Towards pathogen inactivation of red blood cells and whole blood targeting viral DNA/RNA: design, technologies, and future prospects for developing countries

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Abstract

Over 110 million units of blood are collected yearly. The need for blood products is greater in developing countries, but so is the risk of contracting a transfusion-transmitted infection. Without efficient donor screening/viral testing and validated pathogen inactivation technology, the risk of transfusion-transmitted infections correlates with the infection rate of the donor population. The World Health Organization has published guidelines on good manufacturing practices in an effort to ensure a strong global standard of transfusion and blood product safety. Sub-Saharan Africa is a high-risk region for malaria, human immunodeficiency virus (HIV), hepatitis B virus and syphilis. Southeast Asia experiences high rates of hepatitis C virus. Areas with a tropical climate have an increased risk of Zika virus, Dengue virus, West Nile virus and Chikungunya, and impoverished countries face economical limitations which hinder efforts to acquire the most modern pathogen inactivation technology. These systems include Mirasol® Pathogen Reduction Technology, INTERCEPT®, and THERAFLEX®. Their procedures use a chemical and ultraviolet or visible light for pathogen inactivation and significantly decrease the threat of pathogen transmission in plasma and platelets. They are licensed for use in Europe and are used in several other countries. The current interest in the blood industry is the development of pathogen inactivation technologies that can treat whole blood (WB) and red blood cell (RBC). The Mirasol system has recently undergone phase III clinical trials for treating WB in Ghana and has demonstrated some efficacy toward malaria inactivation and low risk of adverse effects. A 2nd-generation of the INTERCEPT® S-303 system for WB is currently undergoing a phase III clinical trial. Both methodologies are applicable for WB and components derived from virally reduced WB or RBC.

Keywords: blood, red blood cell, virus, pathogen, inactivation.

Introduction

Each year, over 110 million units of blood donations are collected worldwide. Nearly half of these donations come from developed countries that are home to less than 20% of the global population. In high-income countries, products from transfusion, including red blood cells (RBC), platelets and plasma, are most commonly used in aiding cardiovascular and transplant surgery, and to provide therapeutic support in heavy trauma and haematologic malignancies. In lower income countries, blood transfusions are used more frequently in managing malaria, anaemia, trauma and pregnancy-related complications. Each unit of blood has the potential to benefit several types of pathologies, but each infected or contaminated blood product can severely harm its recipients[1]. In the absence of efficient and cost effective donor screening, viral testing and pathogen inactivation, the risk-benefit ratio of the blood products is directly dependent on the infection rate of the donor population[2].

Although generally following international guidelines and guides, different countries can tailor their specific donor selection criteria and process guidelines based on their own epidemiological and economical standards. As a result, risk levels vary between countries. For example, in Italy, transfusion recipients face an estimated residual risk of 1 in 72,000 for hepatitis B virus (HBV) after blood screening by nucleic acid testing (NAT), whereas in Germany the respective remaining risk is approximately 1 in 620,000[3]. For hepatitis C virus (HCV), in Italy, it is approximately 1 in 5,000,000 as compared to approximately 1 in 1,150,000 in the USA[3]. The risk of receiving a human immunodeficiency virus (HIV)-infected blood product in Spain is almost 7 times more likely than in Germany[2] and over 14 times more likely than in Canada[3]. Malaria is prevalent in sub-Saharan Africa. In Ghana, the blood recipient and donor prevalence for being parasitemic for at least one of four species of plasmodium is over 50%[4].

Fortunately, current pathogen inactivation technologies (PITs) are effective against parasites,

including plasmodium. In an effort to maintain a global standard, the World Health Organization (WHO) published a set of guidelines on good manufacturing practices to regulate the collection and processing of blood products in 2011[5]. Since then, there have been major developments regarding donor screening, blood processing, testing and storage procedures. Because of the great demand for access to quality blood products, a number of safety nets have been set in place to minimise the contamination of blood products by viruses, and adventitious bacteria, parasites or other harmful agents. These include strict donor selection and screening procedures, and blood handling protocols, but these do not completely eradicate the threat of unwanted pathogens. Some countries have begun implementing new forms of PIT to lessen the occurrence of disease transmission by transfusion. There has been remarkable progress in the safety of transfusions over the past few decades due to new measures targeting infectious threats. The continued development of new pathogen safety strategies is critical as new viruses emerge and the demand for transfusions continues. In the United Kingdom, there were major problems with the risk of prion transmission through transfusion. This led to the development of a prion removal filter.

Relevant blood-borne pathogens, and relative degree of risks

HIV, HBV, HCV, and to a lesser extent West Nile virus (WNV), Zika virus (ZIKV) and Dengue virus (DENV), are among several viruses potentially associated with blood transfusions and are targeted for removal by testing and/or inactivation. Syphilis is still a significant bacterial blood-borne target in developing countries, though not as much in developed countries, and plasmodium is a major parasite monitored for removal[6].

HIV I and II

The HIV/AIDS pandemic is among the greatest health challenges in modern history[7]. HIV-1 progresses faster and is more transmissible than HIV-2[8]. The combination of a large regional demand for blood products, inadequate testing of blood products, and the extremely high (95-100%) efficiency of HIV transmission through unsafe blood products contribute to the high risk of transfusion-associated HIV[9]. In addition, HIV is one of several viruses that can infect multiple cellular and plasma components of blood products[6]. An analysis testing HIV-positive blood samples conducted with relatively modern amplification procedures and sequencing techniques demonstrated that HIV viral sequences could be found in 99% of peripheral blood mononuclear cells (PBMNC). HIV-infected plasma samples were shown to be negative for HIV RNA sequences. However, HIV-1 RNA was present in over 85% of the samples[10]. This is consistent with a previous study which indicated that HIV RNA is highly associated with PBMNCs, plasma and platelets[11].

HBV

Data on the seroprevalence of HBV are limited for many developing countries[12]. The WHO reported that approximately 240 million people live with chronic hepatitis B[13]. HBV is primarily spread through mucosal exposure to infected blood or body fluids. Once infected, the subject may clear the infection and develop immunity or become a chronic carrier and be at risk of developing liver cirrhosis or hepatocellular carcinoma[12]. To lower the risk of HBV transfusion transmission, donor blood is screened for hepatitis B surface antigen (HBsAg) and, in some countries, anti-hepatitis B core (HBc) antibodies[14]. HBV can be found in approximately 0.03% of blood donations in high-income countries, as compared to close to 0.1% in mid-income countries and 3.7% in some countries of lower income[15]. Interestingly, HBV had the highest prevalence of all transfusion-transmittable diseases in Australia in 2014 and previous years[16]. HBV can be transmitted by non-virally inactivated plasma components and cellular blood components[6]. Seed *et al.* determined the statistical transmission rate of donor blood components from patients with occult HBV infections (OBI), defined as having anti-HBc reactivity as well as low quantities of HBV DNA and without traceable HBsAg, and patients who tested "inconclusive" (donor individuals with anti-HBs <100 IU/I) for HBV. The combined results showed an anti-HBc rate of 2.86% in RBC, 3.01% in plasma components, and HBV was completely absent (0%) in cryoprecipitate or platelets[17]. As with other viruses, the possibility of HBV being hidden in a window-period increases the challenge in detecting it during screening processes.

HCV

Hepatitis C virus is a member of the family *Flaviviridae*[18]. Between 170 and 184 million people (2-3% of the global population) are infected with HCV[18-20]. Most HCV cases become chronic, potentially leading to progressive liver cirrhosis, fibrosis and liver cancer[18,21]. There are at least 6 major genotypes of HCV which can be further divided into 83 subgroups[22]. HVC-1 can be found globally, including Europe and North America[18,23].The wide distribution of HCV-1 is, in part, the result of blood transfusions and needle sharing, such as among drug addicts[18]. In West Africa, HCV infections are predominantly caused by HCV-2, whereas HCV-1 and HCV-4 are more common in central Africa, sub-Saharan Africa, with HCV-4 having a heavy presence in Egypt[21]. HVC-3 and HCV-6 is often identified in South-East Asia and India, and HCV-5 is common only in South Africa[18]. The spread

of HCV by blood transfusion used to be common in the USA, but became rare once anti-HCV screening became available in 1992[24]. Similarly to HBV, HCV may be transmitted through blood transfusion in cellular blood components as well as plasma products[6]. Although HCV can enter cells through the CD81 receptor, which is absent in platelets, according to an *in vitro* study using HCV-negative blood, HCV can infect platelets through alternative means[25]. However, HCV-RNA levels are higher in serum compared to platelets[26]. A study ranking the general distribution of HCV-RNA in infected blood components found whole blood to have the highest amount of HCV-RNA and significantly more than in plasma, which was significantly more than in PBMC, which was more than in neutrophils, which contained the least amount of HCV-RNA[27]. Although screening criteria are in place to prevent transfusion transmission, HCV can have a window period of roughly two months, making it extremely difficult to detect by anti-HCV antibody screening at early stages of infection[28], and justifying efforts to introduce NAT.

WNV

In addition to ZIKV and DENV, WNV is also a flavivirus transmitted by *Aedes* mosquitos. A major difference is that the primary hosts of WNV are birds. Although WNV can also infect humans and other vertebrates, the amount of virus required to cause successful transmission is unknown[29]. WNV has infected over 1 million people in the USA. While WNV is generally asymptomatic, it is the greatest cause of viral encephalitis in the USA[30], despite the fact that less than 1% of those infected with WNV develop neurodegenerative diseases[31]. More common symptoms of WNV include fever, headaches, chills and fatigue[32]. WNV has been demonstrated to bind to circulating RBC and is transmissible through blood transfusion[29]. The detection of WNV culture-positive plasma suggests the potential for fresh frozen plasma (FFP) to also be a means of transmission of WNV[32].

DENV

Similarly to ZIKV, DENV is a flavivirus transmitted by the *Aedes* mosquitos. There are 4 serotypes of the virus (DEN-1 - DEN-4). DENV presents a broad spectrum of symptoms ranging from asymptomatic to fatal[33]. A large proportion of infections, as much as 87%, do not advance to a clinical stage[34]. However, the clinical symptoms can cause dengue fever, haemorrhagic fever, or febrile illness. There have been reports of DENV outbreaks in many of the same regions where Zika virus is present. The spread of DENV, in combination with the high dangers associated among a proportion of those infected, has led to an increased concern for DENV transmission *via* blood products. Studies have indicated that DENV can bind to human platelets in the presence of virus-specific antibody[35] as well as replicate in peripheral blood mononuclear cells[36]. DENV is known to associate with blood components as well as plasma products[6].

Chikungunya virus

As is the case with many viruses in tropical regions, the primary method of transmission of chikungunya virus (CHIKV) is through various *Aedes* mosquitos[37]. First identified in Africa in the 1950s, reports of CHIKV transmission through transfusion have so far been rare[38]. However, over the past decade, significant outbreaks of a mutated form of CHIKV have occurred in islands in the Indian Ocean, in the Caribbean and in Europe, in large part due to tourism and international shipping around the world[37-39]. CHIKV is an RNA-enveloped virus that produces symptoms including rash, fever, and arteritis[37]. Severe cases can cause uveitis, dendritic lesions, and various forms of neuritis[40,41].

ZIKV

ZIKV, an arthropod-borne flavivirus primarily transmitted by the *Aedes* mosquitos, induces temporary, mild symptoms including rash, headaches and fever in infected individuals[42,43], but is often transmitted without producing symptoms, leaving infected individuals unaware of their condition. Seemingly healthy pregnant mothers have reported more serious fetal abnormalities, including intrauterine growth restrictions and microcephaly, as well as pregnancy complications including miscarriages and stillborn deliveries[42-44]. Zika outbreaks have been recorded in Africa, North and South America, and Asia[43]. In 2013-2014, French Polynesia experienced the largest Zika outbreak to date[45]. During that outbreak, almost 3% of blood donors were shown to be positive for ZIKV[46]. The recent ZIKV epidemic has led to many new concerns over transfusion safety. The impact of ZIKV on blood products is still under investigation by the WHO. However, recent reports indicate platelet transfusion as a means of transmission for ZIKV[47]. The FDA now recommends testing blood components of all donated blood in the USA for ZIKV[48].

Syphilis

Also nicknamed "the great imitator" and "the great pox", syphilis is a disease caused by the spirochetal bacterium *Treponema pallidum*[49]. Syphilis progresses through 3 lifelong stages, which ultimately lead to breakdown of the nervous system and heart failure[50]. Primary syphilis typically occurs within the first three months after exposure with symptoms such as painless lesions and chancres in the external genital regions, hands or lips. Secondary syphilis often occurs 2-8 weeks after the disappearance of primary symptoms,

but occasionally primary symptoms remain. Secondary syphilis symptoms include fever, rash, headaches and pharyngitis. Tertiary syphilis has become far more rare as a result of modern antibiotics, but symptoms include cellular necrosis, fibrosis, and neurological and cardiovascular problems[49]. Syphilis is mostly transmitted sexually, but can also be transmitted congenitally, or rarely as a result of transfusion[51-53]. Pregnancy complications occurring as a result of syphilis include stillbirths, miscarriages and newborns with congenital syphilis. Despite recent increases in syphilis cases in the USA, syphilis is a greater problem for the developing world[49,51]. This is a cause for concern with regard to the generation of blood products. According to a study carried out in Burkina Faso, the seroprevalence of syphilis among first time blood donors is approximately 1.5%[54]. Syphilis can be associated with transfusion transmission through cellular component-based products, but not plasma products[6].

Malaria

Malaria is believed to be the most important parasitic disease currently facing humans[55]. In 2015, 95 countries and territories had active cases of malaria transmission. Today, approximately 3.2 billion people, or half the world's population, is at risk for malaria. *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* are protozoans responsible for the disease known as malaria[56]. *Plasmodium falciparum* is the main parasite associated with severe clinical malaria and is the most fatal of the malaria plasmodia[55]. Malaria is typically transmitted to humans by the female anopheline mosquito during bloodmeal[57]. In sub-Saharan Africa, Malaria infects approximately 150 million people and leads to between 660,000 and 3 million deaths annually[55,58,59]. The first symptoms include vomiting, chills, headache and fever[59]. If not treated immediately, the disease may progress to severe anaemia, respiratory distress, impaired consciousness, abnormal bleeding and cerebral malaria[55,60]. Currently, it is estimated that only 10% of global malaria cases are detected[59]. Malaria infection involves the plasmodium invading RBC[61]. Individuals with sickle cell anaemia tend to be less susceptible to the effects of malaria due to the structure of their erythrocytes, making it difficult for malaria replication. Due to a combination of malaria prevalence, inefficient methods of blood screening, limited resources, and the speed at which blood products are received and transfused, there is a persistent risk of transfusion-transmitted malaria in sub-Saharan Africa. Unfortunately, routine serological tests that are typically effective at limiting the risk of transmission of HIV, HBV, HCV and syphilis are not as successful against malaria[53]. PIT to inactivate plasmodium in whole and red blood cells exists, but is not yet commercially available[62].

Prions

Infectious improperly-folded proteins known as prions are capable of producing infection. The free or cell-bound form of prions can contribute to a number of serious diseases. Among them are kuru, Gerstmann-Straüssler-Scheinker syndrome, fatal familial insomnia and transmissible spongiform encephalopathy (called Creutzfeldt-Jakob disease [CJD]), which is an untreatable, potentially fatal neurodegenerative disease[63]. Since the first reported iatrogenic transmission of CJD in 1972, there have been 4 documented cases of individuals acquiring the variant form of CJD (vCJD) from non-leucocyte reduced red cells[64]. A filter containing an affinity resin capable of adsorbing prions from RBC concentrate has had CE marking since 2006. The decision as to whether to require prion removal as part of screening procedures on collected RBC in the United Kingdom is still pending. (The issue is under the scrutiny of the advisory committee on the safety of blood, tissues and organs [SaBTO]).

Transfusion-transmitted sepsis

While blood-borne infections are a critical concern for blood transfusion safety, transfusion-transmitted sepsis is a less, but still significant, concern occurring in approximately 1 out of every 2,000-3,000 transfusions according to the CDC[65]. The most frequent contaminant of blood for transfusion is the gram-positive skin-borne bacteria called *Staphylococcus aureus*. Contamination typically occurs as a result of the skin surface not being properly cleaned and bacteria from the skin passing into the donated blood through the collection needle[65]. This is the most common cause of bacterial contamination of platelets[66]. Gram-negative bacteria such as *Escherichia coli* (E. coli), *Acineobacter* and *Klebsiella* can be present in blood donations from symptomless donors and cause infections such as pneumonia, meningitis or other severe illnesses in blood recipients[65]. In addition, *Staphylococcus aureus* and *Bacillus cereus* are largely implicated in the contamination of contamination of platelets. Platelets in plasma stored at room temperature provide an ideal media for bacterial growth[66].

Other emerging pathogens

In addition to the well-established pathogens mentioned above, it is critical to consider the threat of emerging pathogens when discussing the risks of transfusion-transmission infections. Among newly emerging pathogens is the Ross River virus (RRV), in which the first reported transfusion-transmitted case was confirmed by the Australian Red Cross on May of 2014[67]. The patient had received RBC from a donor who was subsequently diagnosed with RRV less than a week after donating blood. Other new TTI threats include *babesia*, which are intraerythrocytic protozoan

parasites more commonly transmitted by tick when humans are accidental hosts. Although many people infected with babesia do not develop symptoms, babesia can be life-threatening for the elderly or those with weakened immune systems. Chagas disease, produced by a protozoan parasite called *Trypanosoma cruzi* is another parasite typically transmitted to humans by insect. The pathogen for Chagas disease can exist freely in blood and consequently can be transmitted *via* blood transfusion. Chagas disease can produce severe cardiac and gastrointestinal dysfunction. It is difficult to assess individual risks for the many TTIs because many of them depend on a number of factors that have not been fully established and/or that vary from country to country[68].

Methods of pathogen inactivation licensed or under development for cellular components and plasma for transfusion

Historically, the first blood products to have successfully been subjected to efficient viral inactivation treatments are the fractionated plasma derivatives[69]. In efforts to heighten safety, improve blood product quality, and decrease the rate of TTIs, various technologies have subsequently been designed to inactivate pathogens also in blood components and supplement the existing donor screening and donation testing processes. Pathogen inactivation (PI) refers to technology that applies a physical, chemical, photochemical process to inactivate or kill blood-borne pathogens. These can include methods based on solvent and detergent techniques, photochemical inactivation techniques, such as Mirasol® (Terumo BCT, Lakewood, CO, USA) and INTERCEPT® (Cerus Corporation, Concord, CA, USA), and others[70]. Some techniques focus on plasma for transfusion or specific blood components, while others are under assessment for whole blood pathogen reduction. Some PI technologies for blood products are already being implemented, while newer alternatives are currently under development. PI technologies are met with several different challenges and expectations. They are expected to inactivate enveloped and non-enveloped viruses, gram-negative and gram-positive bacteria, various different parasites and white blood cells while simultaneously not damaging the blood product or otherwise posing a threat to the recipient. Most PI technologies focus on targeting the nucleic acids of pathogens as RBC and platelets do not have a nucleus. However, there is a risk of damaging important blood membrane molecules and proteins, which can compromise the quality of the blood product[71].

Solvent/Detergent

Solvent/Detergent (S/D) works to inactivate pathogens *via* membrane-disruption[70]. The typical S/D treatment, such as the one used for fractionated coagulation factors concentrates, involves a combination of an organic solvent, such as tri(n-butyl)phosphate (TNBP), and a non-ionic detergent (such as sodium cholate, Tween 80, Triton X-45 or Triton X-100) at 24 followed by removal of compounds *via* oil extraction and/or chromatography[70,72]. S/D techniques using TNBP and Triton-45 and applied to mini-pool or individual plasma donations have demonstrated efficacy in the inactivation of HCV and DENV in blood plasma[73-75]. Octaplas (Octapharma AG, Lachen, Switzerland) is an example of industrial-scale S/D-treated human plasma that replaces multiple coagulation factors in patients undergoing liver or heart transplant. Octaplas methodology can be applied for the treatment of either apheresis or whole blood derived-plasma. SDR HyperD® and C18 packings are chromatographic sorbents designed to remove solvent and detergent from blood products and other biological fluids after pathogen inactivation. Octapharma S/D technology was licensed for use in plasma in the United Kingdom in 1998 and as early as 1985 in other countries, and VIPS S/D technology received *Conformité Européenne* (CE) mark certification in 2009 and is licensed in Egypt[76]. It should be kept in mind that this process does not inactivate non-enveloped viruses, but was shown to affect some bacteria[77].

THERAFLEX® MB-Plasma System

The THERAFLEX® (Macopharma, Mouvaux, France) pathogen reduction system incorporates filtration, methylene blue (MB) and 630 nm visible light for pathogen inactivation in single units of plasma[3,78]. This system differs from the technology used on platelets, which uses short-wave, or narrow-bandwidth UVC light and agitation to create pyrimidine dimers[79]. MB interacts with the nucleic acids. Because MB alone is ineffective against intracellular pathogens, plasma is additionally subject to filtration prior to treatment[80]. Fifteen countries currently use THERAFLEX® MB-Plasma technology[78].

INTERCEPT® Blood System (Psoralen/UVA)

In the INTERCEPT® system, psoralen amotosalen is used in combination with UV light to penetrate the nucleus of pathogens and form non-covalent bonds between pyrimidic bases of DNA or RNA. UV light energy between 320-400 nm (UVA) transforms the non-covalent bond into a covalent bond preventing DNA from being replicated and blocking RNA from being transcribed. The INTERCEPT® Blood System can be used for the treatment of plasma and platelets, but potentially results in the loss of 10% of treated platelets. However, the efficacy of INTERCEPT® at treating platelets has been well documented. Although routinely used against bacteria, enveloped and non-enveloped

viruses and protozoa, the INTERCEPT® Blood System is less effective against some viruses and ineffective against prions. As of July 2013, at least 20 countries have implemented the use of the INTERCEPT® Blood System, including several areas in Europe[81,82]. More specifically, 13 countries currently use the INTERCEPT® System to treat blood plasma and 22 countries use the INTERCEPT® Blood System to treat platelets[78].

Mirasol® PRT system (Riboflavin/UV)

Mirasol® Pathogen Reduction Technology (PRT) is a treatment based on the application of riboflavin (RB), also called vitamin B2, followed by illumination with ultraviolet light (UV) with light energy in the range of 265-370 nm for approximately 5 minutes[2,83]. UV light can activate RB resulting in the formation of free oxygen radicals and selective destruction of the DNA and RNA of bacteria, viruses and other potential blood-borne pathogens[81,84]. It can also inactivate leucocytes with minimal damage to blood products[84]. Because RB is an essential nutrient in blood, and is regarded by regulatory authorities as a "GRAS" (generally regarded as safe) substance[85], it has been thought that there is no need to remove it from the blood after the treatment[2]. Mirasol® PRT is effective in the treatment of plasma, platelets, RBC and whole blood, and has demonstrated the potential to reduce heavily harmful bacterial infections with up to 98% efficacy after transfusion[86,87]. A separate study conducted for the African Investigation of the Mirasol® System (AIMS), determined Mirasol® PRT was significantly effective at reducing transfusion-transmitted malaria in a hospital in sub-Saharan Africa[88]. This technology has been approved for use in Europe and several locations in the Middle East[2,83]. However, it has not yet been approved in the USA or for plasma and platelets. It has not been licensed anywhere for whole blood or RBC.

THERAFLEX® UV-C Technology

For pathogen inactivation, platelet concentrates are suspended in an additive solution and agitated to allow for increased mixing and higher penetration of UVC light[3]. The lack of photoactive chemicals reduces toxicological risks[79]. The THERAFLEX® system has demonstrated efficacy in the inactivation of bacteria, viruses and protozoa. THERAFLEX® UV-treated platelets are currently under phase 3 clinical trials; one concern is that HIV has been shown to be resistant to UVC light, which may be because HIV uses reverse transcriptase for replication and possibly its repair mechanism[3].

Unfortunately, PITs are highly ineffective at removing vCJD disease from blood components[89]. Consequently, there are no licensed PITs for vCJD[90]. Currently, the most successful approach to reducing prions from blood products is by filtration. However, despite the aid of filtration, there are no effective testing procedures to determine residual prion infectivity levels beyond filtration[91]. It should be stressed that no PITs applied to blood components provide absolute guaranteed protection against all blood-borne pathogens, but, in the absence of large pooling, they do contribute substantially to their margin of safety.

Towards pathogen inactivation in RBC and whole blood

For pathogen inactivation in RBC or whole blood, there are two general approaches: 1) whole blood can be fractionated into its components and then PI processes can be applied to the RBC separated from the other components; 2) the PI treatment can be directly applied to the whole blood[62].

Early attempts were made in 2002 to virally-inactivate RBC using PEN 110 (INACTINE) by reacting with nucleic acids of pathogen DNA and RNA without light activation[87,92]. After successfully passing phase I and phase II trials, PEN 110 was halted due to antibody responses during the phase III trial[93]. Although it did not apparently harm erythrocytes, formations of antibodies against RBC did occur in patients receiving PEN 110-treated RBC[87], eventually leading to its discontinuation[93].

Mirasol®

Having already successfully proven its efficacy for pathogen inactivation in platelets and plasma, Mirasol® has recently been developed to treat whole blood[70]. The development and studies on the efficacy of Mirasol® technology had previously completed a phase III clinical trial for the inactivation of malaria in whole blood transfusion in Ghana. The phase III trial indicated clinically significant reduction of malaria in Mirasol®-treated blood samples compared to control on untreated whole blood, as well as a lower incidence of transfusion-related adverse events[88]. According to one report, Mirasol® technology has recently received CE approval for whole blood[94].

Cerus S-303 INTERCEPT® (FRALE)

Designed by Cerus Corporation, the S-303 system was granted its CE mark in 2002[95]. Rather than using UV light, the S-303 system applies a fully chemical approach by utilising the S-303 frangible anchor (mustard hydrochloride moiety) linker (alkyl chain) effector (acridine moiety), also known as the "FRALE" method, to form irreversible crosslinks in DNA, preventing replication and resulting in pathogen inactivation[2,76,95]. The compound S-300 and heteroalkyl compounds are generated as an end product of the S-303 reaction. This procedure incorporates the addition of glutathione to prevent protein damage in the process[80]. Although previous studies did not measure S-300 deposits in the body after transfusion treated with the S-303 FRALE

system, it was believed that short-short term studies on toxicology will not reveal carcinogenicity or other genotoxic consequences[95]. A phase III trial on S-303 conducted over a decade ago demonstrated a significant increase in patients developing constipation. Also, 2 of the 148 subjects developed positive indirect antiglobulin tests[96,97]. This resulted in the S-303 development programme being halted[80]. However, Cerus recently developed a 2nd-generation treatment for RBC using 10 times more glutathione and an adjusted pH, and has recently started recruiting patients for its phase III clinical trial in Europe[70,79,80]. The application of S-303 FRALE toward pathogen inactivation in RBC does not appear to yield antibodies against red cells. However, there is a concern that S-303 FRALE might alkylate proteins, causing neoantigen formation after repeated treatments of patients[87]. More research is needed to determine adverse effects that may include genotoxicology, immunotoxicology and carcinogenicity[95]. Once concerns are addressed, S-303 PI technology has a potential to move toward commercialisation[97].

With the exception of INTERCEPT® technologies and Octaplas S/D, the US Food and Drug Administration (FDA) have not approved most PITs in the USA due to concerns as to whether PIT can modify or degrade transfusion products[80]. To overcome restrictions from the USA, PITs must demonstrate safety as well as direct evidence of effective pathogen elimination. In Europe, most countries allow the implementation of PI technologies with pharmaceutical license or CE mark certification, depending upon the procedure used and pooling. So far, S/D, INTERCEPT®, Mirasol® and THERAFLEX® MB are approved for plasma and INTERCEPT®, Mirasol® and THERAFLEX® UVC have CE mark certification for platelets[76,79,80] (Table I). The CE mark determines the readiness with which a particular technology can be introduced on the market[79]. The Mirasol® system received a CE mark class IIb, which requires self-certification by the manufacturer and INTERCEPT® (amotosalen) system received a CE mark class III, meaning that it requires the clinical data approved by a national authority[79]. In developing countries, blood screening in the presence or absence of PI technology already represents an economic challenge. Currently, many poorer areas are in need of an inexpensive alternative to existing PI technologies.

Cost considerations

The impact of cost on PI technology implementation may dictate how much risk of product contamination certain countries are willing to accept[78]. The financial burden associated with some PI technologies may be considered an unaffordable luxury. Cost concerns with implementing PIT include, in addition to the purchase of equipment and kits, expenses to ensure training on proper maintenance, safe and proper usage as well as quality testing. An anticipated key limitation, for example, involves the cost of combining new technologies with current testing routines. This challenge would be a consequence of the long-standing traditional methods of testing that blood

Table I - Current pathogen inactivation technologies, mechanisms, applications and licensing status.

Manufacturer	Technology	Key mechanisms	Transfusion components	Licensing	Countries
Cerus	INTERCEPT®	Amotosalen + UVA Light (320-400 nm)	Platelets (apheresis or whole blood-derived)	CE marked (class III) 2002	22
	INTERCEPT®	Amotosalen + UVA Light (320-400 nm)	Plasma (apheresis or whole blood-derived)	CE marked (class III) 2006	13
	INTERCEPT®	S-303 (FRALE)	Red cells	n/a	-
	INTERCEPT®	S-303 (FRALE)	Whole blood	n/a	-
Terumo BCT	Mirasol®	Riboflavin + UVB Light (280-360 nm)	Platelets (apheresis or whole blood-derived)	CE marked (class IIB) 2007	18
	Mirasol®	Riboflavin + UVB Light (280-360 nm)	Plasma (apheresis or whole blood-derived)	CE marked (class IIB) 2008	11
	Mirasol®	Riboflavin + UVB Light (280-360 nm)	Whole blood	CE marked 2015	-
Macopharma	THERAFLEX®	UVC light	Platelets	CE marked (class IIB) 2009	-
	THERAFLEX®	Filtration + Methylene Blue + visible light (400-700 nm)	Fresh frozen plasma (apheresis or whole blood-derived)	CE marked (class III) 2004	15
Octapharma	Octaplas (S/D)	Solvent/Detergent	Large-pool of plasma (apheresis or whole blood-derived)	Licensed (in UK) 1998	32
VIPS	n/a	Solvent/Detergent	Single donation or mini-pool of plasma (apheresis or whole blood-derived)	CE marked 2009	At least 3
Vitex	INACTINE	PEN 110	Red cells	n/a	-

n/a: not available; UVA/B/C: ultraviolet A/B/C.

bank workers are accustomed to[95]. Opportunity cost may be a large factor in the decision-making process, taking into consideration that PIT may be used to inactivate rather than test for emerging infectious agents. It is important to note that the cost of opting to use PI technology may, therefore, reduce current expenses associated with donor screening procedures, risk-based decisions, and costs associated with treating TTI-affected patients. One report claims that the World Bank estimated that a $ 26 million public health investment could be enough to prevent 90% of Ebola cases in West Africa. A worthy challenge for researchers would be to merge all existing data investigating costs and risks to establish a model for acceptable risk. Missing the opportunity to do this led to expenses of $ 1.6 billion for emergency responses combating Ebola outbreaks in the region[78]. Although this particular instance was unrelated to transfusion, it serves as an indication of the potential increased expense related to the failure of investing in medical technology and knowledge directed at preventing the spread of disease.

Although PI technology is known to be expensive, there have been few studies that directly compare cost and benefit among the various PI technology options[97].

Conclusions

Efforts to address the spread of frequent and serious transfusion-transmitted reactions of the 20th century have led to strict regulations and guidelines being put in place, resulting in a 1,000-fold decrease in the transfusion-transmission of infections such as HIV, HBV and HCV[98]. The associated risks for RBC and whole blood could be significantly further reduced with the approval of adequate PI technology. Before wide scale implementation of PI technology for RBC and whole blood, that technology must be proven to be safe, cost-effective, and efficient at inactivating pathogens[2]. Currently, clinical trials and policies dictating the application of PI technology may act as a barrier to the use of PI systems in some countries. The impact of global warming may have a very serious impact on the necessity to implement effective PI technology. As temperatures rise, the transmission of vector-borne pathogens may increase dramatically. This may lead to an increase in the transmission of diseases like WNV, DENV, ZIKV, CHIKV and others. As a direct consequence, the implementation of PI technology may be an increasingly attractive proposition[97].

There have been tremendous advances made towards the safety of blood transfusion over the past few decades as a result of the technologies developed to target immunological and infectious risks. Many of the existing PI systems are easy to apply and take only a matter of minutes[80]. There has not yet been a consensus on the most effective practices in the treatment and management of blood among Blood Establishments, although conferences may promote future agreements[99]. Viral inactivation for plasma products is almost a universal standard, but as of today, there are still no universal PI technologies applicable to all transfusion products, although some are promising. Also, every current PI technology has difficulty dealing with spore-forming bacteria in platelet concentrates[71]. A separate challenge is that blood transfusions are regarded as expensive treatments among less wealthy countries[80,99]. Infrastructure would add to the investment challenge for developing countries intending to incorporate the newest PI technology[80]. Therefore, options that may be ideal for a wealthy country may not be as desirable for a lower income country. Cost is regarded as an important factor in determining the most preferred solution in blood treatment processes. An ideal PI system would be affordable for developing countries, effective at killing a wide range of pathogens, and have no toxicity or carcinogenicity[76]. Authorities on the topic of blood safety are tasked with finding a balance between affordable, effective PI technology and acceptable quality standards of blood products. Potential benefits of such a system include reducing the risk of current TTIs to zero, eliminating "blind spot" areas of modern testing procedures, and wiping out the need for donor screening questions[62]. The previous studies have been encouraging indicators of the significant progress in pathogen inactivation and reduced risk of whole blood products possible in the near future.

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