Past, present and forecast of transfusion medicine: What has changed and what is expected to change?

Amy E. Schmidt, Majed A. Refaai, Neil Blumberg

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University of Rochester medical center, department of pathology and laboratory medicine, 14642 Rochester, NY, USA

Correspondence:
Neil Blumberg, university of Rochester medical center, department of pathology and laboratory medicine, 14642 Rochester, NY, USA.
neil_blumberg@urmc.rochester.edu

Summary

Blood transfusion is the second most used medical procedures in health care systems worldwide. Over the last few decades, significant changes have been evolved in transfusion medicine practices. These changes were mainly needed to increase safety, efficacy, and availability of blood products as well as reduce recipients’ unnecessary exposure to allogeneic blood. Blood products collection, processing, and storage as well as transfusion practices throughout all patient populations were the main stream of these changes. Health care systems across the world have adopted some or most of these changes to reduce transfusion risks, to improve overall patients’ outcome, and to reduce health care costs. In this article, we are going to present and discuss some of these recent modifications and their impact on patients’ safety.

Introduction

The field of transfusion medicine has changed greatly over the last century. In 1900, Dr. Karl Landsteiner discovered that the blood of two people agglutinates when combined. He then went on and discovered the blood groups A, B, and O, which he labelled initially as group C. Later on, he discovered that blood transfusion between people of the same blood group did not lead to blood cell destruction whereas blood cells were destroyed when blood was transfused between people with different blood groups. In 1907, Dr. Ottenberg performed the first successful blood transfusion at Mount Sinai Hospital in New York. Nowadays, blood transfusion is one of the most frequently performed procedures in hospitals. Additionally, most blood transfusions performed now are actually component therapy: red blood cells (RBCs), platelets, plasma, and cryoprecipitate. Over the last few decades, the field of transfusion medicine has jumped to the forefront of medicine with areas such as massive transfusion protocols, blood management, and cellular therapies rapidly evolving. This article will address these and other current topics in transfusion medicine and examine them from a historical perspective to understand how each area has changed and what changes to expect in the future.
**Universal leukoreduction**

White blood cells (WBCs) in blood products are an important cause of transfusion complications, such as febrile nonhemolytic transfusion reactions (FNHTR). The rate of WBC derived pro-inflammatory cytokines increases over the storage period [1-4]. Additionally, the transfused WBCs may be the target of recipient derived antibodies that can stimulate the WBCs to release cytokines. WBCs contain class I HLA antigens that sensitize patients and cause them to become platelet refractory. WBCs may also contain cytomegalovirus (CMV) and are associated with increased incidence of transfusion-transmitted CMV.

Universal leukoreduction (ULR) has been shown to have several benefits including reduction of FNHTRs [5-7], decreased platelet refractoriness caused by alloimmunization against leukocyte antigens [8-10], and decreased transmission of CMV [11,12]. Two forms of ULR were clinically adopted, prestorage and post-storage leukoreduction. The former has been shown to be advantageous as compared to the later form, which has been reported to result in leukoreduction failures in more than 5% of the time [13-15]. In addition, higher concentrations of WBC fragments and microparticles, which accumulate during storage and may not be removed by the standard filtration, were associated with post-storage leukoreduction [16].

Observational studies in critically ill patients and randomized trials in cardiac surgery of leukoreduced transfusions showed a decreased mortality as compared to non-leukoreduced transfusions [17,18]. Additional prospective and retrospective studies found that prestorage leukoreduction of RBCs abrogated detrimental effects (increased likelihood of multi-organ dysfunction syndrome (MODS) and increased risk of infection) seen with non-leukoreduced blood [19,20].

ULR has also been studied as a means to reduce transmission of variant Creutzfeldt-Jakob disease (vCJD). Viable leukocytes have been shown to very efficiently transmit prion disease [21,22]. Animal models of spongiform encephalopathy have shown that leukoreduction effectively reduces transmission via blood transfusion [21,23]. In the UK, 4 cases of blood-born vCJD have been reported in patients who received non-leukoreduced RBCs [24-27]. However, since implementation of ULR in Europe, there have been no cases of transfusion acquired vCJD [28]. This should be tempered by the fact that a large portion of blood transfusion recipients do not live long enough to develop vCJD. Although ULR reduces the risk of prion transmitted disease, it does not completely eliminate it. A new bifunctional filter (Sepacell Prima, Asahi Kasei Medical) has been developed that is comparable to current leukoreduction filters but also effectively reduces exogenous prion proteins by greater than 3 logs [29]. Thus, additional procedures such as treatment with a bifunctional filter or an affinity ligand gel to prions as is done in Octaplas LG (Octapharma, Lachen, Switzerland), which is described in a later section, are most likely to be implemented in the future, particularly in the UK where there is believed to be a high potential subclinical vCJD prevalence in potential blood donors [30].
ULR has also been found to affect microparticles (MPs). MPs are small vesicles that are < 1 μm that resemble their parent cell in terms of similar surface proteins and membrane lipids. Interestingly, all blood cells can release MPs. The most plentiful MPs in the blood are platelet MPs (PMPs). PMPs have been found to have 50- to 100-fold higher procoagulant activity than activated whole platelets [31]. Chan and Sparrow examined the effect of whole blood hold-time and leukoreduction on MPs as well as on the hemostatic potential of fresh frozen plasma (FFP) [32]. They found that leukoreduced-FFP had fewer MPs overall as well as less PMPs and less phosphatidylserine (PS) expressing MPs. They also found that leukoreduced-FFP had lower maximum amplitude (MA) and slower clot formation time (R time) as assessed by thromboelastography (TEG) [32]. Silliman et al. examined the effect of leukoreduction of plasma and RBCs from donors known to have human leukocyte antigen (HLA) antibodies and human neutrophil antigen (HNA) antibodies on levels of immunoglobulins, levels of HLA antibodies, neutrophil priming activity, and transfusion related adverse events (TRALI) induction in an animal model [33]. They found that filtration of the plasma with a standard leukoreduction filter removed > 96 % of IgG, HLA antibodies, and HNA antibodies. It also significantly decreased neutrophil priming activity and mitigated TRALI induction [33]. Similarly, filtration of RBCs resulted in removal of HLA antibodies as well as inhibited lipid priming activity and lipid mediated TRALI [33]. Notably, following implementation of ULR, Blumberg et al. reported a decrease in TRALI events seen at a large medical center [34]. Thus, leukoreduction appears to play a role in mitigating TRALI risk; however, for unknown reasons this effect is not detectable on TRALI incidence worldwide [35].

ULR has also been shown to decrease the risk of transfusion related immunomodulation (TRIM) [36]. Animal and human studies have shown that allogeneic blood products cause down regulation of cellular immunity and dysregulation of inflammatory innate immunity [37]. Despite ULR, RBC and platelet products accumulate what is referred to as the "storage lesion" as well as release lipid mediators and MPs as they age. The "storage lesion" and these age related changes are discussed in great detail in the "Age of Blood" section. It is possible that the storage supernatant and/or the actual aged cells themselves are causing TRIM. Most data suggests that the storage supernatant is a large contributor. A randomized trial of adults with leukemia has shown that patients receiving washed, leukoreduced, ABO identical products had substantially improved survival as compared to patients receiving unwashed, leukoreduced, ABO identical products [38,39]. Additionally, Cholette et al. found that children undergoing cardiac surgery who received washed, leukoreduced blood products had lower levels of inflammatory markers when compared to children receiving unwashed, leukoreduced products [40]. Washing of blood products would also be expected to dramatically reduce the incidence of TRALI. Thus, further experiments and clinical trials are needed to explore the role, feasibility, and cost of washed blood products in reducing TRIM and TRALI as well as assessing the role of washing in reducing overall patient morbidity and mortality.

ULR of all cellular blood products was implemented in the UK in 1999 and in Europe and Canada after 2000. ULR was started in Japan in 2004 and completed by 2007 [9]. However, ULR has not yet been fully implemented in the US as some hospitals and transfusion centers still use non-leukoreduced products. It is predicted that prestorage ULR will be implemented in 100 % of donor centers and that only ULR products will be available in the US as the benefits are clear. ULR is also expected to be implemented in many other countries as costs allow. Unfortunately, the additional costs of leukoreduction are prohibitive of its implementation in many places.

**The age of blood**

Depending upon the storage solution used, RBCs can be stored for up to 42 days. As blood ages, RBCs are said to develop a "storage lesion" which includes leakage of potassium and chloride from the RBCs, depletion of 2,3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP), loss of phospholipids and cholesterol as well as exposure of phosphatidylserine (PS), elaboration of lipid mediators, loss of glutathione (GSH), autooxidation of hemoglobin to methemoglobin, decreased blood flow viscosity and adherence to endothelial cells, increased microparticle formation, and disruption of NO-mediated vasodilation [41-47]. Oxidative stress is thought to be a major driving force in development of the storage lesion, which is characterized by functional and structural changes to the RBCs [48-51]. The effects of RBC storage lesion on patient morbidity and mortality have been studied vigorously with mixed results [52].

A variety of retrospective observational studies associated older RBC transfusion with nosocomial infection, increased LOS, increased days on mechanical ventilation, increased risk of multiple organ failure (MOF), renal failure, and increased mortality [53,54]. However, up until now, there is no conclusive evidence supporting transfusion of fresh RBCs. In 2010, Eikelboom et al. reported an association between transfusion of older RBCs and in-hospital mortality in cardiovascular patients between 2002 and 2006 [55]. They expanded their cohort of cardiovascular patients to include patients from 2002-2011 and found no association between age of transfused RBCs and inhospital mortality [56]. Spinella et al. found that trauma patients transfused with older RBCs (> 28 days) had an increased incidence of deep vein thrombosis (DVT) as well as increased incidence of death from multi-organ failure in addition to an independent association with mortality [57]. However, a later study by Katsios et al. found no association between the age of RBCs and DVT in medical and surgical intensive care unit (ICU) patients [58]. Similarly, Dunn et al. performed a retrospective
observational study of the effect of RBC age on orthotopic liver transplant outcome and found that the age of the blood was not associated with organ rejection, infection or mortality; however they did find that the number of RBCs administered was associated with increased 2-year mortality [59]. Later, Cywinski et al. performed another retrospective observational analysis of the effect of RBC age on graft survival in orthotopic liver transplant patients and found that patients who received intraoperative transfusions of older (> 15 days) RBCs had decreased graft survival [60].

Yet another retrospective study looked at the effect of age of RBCs on cancer recurrence and overall survival (OS). They compared fresh RBCs (< 14 days) to intermediate RBCs (14-28 days) to old RBCs (> 28 days) and found that the age of blood had no effect on cancer recurrence or OS [61]. Lastly, Manhiot et al. studied the effect of age of RBCs on pediatric cardiac surgeries and found that transfusion of older blood to patients who received high transfusion volumes was associated with increased risk of bleeding complications, renal impairment, higher inotrope score after surgery, increased chest tube drainage, prolonged hospitalization, and increased un-hospital mortality [62]. A study in adult patients undergoing off-pump coronary artery bypass surgery found that transfusion of older blood was associated with increased wound complications and length of stay [63]. Other studies looking at the effect of blood age in adult coronary artery bypass graft (CABG) surgery patients found that the age of the RBCs did not affect the use of inotropes, LOS, or mortality [64,65]. Thus, there is a plethora of contradictory data regarding the age of blood.

In an attempt to bring clarity, three large clinical trials investigating the age of blood and its effects on recipient health were undertaken; ABLE, RECESS, and ARIPI. The Age of Blood Evaluation (ABLE) study, which was a randomized, blinded trial conducted at 64 locations in Canada and Europe, looked at 90-day mortality in critically ill patients receiving fresh RBCs (< 8 days old) versus older blood (oldest unit available in blood bank, mean = 22 days old). They found essentially no difference in 90-day mortality between the two groups [66]. The Red-Cell Storage Duration Study (RECESS), a randomized, blinded trial conducted at multiple centers across the US examined clinical outcomes following cardiac surgery in patients (> 12 years old) who received RBCs < 10 days old versus older RBCs (> 21 days old). Notably, they did not observe any statistically significant differences in Multiple Organ Dysfunction Scores (MODS), 7-day or 28-day mortality [67]. The Age of Red Blood Cells in Premature Infants (ARIPI) trial examined length of stay, nosocomial infections, and organ dysfunction in premature infants weighing < 1250 g who received fresh blood (< 7 days) versus standard issued blood (mean: 14.6 days). This study found no statistically significant differences in the measured outcomes based upon the age of the blood [68].

These three large trials support that RBC age has little to no effect on outcome. However, it is likely that studies investigating the effect of RBC age on various conditions and surgeries will continue to appear in the literature for years to come and this debate will continue for several more years. The most likely outcome is that RBCs of all ages will continue to be used for all patient populations. It is more likely that better storage solutions and additives will be implemented to reduce the storage lesion. Moreover, rejuvenation of older RBCs prior to release for transfusion may become a routine procedure (see Rejuvenation of RBCs section).

Rejuvenation of RBCs

RBCs with decreased 2,3-DPG result in hemoglobin with an oxygen dissociation curve shifted to the left and increased affinity for oxygen. This leads to decreased oxygen delivery to tissues. Notably, during storage for 35 days, the 2,3-DPG concentration was found to decrease from 13.2 μmol/g Hb to 0.7 μmol/g Hb [69-71] and after storage for 42 days, the ATP concentration was found to decrease from 4 μmol/g Hb to 2 μmol/g Hb or less [72]. Stored RBCs have also been found to have increased adherence to blood vessel wall endothelial cells which can be problematic by impeding blood flow [73-77]. This is particularly important in infants and patients with sickle cell anemia. Neuman et al. compared brachial artery flow-mediated dilation in patients receiving fresh (< 14 days old) RBCs to old (> 21 days old) RBCs. They found that transfusion of older RBCs resulted in a statistically significant decrease in NO-mediated vasodilation [78]. Notably, they also observed decreases in 2,3-DPG levels in patients transfused with older RBCs. Additional studies are needed to confirm this finding.

Following RBC transfusion, approximately 50 % of the depleted 2,3-DPG is restored within 7 hours [79]. In several studies, RBC in vivo recovery has also been linked to ATP levels [72,80]. Rejuvesol (Cytosol Laboratories, Braintree, MA), which contains sodium pyruvate, inosine, adenine, dibasic sodium phosphate, and monobasic sodium phosphate, has been shown to restore both 2,3-DPG and ATP levels in RBCs stored for up to 120 days [81,82]. In addition to Rejuvesol, a new additive solution (AS) is also being tested. The new AS is called phosphate-adenine-glucose-guanosine-glucanate-mannitol (PAGGM). Notably, PAGGM is more alkaline, has decreased osmolarity, and no chloride as compared to normal AS’s. PAGGM differs from the currently available AS in that it is able to maintain the 2,3-DPG and ATP levels in RBCs stored for 35 days [83]. PAGGM is believed to exert its effect via increasing phosphofructokinase activity [84].

Microcirculatory disorders associated with diseases such as sickle cell anemia and thalassemia have been attributed in part to increased RBC adherence to endothelial cells [85,86]. The increased adherence to endothelial cells is thought to be driven in part by oxidative stress, which causes numerous changes in
RBCs including translocation of PS to the surface of the RBCs. The presence of PS on the surface of the transfused RBCs has been shown to enhance the adherence of RBCs to endothelial cells [77]. Notably, prestorage leukoreduction has been shown to reduce translocation of PS in stored RBCs [87]. Unfortunately, as discussed previously, ULR is not yet standard in the USA. Koshkaryev et al. found that rejuvenation of stored RBCs reversed accumulation of reactive oxygen species, calcium and PS and suppressed the enhanced RBC adhesion to endothelial cells [88]. In another study, Barshtein et al. found that post-storage rejuvenation of non-leukoreduced RBCs only reversed 40–70 % of the RBC alterations with rejuvenation having a decreased effect as RBC alterations worsened [89]. Storage-induced RBC damage is not only affected by the length of storage but also by the storage media and leukoreduction. Thus, further studies are needed to clarify if alterations of prestorage leukoreduced RBCs in AS can be fully restored with rejuvenation. Rejuvenation with Rejuvesol and use of PAGGGM are both being evaluated for use in sickle cell patients as well as other patients with microcirculatory disorders in which increased RBC adherence to endothelial cells could be problematic. Additionally, use of PAGGGM and/or Rejuvesol RBCs for these and other patients may be beneficial due to the increased 2,3-DPG stores and ability to deliver oxygen to tissues. Use of these modalities and/or newly developed alternatives may be the wave of the future in helping improve oxygen delivery and decrease sluggish flow caused by stored RBCs in at risk populations.

**Trauma and massive transfusion**

Injury is the leading cause of death in people 1–44 years old in the US [90]. Notably 20–40 % of trauma deaths that occur after hospital admission involve massive bleeding [91]. Hemorrhage is the second most common cause of early in-hospital mortality accounting for a large portion of trauma deaths that occur within the first 24 hours [92]. Resuscitation has dramatically changed over the last decade or so. The early administration of blood products during resuscitation is referred to as damage control resuscitation (DCR) [93]. The goal of DCR is to prevent and immediately correct trauma associated coagulopathy. DCR was initially practiced in the military where a balanced ratio of FFP:RBC:platelet of 1:1:1 was employed. DCR has resulted in improved outcomes in both the military as well as civilian trauma setting as compared to previous resuscitation patterns [94-100]. Despite the early use of a 1:1:1 ratio, a 2:1:1 ratio also became commonly used. Thus, the Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPR) study was done to investigate the best ratio of products to use during resuscitation. This study found no differences in mortality between the 1:1:1 and the 2:1:2 groups at 24 hours or at 30 days [101]. Additionally, there was no difference in complications between the two groups. Significantly, less exanguination was observed in the 1:1:1 group and more patients reached hemostasis [101].

The Prospective Observational Multicenter Major Trauma Transfusion (PROMMTT) study showed that early transfusion (within minutes of arriving at hospital) was associated with improved 6-hour survival [97,100]. Additionally, patients with increased plasma to RBC ratios (> 1:2) were found to have improved 30-day survival as compared to patients who received lower plasma to RBC ratios (< 1:2) [102-105]. Notably, 1-day and 30-day survivals were found to be increased when patients received higher ratios of platelets to RBCs [95,106,107]. Delayed but balanced transfusion ratios did not have the same protective effect as receiving plasma early [108].

Several studies have shown that warm fresh whole blood is more efficacious than using component therapies in a 1:1:1 ratio [66,107,109]. Patients with massive bleeding who receive fresh whole blood have improved survival and reduced complications due to storage effects. However, there are several difficulties in obtaining fresh whole blood. Most blood centers do not stock fresh whole blood and thus it is not readily available at trauma centers. Additionally, it is difficult to properly screen fresh whole blood for infectious diseases and still have it available for use in trauma situations. In the future, fresh whole blood may be able to be used if rapid and cost effective infectious disease screening tests become available.

In the PROMMTT study, patient were found to have increased levels of MPs derived from endothelial cells, RBCs, and leukocytes as well as increased levels of tissue factor bearing MPs (TFMPs) [110]. Notably, coagulopathic trauma patients were found to have much lower levels of PMPs, TFMPs, and thrombin generation in addition to more bleeding and increased mortality [110]. Thus, it is likely that PMPs are important in TAC.

To further understand the role of PMPs, Matijevic et al. examined PMPs in plasma. They compared thawed plasma at day 5 to freshly thawed FFP at day 0. They found that the majority of MPs were indeed PMPs and that day 5 plasma had a 50 % reduction in MPs and a 29 % decrease in procoagulant activity [111]. A recent prospective observational study of trauma patients found that patients with low levels of phosphatidylserine (PS) positive PMPs had impaired clot formation and were more likely to receive more RBC transfusions during the first 24 hours following injury [112]. These studies suggest that PMPs may play an important previously overlooked role in TAC. Further studies are needed to further evaluate the role of PMPs in TAC and in the future, PMP administration early in the resuscitation may improve morbidity and mortality.

Hyperfibrinolysis has been shown to be present in a significant number of trauma patients and may contribute to mortality [113]. The Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage (CRASH-2) study showed that early administration of tranexamic acid (TXA) decreased the overall mortality in trauma patients (4.9 % TXA versus 5.7 % placebo; \( P = 0.0077 \)) as well as the risk of death by hemorrhage [114,115]. Similarly, the Military Application of TXA in Trauma
Emergency Resuscitation (MATTERs) study found that TXA administration reduced overall mortality by 6.5% as compared to placebo [116]. Following these studies, most major trauma centers have instituted the use of TXA in trauma patients within the first 3 hours following injury. The CRASH-2 study found that administration of TXA after 3 hours was associated in an increased risk of thrombosis [115] and the MATTERs study found that pulmonary embolism (PE) and deep vein thrombosis (DVT) were 9 and 12 times more frequent in patients receiving TXA [116]. Currently, the Pre-hospital Antifibrinolytics for Traumatic Coagulopathy and Haemorrhage (PATCH)-Trauma study is ongoing to investigate the safety and efficacy of TXA administration [117]. Additional studies are ongoing to develop drugs that can be paired with TXA and administered to trauma patients as part of a “cocktail” to prevent, control, and/or treat TAC. Trauma patients with low fibrinogen have been shown to have increased blood loss, increased transfusion needs, and poor outcome [118-120]. Notably, European Trauma Guidelines recommend treatment with fibrinogen concentrate or cryoprecipitate if significant bleeding is present along with thromboelastographic signs of a fibrinogen deficit or a fibrinogen level < 1.5-2 g/L [121]. In Europe, fibrinogen concentrates are more commonly used during trauma than in the US, this is likely due to the high cost of fibrinogen concentrate in the US. Several studies have shown an association between administration of fibrinogen/fibrinogen concentrates and improved outcome [104,122]. Thus, additional studies are needed to clarify the use of cryoprecipitate/fibrinogen concentrates.

In conclusion, the trauma field is rapidly evolving particularly as it relates to resuscitation and transfusion medicine plays an important role. Hospitals of all sizes are instituting MTPs; however, these protocols are not standardized and a more homogenous ratio of blood products is needed. Additionally, it is possible that fibrinogen concentrates will become more prevalently used in the US. Lastly, it is quite likely that we will see the creation and implementation of a “cocktail” of drugs that will be administered early on to trauma patients to control TAC.

**Blood management programs**

Blood management programs have sprung up at medical centers across the US. The goals of these programs are two-fold: (1) to improve patient safety and outcomes and (2) to reduce blood transfusion and health care costs. Based upon a variety of studies, restrictive transfusion parameters have been deemed safer and preferred over liberal transfusion parameters. Restrictive transfusion parameters have been found to be safe in nearly all clinical situations [123,124].

One of the largest trials looking at transfusion triggers was the Transfusion Requirements in Critical Care (TRICC) trial. These investigators found that maintenance of hemoglobin between 7 and 9 g/dL was at least equivalent and in some instances superior to liberal transfusion to maintain hemoglobin >10 g/dL [125]. Higher hemoglobin concentrations were only required in patients with cardiac disease [125]. Another study found that critically ill patients can tolerate hemoglobin levels of 7 g/dL and that liberal transfusion strategies are associated with worse clinical outcomes [126]. In the Transfusion Trigger Trial for Functional Outcomes in Cardiovascular Patients Undergoing Surgical Hip Fracture Repair (FOCUS) study, investigators examined whether liberal transfusion (maintain hemoglobin > 10 g/dL) as compared to restrictive transfusion (transfuse at hemoglobin < 8 g/dL) had any effect on recovery or mortality [127,128]. Notably, they found that liberal transfusion as compared to restrictive had essentially no effect on the patient’s recovery or long-term mortality [127,128]. Additional studies by Hardy et al. also found that liberal transfusion is associated with worse outcome in critically ill patients [129]. The CRIT (Anemia and Blood Transfusion in the Critically Ill) study is a large prospective observational study that looked at transfusion practices in critically ill patients. This study found that the number of RBC transfusions is independently associated with increased length of stay (LOS) and increased mortality [130]. More complications were also observed in transfused patients [130].

Currently, most blood management programs use a transfusion trigger of 7 g/dL for the majority of patients. However, there is still quite a lot of debate of what transfusion trigger to use in patients with cardiac disease. A recent pilot study looked at liberal transfusion to keep hemoglobin > 10 g/dL versus restrictive transfusion at hemoglobin < 8 g/dL in patients with acute coronary syndrome or stable angina undergoing cardiac catheterization and found that liberal transfusion was associated with a trend toward less cardiac events and decreased deaths [131]. Larger randomized controlled trials are needed to clarify transfusion triggers in this patient population.

Transfusion has also been associated with increased risk of infection. Trauma, burn, and surgery patients have been shown to have an increased risk of infection associated with number of RBC transfusions [132,133]. This is thought to be attributable to TRIM. The worse outcome in patients receiving liberal transfusion may be due to non-leukoreduced products, “storage lesion”, and/or cytokine and microparticle accumulation over time during product storage. Further studies are needed to ascertain this further; however, it is likely a combination of these factors. What is conclusive is that liberal transfusion in adults is not optimal for most clinical situations. Blood management in pediatric populations is more difficult as the literature is not as clear about transfusion triggers. Kneebier et al. found that RBC transfusion was independently associated with risk of death in pediatric intensive care unit (PICU) patients [134]. In a retrospective cohort study, Stone et al. found that pediatric trauma patients receiving RBCs within 24 hours of admission had increased LOS as well as increased mortality [135]. Notably, a confounding factor in both these studies was that patients receiving RBC transfusions were sicker than...
those not receiving transfusions. When differences in illness severity were controlled in another study, Bateman et al. reported that RBC transfusion was associated with increased risk of death, cardiac arrest, nosocomial infection, longer PICU stay, and increased time on ventilator [136]. Similarly to what was found in adults, Jeschke et al. found that increased transfusion number was associated with increased risk of sepsis in pediatric burn patients [137]. Thus, transfusion in pediatric populations is also associated with less favorable outcome, but transfusion triggers are vague and clinical practice varies quite widely [138]. The Transfusion Requirements in Pediatric Intensive Care Units study tried to address transfusion triggers in PICU patients. It looked at 28-day mortality, development of MODS, progression of MODS, ventilator days, adverse events, and nosocomial infections in children receiving liberal RBC transfusion (hemoglobin < 9.5 g/dL) versus restrictive transfusion (hemoglobin < 7 g/dL) [139]. No significant differences were observed in the measured outcomes between the liberal and the restrictive group [139]. Subgroup analysis of pediatric cardiology and general surgery patients showed no increase in MODS in the restrictive group [140,141]. Thus, evidence suggests that a transfusion trigger of 7 g/dL could be used in PICU patients. However, a large, randomized multicenter clinical trial is needed to strengthen and clarify this data.

Restrictive and liberal transfusion parameters have also been studied in neonatal populations. One of the first studies in very low birth weight infants (VLBW) was published by Bell et al. in 2005. They studied 100 VLBW infants (500–1300 g) and found that infants in the restrictive group were more likely to have intraparenchymal brain hemorrhage or periventricular leukomalacia and had more frequent apnea episodes [142]. These children were then followed for 8–15 years and assessed for psychological outcomes [143]. The liberal transfusion group showed less favorable outcome and had decreased intracranial volume as assessed by brain MRI studies [143]. Another study by Kirpalani et al., known as the Premature Infants in Need of Transfusion (PINT) study, looked at the effect of restrictive versus liberal transfusion strategies in 451 extremely low birth weight infants (ELBW) (< 1000 g). Restrictive transfusion parameters showed no significant differences in morbidity or mortality [144]. The infants in this study were followed for 18–21 months and assessed for differences in death or adverse neurodevelopmental outcomes. Similar to previous findings, restrictive transfusion parameters were not found to have a statistically significant effect on death or neurodevelopmental outcomes [145]. Notably, adverse outcomes were more prevalent in the restrictive group infants [145].

The paucity of conclusive evidence for the best transfusion practice in pediatrics and neonates complicates blood management in these populations. This has resulted in a variety of transfusion practices by neonatologists [146,147]. Thus, quite frequently, blood management programs do not have strict guidelines for pediatrics and neonates as in the adult populations. Additional large, randomized, multicenter trials are needed with a long-term follow-up to clarify whether a restrictive or liberal transfusion approach is superior in neonates. The effect of blood management programs can be quite impressive. A recent paper from Goodnough et al. compared the clinical outcomes 3 years before and 3 years after institution of a best practice trigger alert and clinical decision support at physician order entry [148]. The best practice alert was set to trigger if the patients’ most recent hemoglobin level was > 7 g/dL at the time of transfusion request. They reported a hospital wide improved clinical outcomes and significant decrease in mortality rate (from 55.2 to 33.0; P < 0.001), LOS (mean of 10.1 to 6.2 days; P < 0.001), and 30-day readmission rate (136.9 to 85.0 days; P < 0.001) among transfused patients [148]. Moreover, the mean number of RBCs transfused decreased from 3.6 to 2.7 per patient, with a 24 % decrease in RBC use between 2009 and 2013; an estimation of an annual net savings of $1.62 million [148].

Blood management programs are likely to become more widespread and predominant in patient care as further evidence about transfusion-associated adverse events are evolving. However, restrictive transfusion triggers in adults vary widely between practices and patient populations. In a recent survey performed by Sim et al. about transfusion triggers among acute care surgeons, 7 g/dL was used as a transfusion trigger in 45 % of GI bleeding, 75 % of penetrating trauma, 66 % of sepsis, and 62 % of blunt trauma [149]. Therefore, blood management programs should strive to educate practicing clinicians about possible harms of blood transfusion and work with them to reach a greater consensus regarding transfusion practices within each clinical field.

Pathogen inactivation systems
Donor screening has been effective in reducing the transfusion-transmitted infectious diseases; however, it has not been able to eliminate it. In addition, there will always be new viruses, bacteria, and parasites that we are unaware of at present and have no screening tools for. It is also not feasible logistically or monetarily to test donors for every disease that can be transfusion-transmitted. Moreover, in those diseases that are tested for, there is likely to always be a window between infection and test positivity. With all these caveats, pathogen reduction systems have become an important area of development and investigation. Pathogen reduction systems have already been applied to plasma fractionation where heat inactivation, solvent/detergent treatment, and ultrafiltration are employed. However, these approaches cannot be used for cell-based products such as platelets and RBCs. Importantly, even with using pathogen inactivation systems, the risk of infectious disease transmittance is not zero. Current pathogen inactivation systems available and/or being studied for
individual plasma units are: psorales with ultraviolet (UV) light, riboflavin with UV light, methylene blue with visible light, and solvent/detergent. Current pathogen reduction systems available and/or being studied for platelet units are: psorales with UV light, riboflavin with UV light, and UV light alone. Platelets treated with pathogen inactivation have an extended shelf life from 5 to 7 days. Current pathogen reduction systems available and/or being studied for RBCs are: riboflavin with UV light and frangible nucleic acid crosslinkers. The INTERCEPT Blood System (Cerus Corporation, Concord, CA) is one of the only pathogen inactivation systems FDA approved for use in the United States. This system is currently being used in more than 20 other countries [150]. INTERCEPT system uses psorales amotosalen and UVA light and can be used on platelets and plasma products. The amotosalen passes through cellular membranes and intercalates into DNA and RNA; UVA light exposure results in cross-linking of DNA and RNA [151]. Notably, the amotosalen is recaptured which takes between 6-16 hours and only small residual amounts of amotosalen remain [152,153]. Psorales and UV light can inactivate enveloped and nonenveloped viruses, bacteria, protozoa, and leukocytes [151]. This system has been shown to reduce the infectivity of most pathogens tested by 4- to 6-fold log [154-157]. Studies have also shown that the INTERCEPT system results in a greater log reduction in infectivity as compared to the other pathogen inactivation systems [158]. Notably, side effects such as urticaria, platelet activation, and decreased platelet corrected count increment have also been observed with INTERCEPT [159,160]. However, a hemovigilance program in France, Spain, and Belgium studied 7437 platelet transfusions treated with INTERCEPT and found that the rate of undesired events was 0.9 % without instances of bacterial contamination [161].

Another well-studied pathogen inactivation system is Mirasol PRT (Terumo BCT, Lakewood, CO). This system uses riboflavin, which acts as a photosensitizer, to mediate selective damage to nucleic acids upon exposure to UVA/UVB light [162]. Mirasol PRT can be used on both platelets and plasma products. Studies performed using Mirasol both in vitro as well as in animals have shown log/mL. reductions of ≥ 4 for Leishmania donovani infantum and Babesia microti, ≥ 5 for T. cruzi, ≥ 5.1 for West Nile virus (WNV), and 4.5–5.9 for human immunodeficiency virus (HIV) [163]. Mirasol has been effective in reducing the infectious load of both enveloped and nonenveloped viruses. Notably, it has been shown to effect a 1.8 log/mL decrease in hepatitis A virus (HAV), which is very resistant to heat inactivation and other chemicals [163]. Mirasol has also been shown to effectively inactivate numerous strains of both gram positive and gram negative bacteria [163]. In the MIRACLE study, patients who received platelets treated with Mirasol had much lower corrected count increments (CCI) at 1 hour as compared to patients who received standard platelets [164]. Unlike INTERCEPT, Mirasol does not have to be recaptured. Mirasol is not FDA approved for use in the United States; however, it is used in greater than 15 countries [150].

Theraflex-UV (Macopharma, Tourcoing, France) uses UVC light, which acts directly on DNA to cause pyrimidine dimers and impair DNA replication [165,166]. This system can be used on both platelets and plasma products. Currently, Theraflex-UV is only being used for plasma products in approximately 10 countries [150]. The combination of methylene blue and light is also being investigated. Methylene blue is a positively charged dye that intercalates into DNA and when exposed to visible light produces oxygen-mediated DNA damage [167]. Because it cannot get into cells, methylene blue is not effective in inactivating leukocytes. Notably, both methylene blue and Theraflex-UV have been shown to successfully inactivate hepatitis C virus (HCV) [168]. Lastly, is frangible anchor linker effector (FRALE), which inactivates DNA/RNA by forming a covalent bond between nucleic acids using an alkylating agent. The FRALE method of pathogen inactivation has been shown to inactivate viruses, bacteria, protozoa, and leukocytes [169].

Many of these pathogen inactivation systems are approved for and being used for platelets. Increasing evidence is coming out that these systems alter the proteome of the platelets with each system having a different effect. Mirasol affects platelet adhesions and shape change. The INTERCEPT system affects proteins involved in intracellular platelet activation pathways, and Theralf affects platelet shape change and aggregation [170]. Additional studies are needed to further elucidate these effects. Donor leukocytes are known to cause graft versus host disease (GVHD) in immunocompromised recipients or in HLA similar recipients. Currently, the only mechanism to prevent transfusion-associated GVHD is gamma irradiation. Mirasol PRT, Theraflex-UV, INTERCEPT, and FRALE have been shown to be as effective as gamma irradiation in inactivating leukocytes, which would prevent transfusion-associated GVHD [164,171,172]. Octaplas LG, which is a newer version of Octaplas S/D that has been prion-depleted [173] by an additional step of treatment with an affinity ligand gel to prions, was found to be bioequivalent to Octaplas S/D [173,174]. Octaplas S/D (solvent/detergent) is available in over 25 countries [150] and effectively inactivates enveloped viruses; however, it has limited effectiveness in inactivating nonenveloped viruses [173]. In addition, Huismann et al. performed an economic evaluation of Octaplas versus FFP use in the United States using a specific model [175]. They found that patients receiving Octaplas are predicted to have 0.00613 more life years and 0.023 more quality-adjusted life years [175]. The same group performed a similar analysis of Octaplas versus FFP use in Canada and found that Octaplas LG resulted in 0.021 quality-adjusted life years as compared to FFP [176]. Moreover, they assessed that Octaplas LG would cost CA $303.14 less than FFP over a life time horizon [176]. Thus, Octaplas LG was expected to become the predominant plasma
used. Interestingly, with the approval of INTERCEPT and entry of other pathogen inactivation systems, Octaplas may not become as dominantly used as expected. What one can expect to see in the future is that nearly every blood product available in a blood bank will be treated with a pathogen inactivation system.

**Expansion of platelet storage time**

In most countries, platelet storage time is limited to 5 days. Due to this short storage period, there are often shortages of platelets as well as wastage of platelets. Blood Safety and Quality Regulations in the UK, allow platelet storage time to be extended to 7 days if a bacterial detection or pathogen reduction system is used [177]. Several studies have shown reduced platelet recovery and survival for 7-day-old platelets versus 5-day-old platelets [178-182]. Recently, MacLennan et al. looked at the efficacy of bacterial screened platelets stored for 6-7 days as compared to platelets stored for 2-5 days in hematology/oncology adult patients with thrombocytopenia. They found essentially no differences in the CCI, proportion of days with WHO bleeding score of 2 or higher, or median interval to next platelet transfusion between the two groups [177]. Notably, the National Health Service Blood and Transplant (NHSBT) in the UK has implemented bacterial screening and extended the platelet storage period to 7 days in 2011. Since this change, there have not been any reports of transfusion-transmitted bacterial infections attributed to platelet products [177]. Importantly, the NHSBT has seen a 2 % decrease in platelet wastage and improved platelet availability [177]. The AABB has recommended implementation of bacterial screening point of care (POC) testing which would allow the shelf life of platelets to be extended to 7 days in the US as well. To date, very few medical centers and hospitals in the US have implemented this additional screening test due to its higher cost. Nevertheless, additional bacterial testing prior to release of platelets and a shelf life of 7 days is likely to become more prevalent in the near future. Additionally, as discussed above, pathogen reduction systems are also likely to become more widely used which will also result in platelet products with extended shelf life. As a result of implementation of these two processes, platelet availability is expected to increase and platelet wastage to decrease.

**Blood substitutes**

Previous sections discussed ways to increase safety, efficacy, and availability of blood products as well as reduce recipients’ unnecessary exposure to blood. However, one way to address all of these needs is the use of blood substitutes. Blood substitutes are particularly attractive when looking at developing countries with very high levels of HIV, limited donors, and limited ability for infectious disease tests. The search for blood substitutes widely expanded with the contamination of the blood supply by HIV. Currently, with donor screening and testing of the blood, the transmission risk for HIV is down to 1/1,467,000 units in the United States [183]. Notably, this is not the case in developing countries where the HIV risk is much higher. Additionally, there is always the chance of another virus, bacteria, protozoa, or unknown pathogen to again threaten the safety of the blood supply [184]. Presently, there are no FDA approved blood substitutes. One problem that has been encountered is that a non-toxic oxygen carrier has not been identified yet. Various different species of hemoglobin as well as perfluorocarbons (PFCs), a chemical compounds that can carry and release oxygen, have been studied. In one of the first studies in humans using hemoglobin as a blood replacement, Amberson et al. gave lactated Ringer’s solution containing hemoglobin to 14 patients. Notably, five of the patients developed renal impairment [185]. Free hemoglobin is toxic to the kidneys and rapidly scavenges free nitric oxide causing vasoconstriction [186,187]. This toxicity has partially been mitigated by co-transfusion with haptoglobin and use of annelid hemoglobin [184]. PFCs carrying oxygen must be transported by large amounts of phospholipid emulsions, which is problematic as it is somewhat viscous and is similar to adding tremendous amounts of cholesterol to the blood [184].

To date, few blood substitutes have advanced to phase II and phase III trials; HemAssist from Baxter (Deerfield, IL, USA), PolyHeme from Northfield (Mt. Prospect, IL, USA), Hemopure from Biopure (Cambridge, MA, USA), and Hemolink (Hemosol Inc., Toronto, Canada). HemAssist is comprised of diaspirin-cross-linked human hemoglobin (DCLHb) that circulates as 99 % tetramer and has a P50 (partial pressure of oxygen required for 50 % hemoglobin saturation) of 32 mmHg (normal human hemoglobin P50 is 26.6 mmHg) [188]. HemAssist had an in vivo half-life of 6-12 hours and a shelf life of > 1 year [188]. Several studies using HemAssist showed decreased use of RBCs in patients; however, significant adverse events were observed including jaundice, hemoglobinuria, urinary problems, pancreatitis, and increased mortality [188-192]. Thus, several studies were terminated early and Baxter later had to discontinue producing HemAssist.

PolyHeme is a polymerized stroma-free form of human hemoglobin made by cross-linking stroma-free hemoglobin using glutaraldehyde followed by pyridoxalation. PolyHeme circulates as ≤ 1 % tetramer and has a P50 of 26-30 mmHg [193]. Studies showed decreased use of RBC transfusion in PolyHeme treated patients and was well tolerated [193-195]; however, one study showed an increase in side effects such as electrolyte imbalances. Notably, PolyHeme was not approved by the FDA.

Hemopure is highly purified bovine hemoglobin that is polymerized with glutaraldehyde that circulates as ≤ 5 % tetramer and has a P50 of 32-38 mm Hg [186,193]. Several studies performed using Hemopure showed decreased use of RBC transfusion as well as increased oxygen extraction, and increased cardiac adverse effects [196-199]. Hemopure was also denied...
by the FDA. In several phase II and phase III clinical trials, Hemolink, which is a human hemoglobin cross-linked with O-raffinose, was also found to cause hypertension in coronary artery surgical patients [200-202]. Lastly, in a meta-analysis performed by Natanson et al. on all of the blood substitute clinical studies (HemAssist, PolyHeme, Hemapure, Hemospan, and Hemolink), they reported 30 % increase in mortality risk and a 2.7-fold increase risk of myocardial infarction [186].

In an attempt to reduce the vasoconstrictive side effects of these earlier hemoglobin substitutes, OXYVITA Inc. (New Windsor, NY, USA) developed OxyVita, a high molecular weight compound comprised of bovine hemoglobin. In OxyVita, the β globin chains of bovine hemoglobin are cross-linked using bis (3,5-dibromosalicyl)-adipate and polymerized using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide [203,204]. Interestingly, OxyVita did not cause hypertension in animal studies [204]. However, it has high oxygen affinity, which impedes oxygen delivery to tissues [204].

VitalHeme was developed by SynZyme Technologies LLC (Irvine, CA, USA). VitalHeme is comprised of polyoxytrioxilation of αααα-fumaryl cross-linked bovine hemoglobin (PONPH), TEM-POL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), and polyethylene glycol (PEG). VitalHeme was found to be able to transport oxygen, act as an antioxidant, and expand plasma [205]. Notably, it was also found to have neurovascular protective properties [206]. One of its problems is that it is expensive to make.

To combat the problems encountered with the toxicity of previous acellular hemoglobin products, pegylated liposome-encapsulated hemoglobin (LEH) was created (PEG-LEH). PEG-LEH was shown to cause little to no vasoconstriction and hypertension [207] and had a longer circulation half-life [208] as it is cleared through the liver and spleen. Transfusion of PEG-LEH has been associated with increased lipid levels as well as hepatosplenomegaly. The lipid levels were observed to return to normal after ~7 days and hepatosplenomegaly normalized after several weeks [209-211].

Another alternative is annelid hemoglobins. Earthworms (Lumbricus terrestris) and marine worms (Arenicola marina) both lack RBCs and instead use erythrocruorins or hexagonal bilayer hemoglobins as oxygen carriers. Both erythrocruorins are comprised of 144 globin subunits, which contain heme and 36 nonheme linker proteins that are held together by numerous disulfide bonds and electrostatic interaction [212-215]. These erythrocruorins have several advantages over the free hemoglobin substitutes discussed above. Notably, they remain dissolved in the blood and do not sediment or precipitate out. They are also able to bind large amounts of oxygen and display cooperative oxygen binding and transport [215]. Arenicola marina erythrocruorin was used to make HEMOXY Carrier (Hemarina S.A., Morlaix, France) [216]. In several studies, HEMOXY Carrier has been shown to have antioxidant properties and not cause vasoconstriction in rodents [217,218]. Thus, the annelid hemoglobins show some promise in functioning as RBC substitutes; however, more studies are needed.

HemoTech by HemoBioTech Inc. (Dallas, TX, USA) is a newer hemoglobin-based blood substitute. HemoTech is comprised of ATP-adenosine and glutathione cross-linked to hemoglobin [219]. Notably, HemoTech is touted to control vasoconstriction, oxidative stress, and inflammation as well as deliver oxygen [219]. Interestingly, it has also been shown to increase erythropoiesis [219]. Unfortunately, there have been no animal or clinical studies published on HemoTech and all the above effects were derived from biochemical in vitro studies. Thus, it is difficult to assess the true promise of HemoTech.

PFCs also show some promise as RBC substitutes. They are inert and are comprised of fluorine-substituted hydrocarbons. The hydrocarbons have high oxygen and carbon dioxide solubility [184]. PFCs are extremely hydrophobic and are not very soluble in water or blood; hence, they require an emulsifier. Fluosol-DA (Green Cross Corp., Osaka, Japan) is a PFC that was FDA approved in 1989 for use during angioplasty. Unfortunately, in clinical trials, patients had adverse reactions that were thought to be due to complement activation and it was removed from the market in 1994 [220,221]. Several second generation PFCs were subsequently developed: Oxyfluor (HemaGen/PFC, Wal-tham, MA, USA), Oxygent (Alliance Pharmaceutical Corp., San Diego, CA, USA), and Perftoran (SPP-Perftoran, Moscow, Russia). Notably, Oxyfluor and Oxygent showed increased side effects and clinical trials and production were ceased [222,223]. Perftoran is currently approved for use in Russia. However, more research and development is needed prior to PFCs being a clinically viable option.

Another alternative blood substitute is plasma expanders such as polyethylene glycol conjugated albumin (PEG-Alb) by Enzon Inc. (Piscataway, NJ, USA) [224]. PEG-Alb enhances tissue perfusion by increasing blood flow. Thus, it increases oxygen delivery capacity mainly by enhancing the flow without affecting the oxygen carrier capacity [225].

Lastly, as stem cell manipulation and therapy advances, it may be economically feasible to make RBCs for patients requiring chronic transfusion. Giarratana et al. published a proof of concept paper in 2011 in which they generated a homogenous population of RBCs using CD34+ hematopoietic stem cells [226]. The cultured RBCs were then injected into a volunteer subject and the cell survival was measured at 26 days, which was between 41 and 61 % [226]. This half-life is comparable to the half-life of native RBCs. Notably, the cost of this engineered RBC unit was very expensive and prohibitive of wide spread use. However, with time and advances, the costs may decrease. Thus, the future of blood substitutes could go in a variety of different directions and only time and technology will tell.
**Cellular therapies: dendritic cell vaccines, CAR-T, and TRUCK therapies**

Immunotherapy is a growing field, especially in relation to cancer treatment. Three main therapeutic approaches used are dendritic cell (DC) vaccines, CAR-T, and TRUCK therapies. DC vaccines employ DCs loaded with tumor antigen as an anti-cancer vaccine. This system exploits the DC to generate a potent tumor antigen specific T-cell driven immune response [227,228]. The antigen loaded DCs function to allow the immune system to recognize and attack cancer cells thus generating immune memory [229]. Phase III trials of DC vaccine therapies have been conducted in melanoma, glioblastoma, renal cell carcinoma, and prostate cancer [228]. DC vaccines have been shown to be well tolerated with typically only local reactions at the injection site [230]. Side effects such as fever, malaise, and flu-like symptoms have been documented to occur; however, more serious side effects are rare [230]. Currently, only one DC vaccine is FDA approved, sipuleucel-T (Provenge, Dendreon Corporation, Seattle, WA). As compared to placebo, sipuleucel-T was found to increase overall survival by 4.1 months in patients with hormone-refractory metastatic prostate cancer [231]. Notably, only 7.1 % of patients receiving DC vaccine for prostate cancer had an objective response as assessed by imaging or prostate specific antigen (PSA) level [228]. To generate sipuleucel-T, each patient undergoes leukapheresis to collect a large number of peripheral blood mononuclear cells (PBMCs). The PBMCs are then cultured ex vivo for 36–44 hours in the presence of a recombinant fusion protein that is comprised of GM-CSF and prostatic acid phosphatase. The cells are activated and loaded with antigen and then washed and resuspended for infusion [231]. DC vaccines are currently being developed and studied for several additional cancers including acute myelogenous leukemia (AML) [232].

DC vaccines do face criticism due to the limited objective response that has been documented in clinical trials. An objective response rate of 8.5 % was seen in melanoma patients receiving DC vaccines, 15.6 % in glioblastoma patients, and 11.5 % in renal carcinoma patients [228]. These objective response rates are comparable to other therapies currently available for treatment of these cancers. In the DC vaccine trials for melanoma, the overall survival of patients was found to increase 3.3–33.0 months [233-246]. However, two studies did not find an increase in overall survival [247,248]. In trials for glioblastoma, overall survival was found to increase 2.6–25.8 months [249-263]. Lastly, in trials for renal cell carcinoma, DC vaccines improved the overall survival of patients 3.9–17.0 months [264-268]. Thus, there is discordance between overall survival and objective response rate. Despite lack of objective response, patients treated with DC vaccines are surviving longer and there is clinical benefit.

The future of DC vaccines is to improve the vaccine efficacy through the use of combination therapy. Additionally, DCs are being generated that have increased immune stimulatory action. Additionally, Langerhan dendritic cells are being studied as a replacement for DCs due to their improved ability to stimulate cytotoxic T-cells [269]. Several melanoma clinical studies using Langerhan dendritic cells have already started [228,270]. Thus, it is likely that several DC vaccine therapies will be FDA approved in the future and that the DC vaccines will have more therapeutic efficacy as measured in increased survival.

Tumor infiltrating lymphocytes have shown promise in treating cancer. CAR-T therapy takes advantage and builds upon this principle. In CAR-T therapy, a patient undergoes leukapheresis. Their T-cells are then engineered with a chimeric antigen receptor (CAR) which is comprised of a single polypeptide chain containing an extracellular antigen binding domain from an antibody and an intracellular signaling chain typically the CD3ε chain from the T-cell receptor (TCR) [271,272]. The antibody domain allows the engineered T-cells to specifically target a given cancer cell antigen. Additionally, there are 2nd and 3rd generation CARs, which have additional costimulatory domains that improve the T-cell response [273].

The most developed CAR-T therapy currently is targeted at CD19 and is in trials for treatment of B-cell leukemias and lymphomas. Patients receiving treatment with CAR-T therapy directed against CD19 have shown complete responses that have been maintained [274-277]. The literature surrounding CAR-T therapy directed against CD19 is primarily in the field of ALL, where it has been highly effective. However, this therapy has also been shown to be useful in treating chronic lymphocytic leukemia as well [278,279]. CAR-T therapy does have some shortfalls. One large shortfall is that tumor cells quite often lose and gain different antigens and thus become resistant to CAR-T therapy if they lose that particular antigen. Other problem faced by CAR-T therapy is that the stroma surrounding solid tumors is more difficult for T-cells to penetrate and contains numerous other cells such as regulatory T-cells that serve to modulate the T-cell response. Research is ongoing to address these and other issues in CAR-T therapy.

T-cells redirected for universal cytokine killing (TRUCKs) are also a more recent addition to cancer immunotherapy treatments. TRUCKs are essential CAR-T cells that are used to produce and release a transgenic product that accumulates a specific target. Typically, the product produced is a pro-inflammatory cytokine such as IL-12. Early trials using IL-12 showed toxicity in intestinal, hematopoietic, hepatic, and pulmonary tissues [280,281], thus, TRUCKs producing IL-12 were designed that deliver IL-12 directly to the targeted tissue and eliminate the toxicity caused by systemic IL-12. The TRUCKs producing IL-12 were found to have increased efficacy against tumors and increased cytolytic activity [282]. Additionally, more NK cells were found to be localized in...
the targeted tumor which augmented the anti-tumor effect [282]. TRUCKs are advantageous to CAR-T therapy because they are effective against heterogeneous tumors as well as tumors that switch antigens [283]. TRUCKs with IL-12 expression have also been shown to be able to overcome the immunosuppressive milieu of the stroma. This is accomplished by the IL-12 expressing TRUCKs effectively remodeling the stroma and reprogramming the suppressor cells [284,285].

A drawback of TRUCKs is that they are likely to produce more severe tissue damage than CAR-T therapy without cytokine production. Importantly, systemic and localized toxicities in have not been observed in mice models [283]. Several clinical trials with TRUCKs expressing IL-12 are being done. One such trial studied melanoma patients who received IL-12 expressing TRUCKs [286]. This study found that 63 % of patients who received 0.3–3 × 10^6 cells had objective clinical responses [286]. Unfortunately, the responses were short, and the IL-12 expressing TRUCKs rarely persisted at 1 month [286]. Toxicities (liver dysfunction, high fevers, and sporadic life-threatening hemodynamic instability) were observed with high IL-12 levels, which occurred at increasing cell doses [286]. Thus, more research is needed to improve TRUCKs before they are a viable immunotherapy option. In the future, it is quite likely that TRUCKs expressing IL-12 and other cytokines will be utilized in the fight against cancer. TRUCKs are also being studied for use in combating viral infections, metabolic disorders and autoimmune diseases [287].

In the future, it is likely that immunotherapy approaches such as DC vaccines, CAR-T, and TRUCKs will become increasingly more prevalent as well as more therapeutically efficacious. These therapies are also likely to be expanded to address a variety of different cancers as opposed to the select few for which they are currently designed. Lastly, new immunotherapy approaches are likely to be designed and implemented.

Disclosure of interest: the authors declare that they have no competing interest.

References


[27] Leffere JJ, Hewitt P. From mad cows to sensual blood transfusion: the risk of prion transmission by viable blood components in the United Kingdom and in France. Transfusion 2009;49:797-812.


[89] Barshitein G, Gural A, Manny N, Zelig O, Yedgar S, Arbel D. Storage-induced damage to red blood cell mechanical properties can be only partially reversed by rejuvenation. Trans Med Hemother 2014;41:197-204.


