogens in labile blood components have been developed and have entered the advanced clinical trial phase. © 2001 Editions scientifiques et medicates Elsevier SAS

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Currently, prevention of transfusion-associated viral disease depends upon the pre-donation evaluation of potential donors followed by serologic testing for infectious pathogens including: human immunodeficiency virus (HIV- 1 and -2), human T-cell lymphotrophic viruses (HTLV-I), hepatitis B virus (HBV), and hepatitisCvirus(HCV). Cytomegalovirus (CMV) screening is generally performed after blood collection, when CMV sero-negative products are required [1-7]. In addition to these agents, blood is tested for the syphilis pathogen (*Treponema paiidum)* . Testing is not routinely carried out for parvovirus B19, hepatitis A virus (HAy), hepatitis G virus (HGV), hepatitis Evirus (HEY), human herpes viruses (HHV-6 and HHV-8) bacteria, or protozoa. Although continuing improvements in testing have greatly reduced the transmission of viral disease by labile blood components, viruses may still be transmitted because diagnostic tests may be insensitive during the 'window period' before sero-conversion. Even direct tests for a virus, such as the hepatitis B surface antigen test, have a sensitivity threshold that allows contaminated components to escape detection. Recently, a case of HCV transfusion-associated transmission has been documented despite single-sample nucleic acid testing with highly sensitive methods [9]. Prior to the introduction of nucleic acid testing, estimates of the frequency of viral transmission due to transfusion of blood components, per donor,

were 1 in 100 000 for HCV, 1 in 63 000 for HBV, and 1 in 680 000 for HIV [10]. The aggregate risk of receiving a blood component contaminated with one of the viruses for which sensitive tests are in place has been estimated to be 1 in 34 000 [11]. A recent US government report has estimated that the average transfusion episode results in exposure to five donors [12]. Thus, per transfusion episode, the risk of receiving a component contaminated with virus may be as high as 1 in 6,800.

Bacterial contamination of platelet concentrates is a persistent problem due to room temperature (20—24 °C) storage for up to five days prior to use. Bacterial contamination may come directly from the donor or from an external source. A small number of contaminating bacteria can replicate to> 1 ~ per m after five days of storage. A wide variety of bacteria have been cultured from patients with transfusion-transmitted septicemia [6, 8, 13]. Although the number of reported cases of serious transfusion-transmitted sepsis is small, there are no routine laboratory tests to detect bacterial contamination of platelet units. Estimates of the frequency of bacterial contamination range up to 0.4% of platelet concentrate [14]. A 1991 survey by Morrow et al. identified bacterial contamination culminating in a septic response from 6 out of 10 219 transfusions of pooled random donor platelets, a frequency of approximately 1 in 1 700 [13]. A prospective study of 3 *584* platelet transfusions in 161 bone marrow transplant patients demonstrated the risk of symptomatic bacteremia as 1 per 16 patients, 1 per 350 transfusions, and 1 per 2 100 platelet units [15]. These frequencies are significant considering that over 8 million units of platelet concentrates are transfused annually in the United States alone [16].

The logistics and costs of continued expansion of testing processes, for example nucleic acid testing, have been questioned [10]. Testing remains a reactive strategy to insure blood component safety, since new pathogens may enter the donor population before adequate tests can be implemented. Moreover, the sensitivity of all testing methods is limited inherently by the volume of blood that can be analyzed. A complementary approach to improving the safety of blood component transfusion is that of the inactivation of infectious pathogens in blood components by using a process that treats the entire blood component. For example, treatment of plasma fractions with the solvent detergent process has demonstrated the benefits of this approach [17].

Table I. Clinical trials of methods for inactivation of pathogens in labile blood components.

Component	*System*	*No. of Subjects*	*Trial phase*
Buffy coat platelets	S-59	103	III
Single donor platelets*	S-59	671	III
FFP	S-59	12	I
FFP	S-59	27	ha
FFP	S-59		JIb
FFP – STEP CC	S-59	34	lIla
FFP – STEP AC	S-59	120	IIIb
FFP – STEP TTP	S-59	30	IIIc
Red cells	S-303	110	Ia, Ib,Ic
Red cells	PEN- 110	12	I

*Phase I and II trials of S-59 platelets have been completed and are discussed in more detail in the text.

A robust inactivation technology that is compatible with current blood component processing procedures offers the potential for improving transfusion safety beyond that achievable by testing. Moreover, a nucleic acid targeted technology capable of inactivating residual leukocytes may confer additional benefits due to inhibition of cytokine synthesis, lymphocyte proliferation, and antigen presentation. Donor leukocytes are associated with a variety of adverse immune events ranging in severity from febrile transfusion reactions to alloimmunization and graft versus

host disease [18, 19]. Although a number of measures have been implemented to reduce the likelihood of these adverse immune reactions, a robust nucleic acid targeted pathogen inactivation process offers the potential to inactivate all leukocytes, as well as infectious pathogens. Over the past decade a number of laboratories have reported efforts to apply pathogen inactivation technology to platelet and red cell concentrates and single units of plasma. These efforts have now focused on several methods, three of which are in clinical trials *(table I).*

SYSTEMS FOR INACTIVATION OF PATHOGENS IN PLATELET CONCENTRATES

Considerable effort has been devoted to investigations to develop methods for pathogen inactivation in platelet concentrates *(tables II, III).* The potential processes can be divided into two basic groups: psoralen and photo-dynamic methods

Table II. Psoralen methods used to inactivate infectious pathogens and leukocytes in platelet concentrates.

Photo reactive agent	*Target*	*Reference No.*
8-MOP	fd, R17, FeLV, *E. coli, 5. aureu.s*	[24]
8-MOP	MCMV, FeRTY	[57]
8-MOP	HIV	[58]
8-MOP	DHBV	[59]
8-MOP	12 pathogenic bacteria	[60]
AMT	VSV	[25]
AMT	HIV	[26,61]
AMT	VSV, Sindbis	[62]
PSR-Br	bacteriophage	[63, 641
S-59	Pathogenic bacteria	[29]
S-59	Leukocytes	[30]
S-59	HIV, DHBV, BVDV CMV, bacteria	[29]

fd: bacteriophage; R17: bacteriophage; MCMV: murine cytomegalovirus; FeRTV: feline rhinotrachejtis virus; HIV : human immunodeficiency virus; HSV: herpes simplex virus; CMV: cytomegalovirus; VSV: vesicular stomatitis virus; FeLV: feline leukemia virus; Sindbis: Sindbis virus; 8-MOP: 8-methoxypsoralen; AMT: aminomethyltrimerhylpsoralen; PSR-Br: brominated psoralens.

Table III. Photodynamic methods used to inactivate infectious pathogens in platelet concentrates.

Photo reactive agent	*Target*	*Reference No.*
UVB	Poliovirus	[65]
Merocyanine 540	VSV	[66]
Merocyanine 540	HSV, MS2, F6	[25]
Methylene blue	Unspecified	[67]
Phthalocyanines	VSV	[68]

MS2: bacteriophage; F6: bacteriophage; HSV: herpes simplex virus; UVB: ultraviolet B light (280—320 nm: for other abbreviations, see *table II.*

The psoralen mediated processes generally utilize nucleic acid targeted adduct formation, while the photodynamic

processes rely on the production of active oxygen species as the primary mechanism for pathogen inactivation. The photodynamic methods generally do not provide sufficient pathogen inactivation and are associated with unacceptable levels of platelet injury [20]. Psoralen methods have been more extensively investigated and more progress has been made with psoralens than with the other systems.

Early investigations with psoralen mediated pathogen inactivation were conducted with 8-methxyopsoralen (8-MOP) based on the history of prior human use to treat psoriasis and cutaneous T cell lymphoma [21, 22].
These initial studies by Lin et al. established the principle of psoralen mediated pathogen inactivation, but 8-MOP photochemical treatment was not a sufficiently rapid process for treatment of platelet concentrates in clinical use [23, 24].

Several laboratories have investigated the use of AMT, a synthetic psoralen with enhanced nucleic acid binding efficiency [25, 26]. While AMT has increased nucleic acid binding affinity compared to 8-MOP, it exhibits mutagenicity in the absence of light, and thus has an unfavorable toxicology profile. Several classes of new psoralens have been synthesized which offer potential advantages over AMT and 8-MOP. The halogenated psoralens do not appear to be sufficiently effective for viral inactivation, and in preliminary studies have demonstrated adverse effects on platelet viability [27, 28]. A new class of amino psoralens (Helinx Technology) has been synthesized and shown to be highly effective for inactivation of pathogenic viruses, bacteria, and leukocytes in platelet concentrates during a 3-minute UVA illumination [29, 30]. An integrated system (INTERCEPT Platelet System) using the lead compound, S-59, has been developed for large-scale production of platelet concentrates. This device consists of a series of interconnected plastic containers in a closed system for preparation of therapeutic doses of single donor and pooled platelets. The system contains an integral compound absorption device to lower the post-treatment levels of residual S-59 and free S-59 photoproducts. In addition to inactivation of HIV and bacteria [31], Lin et al. demonstrated that human platelet concentrates (300 mL) contaminated with high titers ofHCV (10~~) and HBV (10~~) treated with the S-59 process did not transmit hepatitis after transfusion into naive chimpanzees [32]. Other studies demonstrated that these novel psoralens inactivated high levels of T cells, inhibited leukocyte cytokine synthesis during platelet storage, and inhibited nucleic acid amplification [30]. More importantly, treatment of T cells with the S-59 process prevented transfusion-associated graft versus host disease in both immune competent and immune compromised murine transplant models [30].

Helinx technology with the novel psoralens, S-59, has undergone extensive clinical study [33]. Phase I and II studies using Helinx treated 5-day-old platelets transfused in healthy subjects have shown adequate viability. In these studies, photochemically treated platelets were well tolerated durin~ and after transfusion of full doses (300 mL, 3.0 x 10 1 platelets). The therapeutic efficacy of Helinx treated platelets was examined in a pilot study of profoundly thrombocytopenic patients. This clinical trial was designed to evaluate the hemostatic efficacy of S-59 treated platelet concentrates. In this study, transfusion of Helinx treated platelet concentrates resulted in the shortening of markedly prolonged cutaneous template bleeding times, provided adequate platelet count increments, and acceptable intervals to the next platelet transfusion [34]. The S-59 platelet concentrates were well tolerated.

Two randomized, controlled, blinded, clinical trials to determine the therapeutic efficacy of multiple transfusions of S-59 treated platelet concentrates have been completed. A European trial (EuroSPRITE) using pooled random donor platelets prepared by the buffy coat method enrolled 106 thrombocytopenic patients to receive up to 8 weeks of platelet transfusion support with either Helinx treated or standard platelet concentrates. One hundred and three patients were transfused during this study. The primary endpoint was the platelet count increment 1 hour after transfusion. Secondary endpoints included the platelet count increment 24 hours after transfusion, clinical hemostasis, refractoriness to transfusion, the inter-transfusion interval, and the frequency of acute transfusion reactions. This study demonstrated that platelet pools treated with Helinx technology and stored for up to 5 days provided count increments comparable to those of platelet pools not treated with pathogen inactivation [35]. Furthermore, Helinx treated platelets were comparable to untreated platelets with respect to transfusion intervals, refractoriness to platelet transfusion, the proportion of patients with major hemorrhage, and safety.

A larger US study (SPRINT) has enrolled 671 thrombocytopenic patients to receive up to 4 weeks of platelet transfusion support with either Helinx treated platelets or standard single donor platelets. The primary endpoint in the US trial is the proportion of patients with grade 2 bleeding during the period of platelet support [36]. The secondary endpoints include the proportion of patients with high grade bleeding, platelet count increments, and the same spectrum of endpoints as in the European study. This study will be un-blinded later this year.

SYSTEMS FOR INACTIVATION OF PATHOGENS IN SINGLE UNITS OF PLASMA

The solvent-detergent (S-D) process for the inactivation of enveloped viruses in plasma prepared as fresh frozen plasma has been in clinical use in both Europe and the United States [37]. This product has been shown to be clinically effective [38], although issues concerning the denaturation of anti-thrombotic proteins have been raised [39]. The S-D process requires pooling of 2 500 individual plasma units in a manufacturing process. In order to avoid pooling, several groups have developed a method using methylene blue (MB) for the treatment of single units of plasma prepared as fresh frozen plasma [40]. MB treated fresh frozen plasma (FFP) and cryoprecipitate have shown adequate levels of coagulation factors [41]. Limited clinical trials have demonstrated acceptable tolerability and safety [42]. While this method has been introduced into clinical practice in some European countries, it has not been widely adopted.

The S-59 Helinx technology has been developed for treatment of single (180—300 mL) and jumbo (600—800 mL) units of volunteer donor plasma for preparation of FFP (INTECEPT FFP System) [43]. FFP prepared with Helinx technology has been in clinical trials since 1996. A Phase I escalating dose study of safety and tolerability demonstrated that 1 000 mL transfusions of Helinx treated FFP were well tolerated, and residual S-59 was rapidly cleared [44]. In a Phase II study, Helinx treated FFP provided Factor VII post-transfusion levels and kinetics comparable to those of standard FFP in healthy subjects anti-coagulated with warfarin [45]. A second, small Phase II study demonstrated that Helinx treated FFP corrected prolonged PT and PTT coagulation tests of patients with chronic liver disease and provided effective hemostasis during minor surgery [46].

Three Phase III protocols have been initiated to evaluate the therapeutic efficacy and safety of Helinx FFP for support of patients with congenital coagulopathies, acquired coagulopathies, and thrombotic thrombocytopenic purpura (TTP). A single-arm open label study (STEP-CC) of Helinx FFP for transfusion support of patients with hereditary deficiencies of Factors I, II, V, VII, X, XI, XIII, and Protein C has been completed. Thirty-four patients with the spectrum of congenital coagulopathies treated with FFP received at least one transfusion of Helinx FFP to measure the post-transfusion recovery and clearance of the specific replacement coagulation factor. After this initial study, patients were followed in case of the need for subsequent FFP transfusions to treat or prevent bleeding. Data from this study will be reported this year. One hundred and twenty patients with acquired coagulopathy, primarily due to chronic liver disease were randomized in a double-blinded intent-to-treat, multi-center study (STEP-AC) to receive up to 7 days of either Helinx FFP or standard FFP. The primary endpoint of this study is to determine the response of either the PT or PTT to FFP transfusion. This study has been completed and will be reported later this year. In a third multi-center study (STEP-TTP), 30 patients requiring therapeutic plasma exchange (TPE) for treatment of TTP will be randomized to receive 30 days ofTPE with either Helinx FFP or standard FFP with the option of a second 30-day treatment period depending on clinical response. The primary endpoint of this study is to determine the proportion of patients entering clinical remission for TTP after 30 days of TPE. This study is currently enrolling patients at multiple US study centers.

SYSTEMS FOR INACTIVATION OF PATHOGENS IN RED CELL CONCENTRATES

A number of laboratories have investigated the application of pathogen inactivation to red cell concentrates *(table 11.9.* Red cells present a difficult environment for pathogen inactivation due to the absorption spectrum of hemoglobin and the viscosity of packed red cells. Both photodynamic and nucleic acid targeted methods have been developed *(table IV).* Significant defects of the photodynamic systems have included incomplete inactivation of pathogens, damage to red cells resulting in hemolysis and potassium leakage, increased binding of immunoglobulins, long treatment times, and the requirement to work at a reduced hematocrit or in a thin layer configuration to facilitate light activation. Cellular damage, due to active oxygen species, can be ameliorated by the use of scavengers or quenchers [47, 48]. Wagner et al. have described the use of a new phenothiazine compound, dimethylmethylene blue (DMMB), which exhibits an improved inactivation spectrum compared to MB and demonstrates less hemolysis and immunoglobulin binding during storage after treatment [49].

To date, most efforts have relied on photodynamic methods, but more recently several groups have developed nucleic acid targeted processes. The latter approach offers the potential to minimize non-specific damage to red cell and plasma proteins. Inactine, a stable mono-alkylator compound, has been reported to inactivate a series of model viruses in red cells [50].

Table IV. Methods used to inactivate infectious pathogens in red cell concentrates.

Reactive agent	*Target*	*Reference No.*
Dihematoporphyrin	HIV, HSV, CMV, Sly, *Tcruzi*	[69]
Benzoporhyrin A	VSV, FeLV	[70]
Merocyanine 540	Friend LV	[71]
Merocyanine 540	HSV-1	[72]
Merocyanine 540	*P. falciparum*	[73]
Methylene blue	VSV	[74]
Methylene blue	VSV, (1)6, Sindbis, M13	[751
Phthalocyanines	VSV	[76]
Phthalocyanines	VSV, Sindbis	[68]
PSR-Br	c1)6	[77]
Hypericin	HIV	[78]
Dimethyl methylene blue	VSV, PRV, BVDV, (1s6, R17, EMC	[49]
mactine	Porcine parvovirus, BVDV, HW-1, VSV	[50]
S-303	HIV, DHBV, BVDV, bacteria	[54]

SIV: simian immunodeficiency virus; *T cruzi: T7ypanosoma cruzi;* Friend LV: Friend erythroleukemia virus; (1)6: bacteriophage; PSR-Br: brominated psoralen; PRV: pseudorabies virus; BVDV: bovine viral diarrhea virus; EMC: encephalo-myocarditis virus; Ri 7: bacteriophage; for other abbreviations, see *table II.*

This compound is nucleic acid targeted and does not require light for activation; however, after treatment it requires cell-washing to remove residual compound prior to transfusion. A study was conducted to evaluate the effect of mactine on primate red cell viability [51]. Baboon red cells were treated with the Inactine compound, PEN 110, and stored for 28 days. Following storage, the red cells were radiolabeled with 51-Cr and transfused. Post transfusion recovery and life span of the Inactine treated cells were comparable to that of untreated red cells. A Phase I clinical trial has been completed. This study enrolled 12 healthy subjects in a randomized cross-over design to evaluate the effect of PEN- 110 treatment on red cell viability after 28 days of storage. PEN- 110 treated red cells demonstrated post transfusion recovery and life span comparable to those of untreated red cells [52]. A second study with PEN-110 treated red cells is currently underway. In this study, healthy subjects will receive transfusions of treated red cells stored for 28, 35, and 42 days.

Cook et al. have developed a class of compounds known as anchor linker effectors (ALE) or frangible anchor linker effectors (FRALE) [53]. These compounds are activated by a pH shift after addition to packed red cells suspended in residual plasma and a red cell additive solution. FRALES are nucleic acid targeted and form covalent adducts with DNA and RNA. The FRALE compounds rapidly degrade to an inactive, negatively charged species (S-300) after reaction, thus preventing further binding to DNA and RNA. To provide the highest safety margins for this process, the negatively charged breakdown product (S-300) is reduced to very low levels using a compound absorption device (CAD) which remains in the plastic container over 42 days of storage. The lead compound, S-303 (100 ~tg/mL), upon addition to packed red cells (60% hematocrit) inactivated high titers of cell-free and cell-associated HIV, DHBV, VSV, HSV, BVDV, and both gram-negative and gram-positive bacteria. Using a murine transfusion model, S-303 treated red cells exhibited post transfusion recovery and life span comparable to untreated red cells [53]. In a second series of studies, dog red cells treated with S-303 exhibited post transfusion recovery comparable to untreated red cells [54]. Dogs transfused multiple times with allogeneic treated S-303 red cells failed to develop antibodies to S-303 treated autologous red cells but some dogs did develop allo-antibodies to untreated donor cells, indicating an intact alloimmune response not directed against S-303 treated red cells. Other dogs transfused with S-303 treated red cells (10 mL/kg) 12 times over a 1-month period had no evidence of clinical or histopathologic toxicity. In addition, replacement of 80% of the blood volume of dogs with S-303 treated red cells using treatment concentrations up to 500 ~tg/mL resulted in no toxicity.

The Helinx S-303 technology has been incorporated into an integrated system (INTERCEPT Red Cell System) for treatment of red cell concentrates with pathogen inactivation. Three Phase I clinical trials using S-303 Helinx treated red cells have been completed in healthy subjects. In the first study, 42 healthy subjects donated whole blood that was prepared as packed red cells and treated with either standard methods or S-303 and stored for 35 days at 4° C [55]. After 35 days of storage, the subjects were transfused with an aliquot of radiolabeled red cells to measure recovery 24 hours after transfusion. S-303 red cells were well tolerated and demonstrated an average post transfusion recovery greater than 75%. In a second single-arm study, 28 subjects from the first study were re-enrolled and donated a unit of whole blood that was prepared as packed red cells and treated with S-303 [56]. The treated red cells were placed in storage and on days 7, 14, and 21 after donation, subjects were transfused with 15 mL of S-303 treated red cells. On day 35 after donation, the subjects were transfused with 15 mL of 51-Cr labeled red cells. Before each transfusion during the 35-day storage period, serum samples were obtained and assayed for detection of antibodies directed against S-303 red cells. By paired analysis, the average post transfusion recovery of Helinx red cells in the second study was comparable to the recovery of control and S-303 treated red cells observed in the first study [56]. No detectable antibody against S-303 treated red cells was observed after four or five transfusions. A third Phase I study of 40 healthy subjects has recently completed enrollment. In this study, 30 subjects donated red cells that were treated with either Helinx S-303 technology or prepared as standard red cells followed by storage at 4 °C for 35 days. In contrast to the earlier studies, in this study the S-303 treated red cells were stored with the integral CAD for 35 days. After 35 days of storage, an aliquot of red cells was radiolabeled to measure post-transfusion recovery 24 hours after infusion and red cell life span over 30 days. Using a crossover design, each subject ultimately will receive both types of red cells. In a second protocol to evaluate safety and tolerability, ten subjects have donated and received full unit (250 mL) transfusions of unlabeled, Helinx treated red cells stored for 35 days at 4 °C with the integral CAD device. Data from this study are expected later this year.

CONCLUSION

Considerable progress has been made in the development of technologies to inactivate infectious pathogens in plasma, platelet concentrates, and red cell concentrates. These technologies have now entered the clinical trial phase, and the platelet system has completed Phase III studies in Europe. These inactivation systems have the potential to markedly change the way in which blood components are prepared and to further improve the safety of transfusion support of patients receiving labile blood components.

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