Transfusion Medicine and Hemotherapy

Review Article

Transfus Med Hemother 2014;41:309–325
DOI: 10.1159/000365646

Received: December 23, 2013
Accepted: March 27, 2014
Published online: July 21, 2014

Pathogen Inactivation Technologies for Cellular Blood Components: an Update

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Keywords
Pathogen inactivation · Blood safety · Dengue virus · Chikungunya virus · West Nile virus · Bacteria · Protozoa · Platelet concentrates · Red blood cells · Randomized clinical trial · Hemovigilance

Summary
Nowadays patients receiving blood components are exposed to much less transfusion-transmitted infectious diseases than three decades before when among others HIV was identified as causative agent for the acquired immunodeficiency syndrome and the transmission by blood or coagulation factors became evident. Since that time the implementation of measures for risk prevention and safety precaution was socially and politically accepted. Currently emerging pathogens like arboviruses and the well-known bacterial contamination of platelet concentrates still remain major concerns of blood safety with important clinical consequences, but very rarely with fatal outcome for the blood recipient. In contrast to the well-established pathogen inactivation strategies for fresh frozen plasma using the solvent-detergent procedure or methylene blue and visible light, the bench-to-bedside translation of novel pathogen inactivation technologies for cell-containing blood components such as platelets and red blood cells are still underway. This review summarizes the pharmacological/toxicological assessment and the inactivation efficacy against viruses, bacteria, and protozoa of each of the currently available pathogen inactivation technologies and highlights the impact of the results obtained from several randomized clinical trials and hemovigilance data. Until now in some European countries pathogen inactivation technologies are in in routine use for single-donor plasma and platelets. The invention and adaption of pathogen inactivation technologies for red blood cell units and whole blood donations suggest the universal applicability of these technologies and foster a paradigm shift in the manufacturing of safe blood.

Introduction

Until now, neither artificial oxygen carriers nor hemostatic agents have had the potential to replace manufactured red blood cell (RBC) units or platelet concentrates donated by healthy volunteers. This ‘natural source’ inherently bears the risk of transmitting blood-borne pathogens, although stringent donor selection criteria and very sensitive and specific blood screening tests have substantially increased the safety margin for blood recipients [1–4]. In particular, the successful introduction of nucleic acid amplification testing (NAT) for HIV, HBV, and HCV have led to significantly safer blood products resulting in a residual risk of 1:1 million to 1:10 million [1, 5–8]. Currently, the risk of bacterial contamination, especially in platelet concentrates (1:2,000 to 1:5,000), and transfusion-related bacterial sepsis (1:20,000 to 1:50,000), which is at times fatal (10%), are the most relevant infectious complications [1, 9, 10]. The vast majority of patients receiving platelet concentrates suffer from hematological malignancies, and they are vulnerable to bacteria, leading to higher rates of infectious complications, associated comorbidities, and prolonged hospital stays [11–13]. Furthermore, transfusion-associated bacterial infections may be underestimated due to the fact that fever is a very common complication in such patients or the used antibiotics mask the symptoms of this transfusion reaction. The capacity to inactivate those po-

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tentially contaminating bacteria in platelet concentrates is not in the focus of this article but briefly summarized in the subsection 'Inactivation Efficacy of Current Pathogen Inactivation Technologies'.

In addition, it is still impossible to know or reliably predict if and when emerging pathogens will threaten the safety of the blood supply. To this end, the question how to deal with the appearance of new or reemerging pathogens and how novel technological inventions can be used to further improve blood safety for patients in the European Union and elsewhere is the focus of an ongoing discussion [1, 2].

From a social point of view, it is desirable to pursue a zero-risk strategy to completely prevent transfusion-transmitted infections. It is well known from specific industrial sectors, such as aircraft technology and nuclear energy, that the implementation of programs for continuous quality improvement and risk management is essential to approximate the goal of 'zero-risk' [14, 15]. The impact of an unfortunate event and the probability of its occurrence are the most important determining factors for quantitative risk assessment and root cause analysis [16, 17]. For transfusion-transmitted infections, the most effective stage of hazard prevention is the elimination of the hazard (pathogen), followed by strategies to mitigate the risk of its transmission, e.g. by donor deferral, pathogen screening, or pathogen inactivation [2, 18–20].

This review focuses on the exemplified description of the epidemiology and surveillance of arbovirus infections and their potential risk for blood safety due to the fact of an asymptomatic viremic phase. To the author´s knowledge NAT assays for dengue virus (DENV), chikungunya virus (CHIKV), and West-Nile virus (WNV) have been developed and are partly commercially available [21]. In this context, the invention, pre-commercial development, and introduction into the market of 'universal' pathogen inactivation technologies (PIT) are attractive steps towards the manufacturing of 'sterile' cellular blood components from human sources, which have been a global matter of course for cell-free solutions of human origin (albumin, immunoglobulins and coagulation factors) for decades [1, 22–25].

These PIT imply a proactive, more generalized approach against multiple new and (re-)emerging pathogens, which perpetually challenge the safety of the blood supply and will become an serious alternative to a repetitive implementation of new screening tests (e.g. NAT) [21]. Therefore, this innovation is designated as 'paradigm shift in transfusion medicine' [25]. This review summarizes upcoming PIT with a focus on cellular blood components (platelets and RBCs) and highlights their current progress in clinical evaluations [26].

The Emergence of Pathogens

A pathogen emerges either by de novo mutations or by crossing the species barrier to human beings [2, 18]. HIV is an excellent example of a life-threatening emerging infectious disease. AIDS was first diagnosed in patients with *Pneumocystis carinii* pneumonia and Kaposi's sarcoma, which alerted the US Centers for Disease Control and Prevention to carefully monitor the outbreak of such a new disease for which mainly affected Haitians, homosexuals, hemophiliacs, and i.v. drug users. Two independent research groups (Robert Gallo and Luc Montagnier) published the discovery of a novel retrovirus as the causative agent in *Science* [27, 28]. It is now widely accepted that HIV originated from simian immunodeficiency virus(SIV)-infected chimpanzees and crossed the animal-human barrier. There, it mutated into HIV and spread throughout society by high-risk transmission channels (*www.wikipedia.org*). HIV antibody detection assays have been commercially available since the mid-1980s, and they have been implemented in blood donor screenings worldwide to prevent transmission via blood or coagulation factors of human origin [8].

Dengue Virus

The lessons learned with AIDS have led to the well-established concept of emerging infections, which are relevant to transfusion medicine. It is especially relevant for cases in which the donors are potentially infected (e.g. viremic), yet do not have symptoms, and transmission via blood transfusion is probable or proven. This is true for both DENV and CHIKV, which both are transmitted via mosquitos (*Aedes aegypti* and *Aedes albopictus*) [2, 29]. Dengue fever is an infectious tropical disease that is commonly self-limiting and is accompanied by flu-like symptoms and a skin rash that is similar to measles. However, in rare instances, life-threatening dengue hemorrhagic fever develops.

Since the 1960s, the incidence of DENV has increased dramatically due to urbanization, population growth, and global warming, with more than 50 to 100 million individuals infected annually in more than 100 endemic countries worldwide [30]. In these countries donor deferral is not an appropriate measure for prevention [2, 30]. Neither an approved vaccine nor a specific antiviral drug is currently available. In addition to the elimination of the mosquito or its habitats, protection from mosquito bites by wearing clothes is the only preventive measure. There are several outbreaks in non-endemic regions such as in Texas, Florida, and Australia [29]. Also in France (n = 2), Croatia (n = 15), and Madeira (n = 2,164), individuals who never left their home country suffer from dengue fever [21, 31]. Furthermore, there is evidence that one of the vectors, *Aedes albopictus*, now 'habitats' (non-dengue virus-infected) in Germany, Austria, and Switzerland, stowing away from Italy by commercial transport [32].

DENV can be transmitted by blood transfusions and solid organ transplants in rare cases [30]. Two small outbreaks of transfusion-transmitted DENV in Hong Kong and Singapore have been published, and the incidence of DENV RNA in healthy blood donors was assessed, e.g. in Puerto Rico and

Brazil [33–36]. The rate of asymptomatic DENV RNA-positive individuals was estimated to be 1:250 to 1:1,000 during DENV epidemic outbreaks, and they serve as additional sources of virus dissemination in the community. In Germany, the Robert-Koch-Institut detected more than 600 individuals with dengue fever in 2012, all of whom acquired the infection while traveling (*www.rki.de*).

Chikungunya Virus

Large CHIKV outbreaks have taken place in the Indian Ocean, India, Southeast Asia, and Europe [2, 37]. CHIKV causes a febrile illness with painful and sometimes long-lasting (up to years) arthralgia. In particular, the outbreak in La Reunion (2005 and 2006) affected nearly one third of the island population (265,000 human cases and 237 deaths) [18, 38–40]. The collapse of the regional blood supply was prevented by the import of blood components from France and the urgent implementation of INTERCEPT™ as PIT for sufficient platelet support [41].

Air travelling and shipping around the world contributes to the spread of CHIKV and were recognized as a cause for the unexpected CHIKV outbreak in Northern Italy in the summer of 2007 [42, 43]). The outbreak was limited to 205 patients and was caused by an infectious traveler. Currently, a widespread human-mosquito-human transmission cycle vectored by *Aedes albopictus* is deemed to be unlikely in Europe or the USA because the virus does not persist during cold temperature, mosquito activity decreases in parallel with the virus, there is a well-established socioeconomic framework in place, and there is an international surveillance network [44].

West Nile Virus

The emergence of WNV in the USA can be traced back to first cases, when WNV was imported to New York in 1999 (66 human cases and 22 deaths) [45]. WNV is a mosquito-borne RNA virus whose primary hosts are birds. An epidemic was observed in 2002, with 4,156 individuals affected and a mortality rate of approximately 7% (n = 284) due to severe meningoencephalitis [18]. In 2002, 23 transfusion-transmitted WNV infections were detected, some of which were asymptomatic. In contrast, 6 cases were fatal [46]. Under the leadership of the US Food and Drug Administration (FDA), research organizations, blood donation agencies, and screening test manufacturers build up a task force for the development, industrial scaling-up, and validation of a WNV nucleic acid amplification technology [2]. In summer 2003, WNV NAT test systems with a minipool design were implemented, allowing the screening of viral RNA in a specimen pool of 6–24 donors [47]. Six breakthrough WNV infections have led to a novel concept that provides a seasonal switch to a more sensitive individual donation NAT platform [48, 49]. Occasionally, WNV infections are missed due to a viral titer below the lower limit of detection, as recently reported (Morbidity and Mortality Weekly Report August 9, 2013) by the Center of Disease Control and Prevention (*www.cdc.gov*) for an immunocompromised patient with non-Hodgkin‘s lymphoma who died by diffuse WNV encephalitis.

WNV epidemics occur every year in USA, one of the most severe outbreaks took place in 2012 with 2,734 neuroinvasive cases and 597 viremic donors which was comparable to WNV outbreaks in 2002/2003 (2,946/2,866 patients with neuroinvasive form and 714 viremic donors, WNV-NAT tested for the first time in 2003 [21]. A phylogeographic model was created based on WNV genome sequences of sampled organisms to reconstruct the spatiotemporal diffusion of WNV in North America [50]. It is speculated that bird movements may be responsible for rapid, long-distance movements (1,000 km/year) of the virus. In the European Union and neighboring countries (in addition to others that border the Mediterranean Sea and Eastern Europe, *www.ECDC.europe.eu*), an increasing number of autochthonous WNV infections have been documented. The European Network for Arthropod Vector Surveillance for Human Public Health (VBORNET) supported the European Center of Disease Control (ECDC) to take measures for prevention or control of local outbreaks with a panel of experts in the field of entomology (scientific study of insects) and transfusion medicine [21].

For example, 2010 is the third consecutive year that human cases of WNV infections have been identified in Northeastern Italy, with a trend towards a wider geographic area [51]. Therefore, preventive strategies are currently being discussed by the European Union and the Paul-Ehrlich-Institut in Germany to minimize the risk of WNV transmission via blood (and solid organs), such as the temporal deferral of at-risk donors or the implementation of a sensitive NAT screening test (*www.pei.de*).

Outlook

The emergence of novel pathogens is rather unpredictable, although mathematical models suggest that every 5 years a new transfusion-transmissible infectious agent will emerge [20]. Up-to-date epidemiological data are a prerequisite for proper risk assessment, especially for traveling blood donors who return from endemic areas to Europe [21, 133]. The control of epidemics is of utmost importance and is kept under precautionary surveillance by national and international bodies. Several emerging pathogens are likely transmissible by blood transfusion. Blood safety during the emergence of blood-borne pathogens is a major public health concern, and this is also true for known viruses with genomic mutations susceptible to immune escape mechanisms, new strains of bacteria, or other emerging pathogens or parasites that can spread by international travel or climate change (DENV, *Plasmodium falciparum*, *Trypanosoma cruzi*, *Babesia necroti*) [19, 52, 53]. The highly contagious severe acute respiratory syndrome (SARS) virus in 2002/2003, the H5N1 avian influenza virus (‘bird flu’) since 2003, and, more recently, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV,

H7N9) in 2012 are impressive examples of how rapidly pathogens can emerge and spread worldwide [54–56].

Procedures that address the risk assessment for such emerging infectious diseases that are potentially transmissible by blood and blood products were recently discussed in the USA [20]. Based on strong scientific evidence, policy makers have to carefully consider strategies for optimal risk minimization and for preventing a collapse in the national blood supply. Under- and overestimation of actual risk has undesirable and potentially disastrous consequences. In the absence of available blood screening tests, deferral of blood donors is likely the best alternative to mitigate the risk of dissemination and its amplitude [20]. However, in a case of very high incidence, this strategy implies the disadvantage of paralyzing, at least temporally, the blood supply.

In the future, universally applicable technologies for robust pathogen inactivation may become acceptable strategies to circumvent such risks. These technologies will also have the potential to replace not only routine gamma-irradiation, bacterial testing, and CMV serology but also to scrutinize the usefulness of other valuable test systems for the detection of viral antigens, antibodies, or nucleic acids.

Current Pathogen Inactivation Technologies

Historically, cell-free blood components, such as human plasma, were pathogen-inactivated with solvent-detergent (SD) for large plasma pools or by the addition of methylene blue (MB) for single plasma products in small-sized blood establishments [1, 22–24]. In general, pathogen inactivation resulted in reduced factor VIII activity and fibrinogen content of about 70–80% [57, 58]. More surprisingly, results obtained from randomized clinical trials (RCTs) comparing SD plasma / MB plasma with conventional FFP have not been published to a great extent [24]. The above mentioned methods are not transferable to cell-containing blood components as they heavily damage platelets and erythrocytes.

Therefore, this review will focus on novel PIT, which has become increasingly available for cellular blood components by circumventing these disastrous cell killing properties. Notably, INTERCEPT™ (Cerus Corporation, Concord, CA, USA), Mirasol® (Terumo BCT, Lakewood, CO, USA), and THERAFLEX® UV (Macopharma, Mouvaux, France) represent the currently available technological platforms, and they are each at different stages of marketing readiness. In contrast to the SD/MB method, these technologies all have the capacity to treat cellular blood components, whereas with SD/MB only the treatment of plasma is possible.

INTERCEPT technology is based on supplementation with synthetic psoralen S-59, which penetrates cellular and nuclear membranes and reversibly binds to nucleic acids, especially to pyrimidines in single- or double-stranded DNA and RNA [59]. After UV-A light exposure (320–400 nm, 3 J/cm^2) it robustly cross-links nucleic acids in an oxygen-independent manner. Consequently, this action is independent of potential cytotoxic reactive oxygen species (ROS). Psoralens are naturally occurring substances found in limes and celery. The mode of action has been carefully investigated, especially the high frequency of covalent cross-links (one adduct is formed in about 1 of 83 bp), which inhibit DNA or RNA replication as well as transcription and repair mechanisms. The unbound S-59 and photoproducts are reduced by an adsorption device. The integrated container set for platelets and the UV-A illuminator are licensed for some time and enable ready-for-use application in the routine workflow of blood banks.

Mirasol technology depends on the addition of vitamin B2 (500 µmol/l riboflavin), resulting in a final concentration of approximately 50 µmol/l riboflavin, plus broad-spectrum UV light (280–400 nm, 6.2 J/ml corresponding to 5 J/cm^2) [60]. Riboflavin and its photoproducts are found in natural products (such as foods) and in human blood, albeit at a much lower concentration than in a Mirasol-treated platelet product. Riboflavin serves as a photosensitizer (electron transfer) and promotes the oxidation of nucleic acids, especially guanine residues, without binding to nucleic acids or proteins. This leads to a conversion of riboflavin to lumichrome and other photoproducts. Riboflavin-induced damage is irreversible because both replication processes and RNA/DNA repair mechanisms are strongly inhibited. The frequency of nucleic acid lesions is approximately 1 in 350 bp. However, in context with plasma, a significant role for Mirasol-generated ROS was shown which adversely affected the molecular integrity of avin-labile coagulation factors (FVIII, fibrinogen) and other enzymes like ADAMTS13 [61]. The direct generation of superoxide anion and other ROS may make a reevaluation of the general toxicological assessment necessary.

THERAFLEX UV technology for platelets does not rely on a photodynamic agent and is based exclusively on the force of narrow-bandwidth UV-C light (254 nm, 0.2 J/cm^{2,} irradiation time < 1 min), which induces the formation of pyrimidine dimers [62–65]. To ensure optimal illumination, the platelets are transferred to a large illumination bag that allows for the homogeneous 'penetration' of thin platelet suspensions under frequent agitation (>100/min). Because the addition of a photoactive chemical is unnecessary, there is no need for conventional pharmacokinetic and toxicological assessments. General tolerability and lack of immunogenicity (neoantigen formation) of UV-C-treated platelets have been demonstrated in dogs [66]. In principle, THERAFLEX UV technology is also applicable to FFP. However, a higher UV-C intensity is needed to circumvent the quenching effect of proteins in 100% plasma and to achieve sufficient pathogen reduction rates [25].

Toxicology

The toxicology assessments of all discussed PIT applicable to platelets are summarized in table 1 (modified from [24]). Extensive testing using both in vitro and in vivo assays ac-

Table 1. Toxicology of pathogen inactivation technologies

	INTERCEPT platelets	Mirasol platelets	Theraflex platelets
Phototoxicity	√	√[*a]	photoreagent-free no toxicological assessment necessary[c]
Acute toxicology	√	√	
Repeated dose	√	√	
General pharmacology	√	not applicable[b]	
Reproductive toxicology	√	√	
Genotoxicity	√	√	
Carcinogenicity	√	not applicable[b]	
Neonatal toxicity	√	√	
ADME* studies	√	√	
Occupational safety	√	√	
Neoantigen formation	none	none	none

*Absorption, distribution, metabolism, and excretion.
[a]No phototoxicity of photolyzed riboflavin and lumichrome was observed in acute and genotoxicity studies.
[b]Riboflavin is a food additive and vitamin and a pharmacologic effect of residual riboflavin or its photoproducts is not intended by the manufacturer. In repeated dose toxicity studies as well as in genotoxicity studies there was no evidence of carcinogenicity.
[c]The collateral impact of UVC light on the integrity of plasma proteins should be investigated

Table 2. CE mark approval and status of the clinical evaluation of pathogen inactivation technologies

PIT	Company	Platelets	Plasma	RBC/whole blood
I. CE mark approval				
S59 + UVA INTERCEPT	Cerus	CE class III May 2002	CE class III November 2006	not applicable
S-303 INTERCEPT	Cerus	not applicable	not applicable	not yet
Riboflavin + UV Mirasol	TerumoBCT	CE class IIb October 2007	CE class IIb August 2008	not yet
Theraflex UV	Macopharma	CE class IIb November 2008	alternative technology*	not yet
II. Clinical evaluation				
S59 + UVA INTERCEPT	Cerus	phase III completed routine use	phase III completed routine use	not applicable
S-303 INTERCEPT	Cerus	not applicable	not applicable	phase III initiated
Riboflavin + UV Mirasol	TerumoBCT	phase III ongoing routine use	phase III completed routine use	phase I completed[#]
Theraflex UV	Macopharma	phase I completed	alternative technology*	pre-clinical phase

*Based on methylene blue and visible light.
[#]For escalating UV light energies (22, 33 and 44 J/ml RBC).

cording to the standards of the International Conference of Harmonization for Drug Development is not suggestive of any toxicologically related safety concerns [60, 67–69]. Because of the compound absorption device which is integrated into the INTERCEPT Blood System the patient receives only about 1 µg amotosalen per platelet transfusion (LD50 in rats ~1,000 mg/kg p.o.), whereas the final riboflavin exposure in adults is much higher (~5 mg) per transfusion [59, 60]. However, in consideration of the LD50 (in mice 50–100 mg/kg i.v.) the safety margin was calculated to be about 650–1,300-fold. Consequently, all above mentioned PIT for platelets have passed the licensing procedure (e.g., CE mark) to introduce each medical device into the market (table 2).

Inactivation Efficacy

In addition to the well-known transfusion-transmitted viruses (HIV, HBV, HCV, HTLV) and bacteria, there is a considerable risk of transmitting other pathogens for which blood donations are not commonly tested (e.g., bacteria, Epstein-Barr virus (EBV), Parvo B19) and unknown pathogens that may emerge in the next few years, similar to the appearance of HIV in the early 1980s. It must be noted that the patient's individual burden may be much higher than the estimation of a 'standard risk' (e.g., 1:1 million) usually referring to a single blood transfusion may suggest. This is primarily caused by the transfusion frequency during the entire hospital stay or treatment period and is especially true for patients with chronic

Table 3. Pathogen inactivation efficacy for platelet concentrates [24, 89, 60, 62, 63, 70–79]

	INTERCEPT	Mirasol	Theraflex
Viruses (enveloped)			
HIV-1, cell free	>6.2	5.9	1.4
HIV-1, cell-associated	>6.1	4.5	
HBV	>5.5	2.5a	≥2.8a
HCV	>4.5b	>4.1	≥5.0
HEV (genotype 3)		>3.0	
HTLV-I	4.7	n.t.c	
HTLV-II	5.1	n.t.c	
CMV, cell free		2.1d	ongoing
CMV, cell-associated	>5.9		ongoing
Bovine viral diarrhea virus	>6.0		2.7
WNV	>6.0	≥5.1	3.5-4.0
Chikungunya	>6.4	2.2	ongoing
Influenza A virus	>5.9	>5.0	≥5.3
SARS-CoV	>5.5		
Togavirus		3.2e	≥5.3e
Rabiesvirus		>6.3f	≥6.3[f]
Dengue-virus	>5.2		ongoing
La Crosse virus		>3.3	
Crimean-Congo hemorrhagic fever virus	2.9		
Viruses (non-enveloped)			
HAV	0	1.8	ongoing
Parvo B19	>6.2	>5.0g	5.0g
Blue tongue virus	6.1		
Human adenovirus	>5.9		
Calicivirus	1.7–2.4		
Picornavirus		3.2h	>4.0h
Bacteria gram-negative			
Escherichia coli	>6.4	4.4	>4.0
Enterobacter cloacae	5.9		>4.3
Klebsiella pneumonia	>5.6	2.8	4.8
Pseudomonas aeruginosa	4.5	>4.5	>4.9
Salmonella cholerasuis	>6.2		
Serratia marsescens	>6.7	4.0	≥4.9
Yersinia enterocolitica	>5.9	3.3	
Brucella neotomae		5.4	

Table 3 continued on next page

demand of blood, who require a considerable number of blood transfusions throughout their life (e.g., 100 transfusions corresponding to a drop down of the above mentioned 'standard risk' to 1:10,000).

With regard to platelet and plasma products, the pathogen inactivation profiles for viruses, bacteria, and protozoa are summarized in detail in table 3 for each PIT and updated by literature review and personal communication with the companies (Cerus Corporation: Larry Corash; Terumo BCT: Ray Goodrich, Macopharma: Frank Tolksdorf and German Red Cross Blood Service Springe: Axel Seltsam). [24, 59, 60, 62, 63, 70–79].

Especially the bacterial contamination of platelet concentrates (e.g., with *Staphylococcus epidermidis* and *Bacillus cereus*) and to less extent of RBCs (e.g., with *Yersina enterocolitica* and *Serratia marcescans*) still remains a significant challenge in transfusion medicine with severe impact on the patient's outcome including death [1]. The currently available PIT are able to prevent the majority of transfusion-transmitted bacterial infections. There are some limitations in respect to bacterial spores (e.g., from *Bacillus cereu*s or *Clostridium tetani*) which are produced as survival strategy under extreme environmental conditions [134]. They are defined as a dormant, non-reproductive structure highly resistant to head, desiccation, freezing, UV irradiation as well as chemical and enzymatic destruction. Some but not all enter into vegetative forms in platelet concentrates as shown by spiking experiments [80]. Furthermore, high titers of bacteria under in vitro conditions are not always inactivated sufficiently (e.g., *Klebsiella pneumoniae*) [24]. Results obtained from a direct comparison of the Mirasol technology versus bacterial culture testing were in favor for the PIT in platelet concentrates with

Table 3. Continued

	INTERCEPT	Mirasol	Theraflex
Bacteria gram-positive			
Bacillus cereus (incl. spores)	3.6		4.3
Bacillus cereus (vegetative)	>6.0	2.6	4.3
Bifidobacterium adolescentis	>6.5		
Clostridium perfringens (vegetative)	>6.7		>4.7
Corynebacterium minutissimum	>6.3		
Listeria monocytogenes	>6.3		
Proprionobacterium acnes	>6.2	>2.8	4.5
Staphylococcus aureus	6.6	4.8	≥4.8
Staphylococus epidermidis	>6.6	4.6	≥4.9
Streptococcus pyogenes	>6.8	2.6	
Lactobacillus species	*>6.9*		
Spirochaetes			
Borrelia burgdorferi	>6.8		
Treponema pallidum	>6.8	ongoing[i]	
Parasites			
Babesia microti	>5.3	>4.0	
Babesia divergens			≥5.0
Leishmania major	>4.3	>4.0	
Leishmania mexicana	>5.0		
Orientia tsutsugamushi		>5.0	
Plasmodium falciparum	>6.0	>3.2	≥4.9
Plasmodium yoelli		>4.4[j]	
Trypanozoma cruzi	>5.3	>5.0	2.8 to 4.2

[a]Pseudorabies virus (suides herpes virus). [b]Chimpanzee transfusion studies. [c]HIV is used with some restrictions as model virus also for other retroviruses such as the oncogenic HTLV-I/II. [d]Bovine rhinotrachitis virus, in addition mouse CMV in vivo mouse transfusion model. [e]Sindbis virus, HCV model. [f]Vesicular stomatitis virus. [g]Porcine parvovirus which suitability as model virus for human Parvo B19 remains questionable. [h]Porcine encephalomyocarditis virus, HAV model. [i]In rabbits. [j]In vivo mouse malaria model.

a low initial contamination level (<20 CFU per product) [72]. Nevertheless, this study has shown in parallel a limited capacity of bacteria elimination. Even though the bacterial contamination occurring during blood donation is believed to be very low, bacteria may start to proliferate rigorously during the entire storage period after an initial lag phase of at least 24–48 h [80]. Therefore, we have to take into account that the current PIT are applied immediately after donation (within the 1st day) so that an exponential bacterial outgrowth until the pathogen inactivation takes place is very unlikely and the limited capacity could be neglected in transfusion practice.

In some instances an animal model virus analogous to the human virus has been used which may be of relevance for drawing significant conclusions as recently shown for porcine Parvo B19. Using a real-time PCR inhibition assay, it could be demonstrated that Mirasol reduces intact human parvovirus B19 DNA in plasma 1.7 log compared to 5 log inhibition of porcine B19 in a tissue culture infectious dose assay [81]. Logarithmic reduction with the prefix 'greater than' indicates that a higher infectivity was not given in the in vitro test system applied. The author would like to point out that the virus strain, virus titration method (tissue culture infectious dose), and the calculation platform may differ among the competitors involved.

Therefore, direct side-to-side comparison studies using the same strain of a virus or bacterial species may be desirable to ensure a high degree of comparability and reproducibility. More recently the ISBT Working Party for Transfusion-Transmitted Infectious Diseases published a proof of principle experiment validating the quality, stability and suitability of four PEI bacterial references (*Staphylococcus epidermidis, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli*) in platelet concentrate low-titer spiking experiments which were performed in 14 laboratories around the world. The aims of this international study were successfully fulfilled [80]. To the author's knowledge the UVC-based Theraflex technology is until today the first PIT using the WHO reference bacteria panel for the generation of inactivation data [65]. In case of bacteria spike experiments with high titers one should be aware that robust pathogen killing in vitro may not affect the problem of endotoxin-induced septic shock in patients. So, from a practical point of view the detection of sterility after applying PIT on blood components with reasonable bacterial contamination (simulating the scenario of con-

tamination during donation) is of more clinical relevance [72]. The pathogen inactivation profiles are increasingly complete for the INTERCEPT and Mirasol with its well-known gap for HAV; however, especially for THERAFLEX UV there are still some data missing, and some transfusion-associated viruses have been found to be relatively resistant to UV-C light exposure – this is primarily true for HIV. PIT for RBCs and whole blood is currently being developed for business success because the patient's individual advantage with regard to blood safety mainly depends on the likelihood (up to 100%) of receiving PIT-treated blood components permanently.

Clinical Evaluation and Hemovigilance Data (Platelets)

In vivo Recovery and Lifespan

The pharmaceutical potency of pathogen-inactivated platelets was principally investigated by radiolabeling studies (^{111}In) that involved re-transfusing autologous platelets initially collected from volunteer donors after 5-day storage. The data were corrected for radiolabeling efficiency and pre-injection elution. The minimal FDA requirements for fresh platelets are a mean recovery rate of 65% and a life span of 8 days. The FDA requirements demand a platelet recovery at day 5 of at least 67% (corresponding to 43.5% of the initial concentration of platelets under fresh conditions) and platelet survival of 58% (corresponding to 4.64 days) compared with fresh reference platelets produced in parallel. In contrast to RBC survival in the blood circulation, it is well known that transfused platelets fill up the spleen pool.

In 2004, a mean recovery of 84% (42.5 ± 8.7% vs. 50.3 ± 7.7%) and a lifespan of 80% (4.8 ± 1.3 vs. 6.0 ± 1.2 days) were published for INTERCEPT-treated compared with non-irradiated reference platelets suspended in PASIII [82]. Furthermore, AuBuchon et al. [83] have summarized the results obtained for Mirasol-treated platelets with a mean proportion of 75% (50.0 ± 18.9% vs. 66.5 ± 13.4% recovery) and 73% (4.3 ± 1.0 vs. 5.9 ± 1.1 days lifespan). Very recently, the first results (n = 6) of a phase I study for the THERAFLEX UV technology were published and demonstrated a significant decrease in the platelet recovery and survival rates (74% (37.6 ± 6.5% vs. 28.0 ± 8.2%) and 71% (7.3 ± 0.9 vs. 5.2 ± 1.3 days)), which were comparable to other PIT [84]. Furthermore, Thiele et al. [85] presented preliminary data that the transfusion of UV-C-treated autologous platelets in healthy volunteers met the safety and tolerance criteria.

Upon critical review of these data, the pathogen-inactivated platelets have fulfilled the minimal FDA criteria on in vivo quality independent of the technology applied. However, the raw data consistently indicate a slight but significant alteration of in vivo quality between the test and reference groups in each study. This in vivo phenomenon may be in line with in vitro parameters for assessing the functional integrity of platelets, such as the detection of a higher metabolic state or signs of activation [82, 83].

Clinical Evaluation

Depending on the CE approval mark which enables the free movement of goods in the EU member states and the completion of phase III RCTs, the readiness for market introduction varies for the above mentioned PIT (table 2). Medical devices with a higher risk profile are classified into a higher category. The INTERCEPT device is a CE mark class III product (amotosalen) which requires the critical review of clinical data (e.g., inactivation efficacy, 7-day storage) by the Notified Body to demonstrate compliance to the medical device directive and the approval by the respective National Authority. The Mirasol device is a CE mark class IIb product (riboflavin) which needs in principle a self-certification by the manufacturer. To the author's knowledge, the toxicology profile, inactivation efficacy, and clinical data of the Mirasol technology were also reviewed by a Notified Body prior to approval. The most important data of relevant clinical trials which are already published in peer-reviewed journals are summarized in table 4.

INTERCEPT

For INTERCEPT-treated platelets, results obtained from several clinical trials enrolling more than 1,000 patients have already been published, and they demonstrate strong evidence for general therapeutic efficacy and safety. The five most relevant phase III studies are summarized in detail in table 4 (euroSPRITE [86], SPRINT [87], Janetzko et al. [88], Kerkhoffs et al. [89], and Lozano et al. [90]). A supplemental 'pilot' study [91] and 'phase I/II' study [92] were also published, but not listed in table 4 due to limitations in study design and performance status. Several previously discussed issues have been rather controversial, especially the clinical impact of lower CI/CCI values and the correctness of bleeding assessment according to the WHO or Common Terminology Criteria for Adverse Events (CTCAE) classifications.

The results obtained from the RCT published by Kerkhoffs et al. in 2010 (HOVON study) [89] have especially contributed to the confusion ([89, 93, 94]. The lack of blinding and the evaluations of bleeding complications have been the most important critical comments [95–97]. The observation of grade 2 bleedings below 10% in hematological patients transfused prophylactically is in sharp contrast to previous and recently published data (approximately 60%), making it hard to consider the results (especially bleedings) of this RCT. This must be taken into account when the authors reported a significant increase in all bleedings after the first platelet transfusion (INTERCEPT 32% vs. 19% plasma; p = 0.03) and more grade 3 bleedings (6% vs. 1%; p = 0.044). Interestingly, the authors very recently published data using a rigorous observation and adjudication method to assess bleedings more accurately and consistently [98]. In addition, one other group has

Table 4. Multicenter randomized clinical trials

	PIT	Reference	Study design	Study size (test/reference)	Platelet characteristics (dose in 10^{11}, storage in days)	Primary endpoint (test/reference)	Secondary endpoints (test/reference)
euroSPRITE Van Rhenen et al. 2003 [86]	INTERCEPT	buffy coat T-sol or plasma, all irradiated	multicenter RCT, ITT (56 days of treatment) 80% power to detect ΔCCI-1h ~2,800 or ΔCI 8×10^9/l (α=0.05, 2-sided)	103 patients 52/51 567 products 311/256[a]	dose 3.9 ± 1.0 / 4.3 ± 1.2 storage 3.5 ± 1.1 / 3.4 ± 1.2	CCI-1h[b] 13,100 ± 5,400 / 14,900 ± 6,200 1,800 ⇒ upper bound 95% CI 4,100 LRA[c] dose versus CI-1h Δ1.5 × 10^9/l ⇒ upper bound 95% CI 6.1 × 10^9	CCI-24h[b] 7,400 ± 5,500 / 10,600 ± 7,100 LRA[c] dose versus CI-24h, Δ2.6 × 10^9 any hemorrhagic event 79%/79% any severe hemorrhagic event 6%/6% post-transfusion hemostatic score: 0.28/0.30 acute transfusion reactions (6 h) 6%/5% platelet transfusion interval (days) 3.0/3.4 number of PC 7.5 ± 5.8 / 5.6 ± 5.9 number of RBC 9.3 ± 5.3 / 8.2 ± 6.3 RBC/day of PC support 0.41 ± 0.35 / 0.56 ± 0.68
SPRINT McCullough et al. 2004 [87]	INTERCEPT	apheresis 100% plasma all irradiated	multicenter RCT, ITT (28 days of treatment) non-inferiority design 90% power, α=0.05 Δgrade 2 bleedings (12.5%, 1-sided) Δgrade 3, 4 bleedings (7.0%, 1-sided)	645 patients 318/327 4,719 products 2678/2041	dose 3.7/4.0 storage 3.4/3.6	grade 2 bleeding 58.5%/57.5%	grade 3 or 4 bleeding 4.1%/6.1% CCI-1h 11,100/16,000 CCI-24h 6,700/10,100 acute transfusion reactions (7 days) 3.0%/4.4% platelet transfusion interval (days) 1.9/2.4 number of PC 8.4/6.2 number of RBC 4.8/4.3 RBC/day of PC support 0.31/030
Janetzko et al. 2005 [88]	INTERCEPT	apheresis 100% plasma all irradiated	multicenter, RCT, ITT (28 days of treatment) 80% power to detect ΔCI 13×10^9/l or ΔCCI-1h 5,250 linear regression analysis	43 patients 22/21 218 products 103/115	dose 4.1 ± 1.2 / 3.8 ± 0.4 storage 3.1 ± 1.0 / 3.2 ± 0.8	CI-1h[b] 23,800 ± 18,500 / 31,200 ± 15,500 Δ7,400 ⇒ upper bound 95% CI 17,900 LRA[c] dose versus CI-1h Δ7.2 × 10^9/l	CCI-1h 11,600 ± 7,300 / 15,100 ± 6,400 CCI-24h 7,300 ± 6,200 / 10,400 ± 6,500 LRA[c] dose versus CI-24h, Δ7.4 × 10^9/l number of PC 4.7 ± 3.3 / 5.5 ± 4.7 platelet transfusion interval (days) 2.4 ± 1.0 / 2.8 ± 1.0 any hemorrhagic event: 64%/71% number of RBC 3.7 ± 3.8 / 5.5 ± 5.6 RBC/d at risk 0.4/0.4 acute transfusion reactions(6 h) 6%/5%
HOVON Kerkhoffs et al. 2010 [89]	INTERCEPT	buffy coat T-sol or plasma	multicenter RCT ITT non-inferiority design non blinded[f] 90% power to detect 20% ΔCCI-1h (α 0.025, 1-sided)	295 patients[g] 85/99/(94)[h]	dose: n.d. storage: equally 4 days	CCI-1h (plasma) 11,400 ± 5,300 / 17,100 ± 7,300	CCI-24h 7,900 ± 5,300 / 12,800 ± 7,800 (plasma) any bleeding patient 32%/19% (plasma) bleedings grade 2 7%/6% (plasma) bleedings grade 3 6%/ 1% (plasma) platelet transfusion interval (h) 61 ± 47 / 81 ± 47 acute transfusion reactions 7%/13%

Table 4 continued on next page

Table 4. Continued

	PIT	Reference	Study design	Study size (test/reference)	Platelet characteristics (dose in 10^{11}, storage in days)	Primary endpoint (test/reference)	Secondary endpoints (test/reference)
TESSI Lozano et al. 2011 [90]	INTERCEPT	buffy coat (86%) apheresis (14%) T-sol or SSP, partly irradiated	multicenter RCT ITT non-inferiority design 30% margin	211 patients[e] 101/98	dose: equally 4.2 storage: day 7 (80%) or day 6	CCI-1h 8,200 ± 5,400 / 9,400 ± 5,900	CCI-24h 4,600 ± 3,500 / 6,500 ± 5,200 CI-24h 11,100 ± 8,900 / 15,200 ± 12,200 CI-1h 19,400 ± 13,400 / 21,600 ± 14,600 platelet transfusion interval (days) 2.2/2.3 post-transfusion hemostatic score 84%/77% post-transfusion bleeding (4 days) 23.8%/29.2% RBC transfusion (24 h) 23.8%/21.7% any adverse event (24 h) 31.4%/29.2%
MIRACLE Cazenave et al. 2010 [108]	Mirasol	buffy coat (30%) apheresis (70%) 100% plasma	multicenter RCT, ITT[f] non-inferiority design 20% CCI-1h Δ2,940	110 patients 56/54 541 products 303/238[i]	dose: equally 5.4[b]. storage 2.8 ± 1.1 / 2.6 ± 1.1	CCI-1h[b] 11,700 ± 1,100 / 16,900 ± 1,100[j] Δ5,200 failed to show non-inferiority	CCI-24h[b] 6,700 ± 900/9,900 ± 900, Δ3,200 platelet transfusion interval (days) 2.3 ± 1.9 / 2.7 ± 1.4 RBC/patient during treatment: 2.8 ± 1.7 / 2.6 ± 2.4 any bleedings 59%/43% grade 3–4 bleedings 10.7%/5.6% any adverse event 0.28 ± 0.19 / 0.23 ± 0.19

RCT = Randomized clinical trial; ITT=intent to treat study; LRA = longitudinal regression analysis. [a]Only on-protocol transfusions. [b]For up to the first 8 transfusions, [c]For all transfusions, [d]Caused by the detection of a significant period-by-treatment interaction the first period was validated primarily, however the power to reject non-inferiority was only 20%. [e]199 eligible for CCI-1h. [f]Open labeled. [g]278 eligible for CCI-1h. [h]Two references (plasma and T-Sol). [i]And 65 (18%) / 72 (23%) off-protocol transfusions. [j]SEM instead of SD.

developed a new bleeding severity measurement scale to assess bleedings in patients with chemotherapy-induced thrombocytopenia with a higher level of validity [97, 99].

The impact of the revised meta-analysis, which included the RCT of Lozano et al. [90] and excluded the single RCT using Mirasol technology [100] remains questionable [101] because the data published by Kerkhoffs are still part of the analysis. However, the author now discussed that 'the risk of all bleeding complications is no longer increased', whereas the assessment of 'the risk of clinical significant bleeding complications' is uncertain and strongly depends on the integration or exclusion of a single RCT. In this line, an additional meta-analysis (excluding the HOVON study [89]) did not find any significant differences in bleeding risks [94]. The re-analysis of the SPRINT data has confirmed the equivalency for the prevention of bleedings (grades 2, 3 and 4) and the number of transfused PC and RBC units [102]. In addition to the hemostatic effect, the SPRINT data were further evaluated to assess the adverse event profile. Acute transfusion reactions within 6 h were significantly lower in the INTERCEPT study arm compared to reference platelets suspended in plasma (3.0% vs. 4.4%) [103].

Currently, increasing evidence suggests the clinical non-inferiority of INTERCEPT-treated platelets compared to reference platelets. Post-marketing observational studies confirmed the results which have been obtained by phase III studies [104, 105]. Data from large retrospective studies have also supported the non-inferiority as assessed primarily by these RCTs and have demonstrated a subsequent stable consumption of platelet concentrates in comparable patient cohorts (annual utilization in the same hospital) after INTERCEPT implementation [100, 106–108]. Furthermore, there was no significant increase in RBC transfusions in recipients transfused with pathogen-inactivated platelets. Osselaer et al. [106, 107] have surveyed the adverse events associated with routine transfusion of INTERCEPT-treated platelet concentrates after implementation of a multicenter active hemovigilance program (Belgium, France and Spain) with 5,106 transfusions in the first period and 7,437 transfusions in the second. In these post-marketing surveillance studies, the majority of patients suffered either a hematological malignancy with chemotherapy / stem cell transplantation or underwent cardiovascular surgery or solid organ transplantation. Children and pregnant women were included to receive pathogen-inactivated platelets in a routine workflow. In the latter period, the authors reported an overall infrequent rate (0.7%) of acute transfusion reactions, mainly of mild severity, and only 5 adverse events were considered severe without a causal link to PIT. Furthermore, Cazenave et al. [100] summarized the data from the Etablissement Francais du Sang Alsace after implementation in 2006, comparing the clinical safety and tolerability of more than 13,000 INTERCEPT-treated PC in approximately 2,000 recipients with reference platelet concentrates suspended in 100% plasma or PASIII. The authors stated a

significant reduction in acute transfusion reactions and no evidence of increased RBC consumption in patients receiving INTERCEPT-treated PC. In most of the participating centers, PIT was used to replace gamma-irradiation and anti-CMV serology.

Mirasol

The data volume and level of evidence for the therapeutic efficacy and safety of Mirasol pathogen inactivation technology is less pronounced. Not until 2010, a single multicenter, open-label RCT with non-inferiority design was published by the Etablissement Francais du Sang comparing Mirasol-treated platelet concentrates with conventional products (either apheresis or buffy coat-derived, both suspended in 100% plasma) in hematological patients with thrombocytopenia [109]. Based on the results of the TRAP trial [110], a non-inferiority margin of 20% (equivalent to a mean corrected count increment 1 hour post-transfusion (CCI-1h) difference of 2,940) was defined, and a statistical power of 80% was considered to be sufficient. Six centers enrolled a total of only 118 patients between 2005 and 2007. Four patients in each study arm did not receive any transfusion and were excluded from the intention-to-treat analysis. A total of 678 transfusions were given, 368 in the test group and 310 in the reference group, with a 17.7% and 23.2% frequency of off-protocol transfusions, respectively. The primary endpoint of the study was the CCH-1h on the first 8 transfusions within a 28-day treatment period. The RCT failed to show the hypothesized non-inferiority, with a CCI-1h of 11,725 ± 1,140 (test) versus 16,939 ± 1,149 (reference), resulting in a mean decrease of 5,200 and corresponding to approximately 30%. As secondary outcomes, the CCI24h showed a comparable trend with a mean difference of 3,200 (33%). The difference in the rate of all bleedings did not reach significance (59% vs. 43%; $p = 0.127$), and the frequency of grade 3 and 4 bleedings was too low ($n = 6$ (test) vs. $n = 3$ (reference); $p = 0.490$) to evaluate the clinical safety profile in terms of bleeding complications as this needs a sufficiently powered study design. Even the authors concluded that further clinical studies are required to determine whether the drop in CCI-1h correlates with an increased risk of bleedings.

So far, only small-sized observational platelet studies have been performed or are still ongoing in Spain, Luxembourg, Lithuania, and Serbia. Among others, Antic et al. [111] have shown no statistically significant difference between test and control group corresponding to a CCI-1h value of 7,500 and CCI-24h of 5,000. Yanez et al. [112] has published a CCI-1h of 12,000 and a CCI-24h of 4,900 based on 26 transfusions in 11 patients, and Coene et al. [113] has also shown comparable CCI-1h and CCI-24h values (9,900 ± 5,700 and 5,900 ± 2,900) calculated from a total of 21 transfusions in 8 patients. Although all authors stated that no adverse events were reported, the validity of this information was insufficient due to the small patient size. Furthermore, a similar hemostatic function of Mirasol-treated platelets was suggested by using thrombelastography as in vivo surrogate system [114]. These single observations did not supersede the need to implement an active hemovigilance program for Mirasol-treated platelet concentrates in routine use and to foster further RCTs. In Italy, one additional multicenter RCT with a calculated size of more than 400 patients (IPTAS) has been initiated using Mirasol-treated PC suspended in PAS to address the concern of clinical relevant bleedings. Furthermore, the PREPAReS study (consortium of The Netherlands, Norway, and Canada) is ongoing and has to recruit more than 600 patients receiving Mirasol-treated platelet concentrates suspended in 100% plasma. Both clinical studies will not be completed before 2015 (personal communication Ray Goodrich).

Outlook

Until 2014, only plasma and platelet concentrates derived from pooled buffy coats or collected by single-donor apheresis pathogen-inactivated using INTERCEPT or Mirasol had received marketing authorization approval by European and Non-European National Bodies, while THERAFLEX UV technology and PIT applied on RBC units or whole blood (see below) are still in the early phase of clinical evaluation. Most clinical experiences are available from more than 2 million post-marketing transfusions using INTERCEPT-treated plasma (ca. 40%) and platelets (ca. 60%) in more than 20 countries (personal communication Larry Corash), for which hemovigilance data have been collected and reviewed systematically as mentioned above. The clinical data also include experiences in neonates and pediatric patients. In addition, national hemovigilance data are also available from France (since 2006) and Switzerland (since 2011). Such data are not available from Germany, although the Paul-Ehrlich-Institut released the first manufacturing authorization in 2007 for the University Hospital Luebeck [105]. In the case of Mirasol, the company announces that over 110,000 Mirasol-treated blood components (ca. 60% plasma, ca. 40% platelets) were applied in participating transfusion centers most frequently in routine use without any severe adverse events or an obviously increased rate of adverse events (personal communication Ray Goodrich). However, systematically collected hemovigilance data post marketing authorization have not yet been published. None of the PIT is currently approved by the FDA for the US market. However, the Cerus Corporation has announced the submission of a premarket approval application for INTERCEPT platelets in the first half of 2014.

Taken together, the 'inherent' variability of conventional platelet concentrates (independent of the type of PIT used) must be noted which has been well-accepted over decades. This can be reasonably explained in terms of platelet content by the 'biological source' (e.g., volunteer donors with different platelet concentrations in their peripheral blood) and in terms of clinical efficacy by either the natural aging of platelets during storage or non ABO-identical use (e.g. TRAP

trial). As reported by Slichter et al. [12] in a large randomized multicenter trial, the Prophylactic Platelet Dose Study (PLADO) with 5,466 prophylactic platelet transfusions in 1,272 patients, the platelet dose does correlate with the CCI after transfusion resulting in a higher transfusion frequency, but not necessarily with higher incidence of bleedings. With this in mind, we must ask to what extent lower efficacy (bearing in mind the non-inferiority margins of about 20%) becomes acceptable for a higher safety profile as a result of using PIT rather than to call efficacy and safety of PIT-treated platelets into question aiming at aspects that were accepted with regard to conventional platelet products in the past (e.g., impaired by storage and ABO non-identical use). Furthermore, the very recently published study by Wandt et al. [13] invites to rethink the prophylactic platelet transfusion strategy in some patients not at risk for increased bleeding complications (e.g. autologous stem cell transplantation) because no increase of major hemorrhages could be observed in these patients when platelets have been transfused only therapeutically. However, quality-of-life aspects remain questionable because most of the patients and physicians are seriously affected by routine monitoring of bleedings as they became aware of a permanent latent danger like a sword of Damocles.

Outlook for Red Blood Cells and Whole Blood

INTERCEPT

The PIT applied on platelets is not transferable to whole blood and RBC units because these optically dense blood components need very high doses of UV light. Therefore, Cerus has developed a novel platform technology (2nd generation) that is based on the addition of the chemical compound S-303 (0.2 mmol/l) and glutathione (GSH; 20 mmol/l) as a quencher [115]. S-303 targets and cross-links nucleic acids via a bis-alkylating group to prevent further replication. After the reaction, the non-reactive, negatively charged breakdown product S-300 is formed and rapidly degraded. To minimize the affinity of S-303 with other nucleophiles, especially proteins, glutathione is added and distributed in the extracellular compartment (plasma) to quench these extracellular reactions, whereas S-303 acts as a 'pathogen inactivator' in the intracellular compartment. Treatment solution and breakdown products are removed by centrifugation prior to final storage in approved solutions [116].

During a 1st generation S-303 phase III RCT for chronic anemia patients, two multitransfused participants (of more than 140) developed a low-titer antibody against the acridine moiety of S-303 on the RBC surface that led to positive cross-match reactions in vitro without any cues regarding its clinical impact, such as acute hemolytic transfusion reactions [117]. Nevertheless, this phase III study and a parallel study in cardiovascular surgery patients were halted prematurely, and

Table 5. Toxicology of pathogen inactivation technologies for red blood cells or whole blood

	INTERCEPT S-303 RBC	Mirasol whole blood
Phototoxicity	√	√
Acute toxicology	√	√
Repeated dose	√	√
General pharmacology	√	√
Reproductive toxicology	√	√
Genotoxicity	√	√
Carcinogenicity	√	√
Neonatal toxicity	√	√
ADME* studies	√	√
Occupational safety	√	√
Neoantigen formation	open	√

*Absorption, distribution, metabolism, elimination.

Cerus developed a 2nd generation S-303 technology to minimize the acridine moieties on the RBC surface by increasing the concentration of the protecting glutathione during pathogen inactivation process. Subsequently, the inactivation efficacy against bacteria, viruses, and protozoa of the 2nd generation approach as well as the pharmacokinetic and toxicological assessment of S-303 is principally transferable from the 1st generation (tables 5, 6) [115, 118, 119] (personal communication Larry Corash). This cross-reference was accepted by the FDA because of the identity of substances used. A new phase I study was conducted with a total of 26 healthy volunteers receiving autologous S-303-treated RBC transfusions using a dual radiolabeling approach using ^{51}Cr and ^{99}Tc [116]. The 2nd generation of S-303 did not induce antibody formation. The 24-hour recovery at day 35 was excellent, with a mean of 88.0 ± 8.5%, and demonstrated equivalency with the untreated RBC reference arm (90.0 ± 6.9% recovery). The median lifespan was significantly reduced (32.8 vs. 39.5 days), whereas the in vitro evaluation after 35 days of storage has shown equivalency in hemolysis, extracellular potassium, mean corpuscular hemoglobin concentration, and mean corpuscular volume and a marginal loss of hemoglobin during S-303 treatment.

The S-303 PIT for RBCs is now ready to run in renewed, middle-sized (n < 100 patients) phase III clinical studies in the European Union, in which the patient's enrollment just began for elective cardiac surgery patients with acute blood loss and for thalassemia patients with chronic transfusion demands (personal communication Larry Corash). These clinical studies focus both on the hemoglobin content and in the latter case on the potential immunogenicity with repeat exposure. Furthermore, the company has announced a Cerus whole blood initiative supported by the Swiss Red Cross Humanitarian Fund to pursue the applicability of the S-303 technology on whole blood in developing countries as a humanitarian effort.

Mirasol

The same riboflavin-based PIT which was successfully evaluated for platelets and plasma is now being further developed for whole blood to achieve the ultimate goal of a single PIT platform that is able to 'inactivate' all three blood components simultaneously. Understandably, most of the toxicological assessment and data concerning neoantigenicity and protein binding behavior could be extrapolated to the whole blood system as well [120]. As shown in a baboon study model, riboflavin does not induce a chemical modification on the surface of RBCs such as possessing an immunogenic moiety [121]. Safety and efficacy was assessed in a large animal model (a diffuse, non-surgical bleeding pig model) [122]. The 'drug-related' terminology of phase I–III studies is avoided by the fact that the Mirasol system is rather a medical device than a drug (personal communication Ray Goodrich). However, in context with the generation of ROS this classification has to be reconsidered.

Feasibility data from RBCs (after 42 days of storage) separated from whole blood donations (n = 11) and treated by riboflavin and increasing doses of UV light energy (in this case 22, 33 and 44 J/ml) in the USA have shown high variability (ca. 60–80%) in RBC recovery and RBC half-life on day 42 (24 ± 9 days, range 10–36 days) [123]. Five of 11 subjects met the FDA criteria of treatment success as defined by 75% autologous recovery of radiolabeled RBCs after 24 h (lower limit of 95% CI: 70% recovery), which is, however, in the range of gamma-irradiated RBC units. The rate of hemolysis on day 42 (not day 35) is between 1.0 and 1.5%, and the osmotic fragility increases from 0.8 to 4.1% depending on the UV light dose chosen [123]. As recently reported by the company, the current configuration is now based on the exposure of a higher dose of UV light (80 J/ml) to compensate sufficiently for the UV light absorption by hemoglobin [60]. By choosing comparable UV energy doses, the quality of each separated blood component (e.g. rate of hemolysis) has to be maintained during standard storage period, while there is still an acceptable inactivation efficacy of a broad spectrum of pathogens as summarized in table 6. However some of these data are personally communicated and not yet confirmed by original articles in peer-reviewed journals [120, 124–130]. Riboflavin UV treatment and gamma-irradiation were equally effective in the prevention of transfusion-associated graft-versus-host-disease, resulting in an in vitro 4.7 log reduction of viable T cells which had been subsequently confirmed in immunodeficient murine recipients (NOD-*scid* IL2rγnull) [131].

The company starts the IMPROVE II RCT (comparable to a phase II study with radiolabeled RBCs) in the USA, obviously to validate the impact of the process optimization and UV light dose fixation (80 J/ml) of Mirasol treatment on the RBC quality and loss of inactivation efficacy. In future, one of the focuses will be the potential clinical application of Mirasol-treated whole blood in military scenarios that allow for the immediate substitution of safe RBCs, platelets, and plasma

Table 6. Pathogen inactivation efficacy for red blood cells and whole blood

	INTERCEPT	Mirasol
Viruses (enveloped)		
HIV-1, cell-associated	>6.2	4.5
HIV-1, cell free	>6.5	
HBV	>6.3[a]	ongoing[b]
HCV		ongoing
HTLV-I		
HTLV-II		
CMV, cell free		
CMV, cell associated		ongoing
BVDV[c]	>4.8	
WNV		
Herpes simplex virus	>6.0	
Vesicular stomatitis virus	5.7	>4.5
Chikungunya		
Influenza A virus		
SARS-CoV		
Dengue-virus		
Viruses (non-enveloped)		
HAV		1.7
Parvo B19		4.0[d]
Bluetongue virus	≥6.0	1.5
Adenovirus type 5	>7.4	
Bacteria gram-negative		
Escherichia coli	7.4	
Enterobacter cloacae		
Klebsiella pneumonia		
Pseudomonas aeruginosa	4.5	
Salmonella cholerasuis	4.8	
Serratia marsescens	4.1	
Yersinia enterocolitica	7.4	
Bacteria gram-positive		
Bacillus cereus (incl. spores)		
Bacillus subtilis (vegetative)	>6.3	
Bifidobacterium adolescentis		
Clostridium perfringens (vegetative)		
Corynebacterium minutissimum		
Listeria monocytogenes	>7.1	
Proprionobacterium acnes		
Staphylococcus aureus	>5.1	
Staphylococus epidermidis	>6.9	
Streptococcus pyogenes		
Spirochaetes		
Borrelia burgdorferi		
Treponema pallidum		
Parasites		
Babesia microti	>5.5	>5.0
Babesia divergens		>7.2
Leishmania major		2.3
Plasmodium falciparum	>6.8	>6.4
Trypanozoma cruzi	>5.4	>3.5

[a]Duck hepatitis B model. [b]Human. [c]Bovine viral diarrhea virus (HCV model). [d]Porcine parvovirus which suitability for human Parvo B19 remains questionable.

for cases of life-threatening trauma and trauma-induced coagulopathy (IMPACT). Very interestingly, an additional RCT will take place in Ghana with more than 200 patients to evaluate the prevention of whole blood-associated malaria transmission by Mirasol-based inactivation of whole blood donations [132] (personal communication Ray Goodrich).

Disclosure Statement

PS has received honorarium and travel reimbursement as speaker and chair from CERUS Europe BV.

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