Review Article

Pathogen reduction of platelets: experience of a single blood bank

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Abstract: Pathogen reduction technology (PRT) has the potential to prevent pathogen transfusion transmission from blood donor to patient by impeding the replication of bacteria, viruses and parasites in blood components. Additionally, PRT can help to guarantee blood safety in challenging situations for blood supply, as in the Ebola or Chikungunya epidemics, or in a scenario full of uncertainties such as the current SARS-CoV-2 pandemic. The Balearic Islands Blood Bank (BIBB) is one of the few blood establishments worldwide with more than 10 years of experience in the routine use of amotosalen/UVA (Intercept Blood System) and riboflavin/UVA-UVB (Mirasol PRT system) for platelets (PLTs), the use of riboflavin/UVA-UVB for plasma and with research experience in riboflavin/UVA-UVB applied to whole blood. Over the years, we have had the opportunity to evaluate PRT from different perspectives, such as clinical and hemovigilance research in adults and children, in vitro studies on PRT effects on PLTs and assessing the financial impact of PRT implementation. PRT methods offer remarkable benefits but also have certain limitations, which are important to bear in mind during the decision-making process for PRT implementation. The purpose of this study is to review the current knowledge on PRT for PLTs drawing on our experience acquired over the last decade.

Keywords: Pathogen reduction technology (PRT); platelets; blood safety

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Introduction

Despite increasingly strict donor selection criteria, advances in laboratory testing and procedures for preventing bacterial contamination, such as donor skin disinfection, diversion of the first milliliters of collected blood and bacterial culture, a small risk of bacterial (1,2), viral (3,4) and parasite (5-9) contamination of platelet (PLT) concentrates still remains. There is also a permanent threat of emerging and novel pathogens entering the blood supply, as demonstrated in the recent epidemics caused by the West Nile virus (WNV), Chikungunya virus (CHIKV), Zika and Ebola virus (10-13). Pathogen reduction technology (PRT) for PLTs has the potential to prevent pathogen transmission from donor to patient during PLT transfusion (14). In addition to improving blood safety by preventing the replication of bacteria, viruses and parasites, PRT has other benefits: (I) elimination of the risk of transfusion-transmitted graft-versus-host disease by substituting gamma irradiation with white blood cell inactivation (15); (II) the potential reduction of alloimmunization, which has been described in animal models (16) but still needs to be confirmed by evidence from clinical practice (17); (III) fewer PLT transfusion reactions (18,19); and (IV) the improvement of PLT supply by extending the storage time from 5 to 7 days, which results in a substantial reduction in PLT outdating (20,21).

Our experience in pathogen reduction technologies

The Balearic Islands Blood Bank (BIBB) is responsible for supplying blood to 22 hospitals located in the Balearic Islands, a Mediterranean archipelago in Spain. These hospitals provide a total of 3,675 beds for a population of around one million inhabitants. The BIBB collects around 40,000 whole blood and 5,700 PLT components per year

for transfusion therapy in hematology, oncology, and surgery patients, among others, including those requiring cardiovascular surgery and bone marrow transplant.

In 2008, the BIBB initiated the implementation of PRT for PLTs based on amotosalen plus ultra violet (UV) A light (Intercept System, Cerus Corporation, Concord, CA) (20). In 2012, PRT using UVA and UVB light in the presence of riboflavin (Mirasol System, Terumo BCT, Lakewood, CO) was introduced for treating plasma (22). A year later, in order to treat plasma and PLTs with the same PRT, the BIBB adopted the universal routine use of riboflavin plus UVA and UVB light–treated PLTs for transfusion support in adults (21) and children (23) with thrombocytopenia. In 2017, our blood establishment investigated the effectiveness of riboflavin plus UVA and UVB light in eliminating *T. cruzi* from whole blood units (24). The BIBB is one of the few blood establishments worldwide with more than 10 years of routine application of Intercept and Mirasol PRT for PLTs, the only two commercial PRT systems for PLTs currently available. We also have experience in the routine use of Mirasol PRT for plasma, and in its experimental use for whole blood. Although our research group has previously published papers on the use of these technologies (20-24), the aim of this article is to review current developments in PRT for PLTs drawing on more than a decade of experience in the field.

Pathogen reduction technologies

The three PRT systems developed to date for producing pathogen-reduced PLT concentrates are based on UV light in the absence or presence of a photosensitizer.

The Intercept system (Intercept System, Cerus Corporation, Concord, CA) utilizes approximately 150 µM amotosalen, the synthetic psoralen S-59, as a photosensitizer in combination with UVA light (320–400 nm) at dose of 3.9 J/cm^2. After UV illumination for 3–4 min, the photoexcited amotosalen forms covalent bonds with thymidine bases. This reaction inhibits DNA replication and RNA transcription, which in turn prevents replication of leukocytes and pathogens. After PLT treatment, amotosalen and its photoproducts need to be removed by an in-line compound adsorption device for 6–24 h (25,26).

The Mirasol system (Mirasol System, Terumo BCT, Lakewood, CO) uses on average 50 µM of riboflavin (vitamin B2) as a photosensitizer along with UVA and UVB light (270–360 nm) at a dose of 6.2 J/mL. Upon UV illumination over 4–10 min, the oxygen free radicals generated by riboflavin cause irreversible damage to nucleic acids, which inhibits the replication of pathogens and leukocytes. After illumination, the removal of riboflavin is unnecessary, as this common and essential water-soluble vitamin is considered to be safe (27,28).

The Theraflex-UV PLT system (MacoPharma, Tourcoing, France) uses UVC light (254 nm) at a dose of 0.2 J/cm^2 for 30–60 seconds without the addition of a photosensitizer. Light penetration is achieved by strong agitation and the generated pyrimidine dimers prevent replication of nucleic acids and pathogen proliferation (29,30).

The Theraflex-UV system requires PLT concentrates diluted in a PLT additive solution (PAS), whereas in the Intercept and Mirasol systems PLTs can be diluted either in PAS or plasma. As described in recent reviews, all three PRT systems effectively reduce bacteria and other nucleic acid-containing infectious agents found in PLTs (31-33).

Impact of PRT on PLT functionality

PLT functionality is apparently unaltered by PRT, which only targets dividing life forms. Nevertheless, some researchers have shown that PLTs can be damaged by interactions with the photosensitizer and UV light. It has been demonstrated that PRT treatment can induce deterioration of mitochondrial function, increased metabolism, and spontaneous PLT aggregation and activation, resulting in altered PLT function and quality (34-37). Approaches to studying the impact of PRT on PLTs have gradually changed over the years. In addition to the classical PLT *in vitro* tests, other methodologies such as proteomic (38) and biomolecular (39) profiling are being progressively incorporated into the research field of PRT effects on PLTs.

Most studies using classical tests for evaluating PLT functionality, i.e., swirling, glucose consumption and lactate formation, pH, hypotonic shock response, adhesion assays, aggregation induced by different agonists and flow cytometry detection of PLT membrane markers of activation (CD63, CD62p, CD40L) and apoptosis (annexin V), report a low to moderate loss of PLT *in vitro* function compared to conventional PLTs, which occurs gradually during the 5 to 7 days of PLT storage (34-37). Although in general *in vitro* PLT function assessed by the classical methods has no or scarce correlation with *in vivo* PLT recovery, survival and hemostatic activity (40), some of the *in vitro* tests correspond with *in vivo* parameters. For example, lactate production and pH correlate reasonably

well with PLT recovery and survival time in human subjects, i.e., the lower the pH and the higher the lactate production, the lower the PLT survival and recovery *in vivo* (41). Moreover, PLTs showing swirling as an indicator of discoid shape retention *in vitro* are expected to be functional *in vivo* at transfusion (42).

Proteomic analysis is a very useful approach for studying the quality and function of PRT-treated PLTs, as PLTs have a limited capacity to translate messenger RNA to substitute PRT-modified cellular proteins. Interestingly, proteomic studies have demonstrated that the different PRT systems have different impacts on PLT functionality. Thus, Intercept affects proteins involved in the mechanism of PLT activation and aggregation pathways, Mirasol mainly acts on proteins related to actin polymerization, cytoskeleton organization, adhesion, granule secretion and PLT shape, whereas Theraflex influences proteins associated with changes in PLT shape and aggregation (38).

Studies on the effect of PRT on PLTs at the biomolecular level show that Intercept damages nucleic acids such as RNA, mitochondrial DNA and lipid molecules, resulting in altered membrane packing and defects in PLT signal transduction. The Mirasol system changes proteins by oxidative mechanisms and increases PLT metabolism, leading to an increment in lactic acid and a lower pH. Theraflex also increases metabolism and induces conformational changes in PLT integrin (39).

A long-debated issue is whether the PRT-induced reduction in cell viability only affects a proportion of the treated PLTs (43,44) or results in an overall functional deterioration (45,46). PRT damage (affecting a percentage of PLTs or all of them) can result in increased cellular metabolism, reduced clot strength, and lower PLT increments post-transfusion compared with conventional PLTs. However, patients transfused with PRT-treated PLTs do not necessarily suffer more bleeding events (47).

Our research group has investigated the metabolic activity and hemostatic function of buffy coat PLT concentrates treated with riboflavin and UVA and UVB light. We found that this PRT system accelerates and augments PLT storage lesion, producing glucose depletion, lactate accumulation, PLT acidification and discoid shape loss. Additionally, the clots generated by conventional PLTs at day 14 measured by thrombelastography were still remarkably strong, whereas those produced by PRT-treated PLTs at day 7 were weaker. To confirm these results, clinical trials studying the efficacy of PRT-treated PLTs transfused at the end of the storage period (day 7), when the *in vitro* clot strength seems weaker, are needed (37).

Clinical studies on PRT-treated PLT transfusion

Autologous transfusion studies have found lower PLT recovery and survival in PRT-treated versus conventional PLTs: Mirasol (–25% and –27%) (48), Theraflex (–26% and 29%) (49) and Intercept (–16% and –20%) (50).

The efficacy of PRT-treated PLTs has also been investigated through observational studies, such as those carried out by our research group (20,21). Randomized clinical trials (RCTs) have compared PRT-treated and conventional PLTs in hematology patients: 8 RCTs used Intercept (51-58) and 3 used Mirasol (57,59,60) (Table S1).

Most of these studies found that the transfusion of PLTs treated with PRT resulted in lower post-transfusion PLT count increments, which is consistent with the reduced PLT recovery and survival previously observed in autologous transfusion studies with healthy subjects (48-50). Consequently, to achieve the necessary PLT increment, the use of PRT-treated PLTs implied a higher transfusion frequency (52,58,60).

A meta-analysis of RCTs that evaluated hematology and oncology patients found moderate-quality evidence that PRT-treated PLT transfusion was not associated with statistically significant increments of clinically relevant bleeding and high-quality evidence that it increases PLT requirements (47).

Three RCTs have assessed whether PLTs treated with PRT are non-inferior to conventional PLTs, using as the primary outcome the prevention of WHO grade 2 or higher bleeding in thrombocytopenic patients with hematological disorders. The Italian Platelet Technology Assessment Study (IPTAS) was not able to prove non-inferiority of PRT-treated PLTs due to low statistical power (57). The Evaluation of the Efficacy of Platelets Treated with Pathogen Inactivation Process (EFFIPAP) study found that PLTs treated with Intercept are non-inferior when stored in PAS, but non-inferiority was not demonstrated when they were suspended in plasma (58). The Pathogen Reduction Evaluation and Predictive Analytical Score (PREPAReS) study evaluated the efficacy of PLTs treated with Mirasol compared to conventional PLTs in plasma obtained from whole blood by the buffy coat method, proving non-inferiority in the intention-to-treat analysis, but not in the per-protocol analysis (60).

Unfortunately, the question explored in these studies remains unresolved, especially if we take into account that

the non-inferiority trials had insufficient statistical power to establish a significant difference between WHO grade 2 and 4 bleeding, owing to the infrequency of the more severe events. On the other hand, most studies assessing the safety and efficacy of PRP-treated PLTs have focused on adult hematology and oncology patients.

Insufficient research has been carried out in other patient populations, such as those undergoing massive transfusion in the setting of organ transplantation, surgery or trauma.

Moreover, very few reports have been published about the safety and use of PRT-treated PLTs for transfusion in pediatric patients. A retrospective study in which 240 children received 1,072 Intercept-treated PLTs and 860 conventional PLTs over 21 months found no safety issues and red blood cell utilization patterns were similar. Only febrile nonhemolytic and allergic transfusion reactions were reported over the study period, with the number and type of transfusion reactions similar in both groups. However, there was an increased utilization of PLTs in pediatric recipients aged 1–18 years after the transfusion of Intercept–treated PLTs (61). In another retrospective study, 51 children with a mean age of 11 years were transfused with 141 Mirasol–treated PLTs and showed lower post-transfusion PLT counts compared with 86 children receiving 291 standard PLTs. However, the incidence of bleeding episodes and transfusion-related adverse events was similar in both groups (62). Our research group evaluated PLT use in 379 children up to the age of 15 years, who received 4,236 PLTs treated with Mirasol between 2013 and 2017. While all adverse events in children were mild, we found a significant increase in PLT transfusions in 132 neonates receiving 458 Mirasol-treated PLTs compared with 99 neonates transfused with 176 standard PLTs. We concluded that additional studies are required to assess the efficacy and safety of PRP-treated PLTs in pediatric patients, especially those requiring chronic transfusion therapy (23).

Hemovigilance and PRT-treated blood components

Intercept, which was granted the CE mark in 2002, is commercially available in many European countries (63). According to national hemovigilance systems, there have been no confirmed septic transfusion reactions after the transfusion of 227,797, 167,200 and 214,293 PLT concentrates treated with the Intercept system in Belgium, Switzerland and France, respectively (64). Additionally, a study in Switzerland showed no cases of bacterial infection associated with the transfusion of 205,574 Intercept-treated PLT concentrates (65). There is a potential risk that UV exposure and/or residual photo-products may cause short- or long-term side effects (66,67), especially that residual psoralen may trigger skin rashes in newborns receiving phototherapy for hyperbilirubinemia (68). A retrospective study on transfusion reactions in children failed to find any new rashes after a manual chart review of 11 newborns undergoing phototherapy and receiving Intercept-treated PLTs, although no chart review was done in the control group (61).

In 2014, Intercept PRT was approved for apheresis PLTs diluted in 100% plasma and in 65% PAS-3/35% plasma by the US Food and Drug Administration (FDA) (69). A prospective open label, post-marketing surveillance study following transfusion of Intercept PLT components is currently in progress (PIPER, identifier: NCT02549222) (70). This trial has been designed to detect acute respiratory distress syndrome (ARDS), as Intercept carries a warning to monitor patients for symptoms and signs of ARDS, after a recipient of Intercept-treated PLTs in the SPRINT trial exhibited an increased incidence of the syndrome (1.6% *vs.* 0%) (52).

Mirasol, which obtained CE approval in 2007, is currently in use in some European countries (63), as well as in Russia and the Middle East. A European hemovigilance report, in which our blood establishment participated, described no septic transfusion reactions, virus transmission or any other severe adverse effects after the transfusion of 91,954 Mirasol-treated PLT concentrates in Poland, Spain, Lithuania, Greece, Austria, Luxembourg and Belgium (19). Presently, Mirasol PRT is being assessed in the US in a phase III randomized clinical trial named Efficacy of Mirasol-treated Apheresis Platelets in Patients with Hypoproliferative Thrombocytopenia (MIPLATE, identifier: NCT02964325) (71).

The Theraflex system, which received a CE mark in 2009, is not yet in routine use and is currently under assessment in a phase III clinical trial in Europe (CAPTURE. EudraCT Number: 2015-001035-20) (72).

Limitations of pathogen reduction technologies

PRT has two main limitations. Firstly, despite the broad range of pathogens that can be inactivated by PRT, it is ineffective against some infectious agents. Secondly, the cost of PRT implementation can inhibit its widespread application.

The pathogen inactivation efficacy of the three PRT

methods is widely reported in the literature (73-75). It has been demonstrated that PRT can eliminate the residual risk of transmission of hepatitis C, hepatitis B and HIV infection, as well as prevent transfusion transmission of emerging infectious agents containing nucleic acids. However, the resistance of HIV to the Theraflex-UV system (76) could be an important limitation. The risk of transfusion-transmitted HIV has decreased substantially in most countries, but it can still occur despite negative individual-nucleic acid testing due to the window period (77), the suppression of viral load and delayed seroconversion after the administration of 'on demand' pre-exposure prophylaxis (PrEP), or when there is poor adherence to PrEP (78).

Additionally, PRT cannot protect against pathogenic agents without nucleic acids, such as prions. Non-enveloped viruses are also resistant to inactivation, due to the icosahedral nucleocapsid that maintains viral integrity under hostile environments and acts as a barrier against the penetration of photochemical agents (79). Additionally, endotoxin pyrogenic cell wall components, biofilm-positive isolates and spore-forming bacteria, while exceedingly rare, remain transfusion-transmission infection risks even after PRT treatment (80).

Lastly, the widespread adoption of PRT has been hampered by its cost, which may be further increased by higher PLT requirements in patients transfused with PRT-treated PLTs. However, as our research group has reported (20), the expense of PRT can be partially offset by the extension of PLT storage time from 5 to 7 days, which is associated with a substantial reduction in PLT loss due to outdating. Additionally, implementing PRT may be considered as cost-effective if it eliminates the need to test for new and emerging infectious agents that could challenge blood safety.

PRT and the coronavirus pandemic

As an example of the inactivation of a novel infectious agent, the three PRT methods—Intercept, Mirasol and Theraflex—have the ability to inactivate the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (81,82), responsible for the current COVID-19 pandemic, and the Middle East respiratory syndrome coronavirus (MERS-CoV) (83-85), which has many clinical, epidemiological, and virological similarities with SARS-CoV-2. It has recently been proved that the stability of SARS-CoV-2 IgG and its overall neutralizing capacity is preserved after Intercept treatment of convalescent plasma from patients who have recovered from COVID-19. Therefore, Intercept PRT, as well as mitigating the transfusion-associated risk of viral transmission, does not alter the potential therapeutic potency of convalescent plasma (86).

Conclusions

Over the last decade, we have had the opportunity to study PRT methods in different settings, such as clinical and hemovigilance research in adults and children, *in vitro* studies on PRT effects on PLTs and assessment of the financial impact of PRT implementation. PRT methods offer remarkable advantages but also have certain limitations, which are important to bear in mind during the decision-making process for PRT implementation. Undoubtedly, PRT has the potential to guarantee blood safety in situations that could challenge blood supply, as observed in the Ebola or Chikungunya epidemics, or in a scenario full of uncertainties such as the current SARS-CoV-2 pandemic. Overall, PRT methods are more of an ally than a threat to PLT transfusion safety.

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References

1. Abela MA, Fenning S, Maguire KA, et al. Bacterial contamination of platelet components not detected by BacT/ALERT. Transfus Med 2018;28:65-70.
2. Ramirez-Arcos S, DiFranco C, McIntyre T, et al. Residual risk of bacterial contamination of platelets: six years of experience with sterility testing. Transfusion 2017;57:2174-81.
3. Weusten J, van Drimmelen H, Vermeulen M, et al. A mathematical model for estimating residual transmission risk of occult hepatitis B virus infection with different blood safety scenarios. Transfusion 2017;57:841-9.
4. Tedder RS, Ijaz S, Kitchen A, et al. Hepatitis E risks: pigs or blood-that is the question. Transfusion 2017;57:267-72.
5. Jimenez-Marco T, Riera C, Fisa R, et al. The utility of pathogen inactivation technology: a real-life example of Leishmania infantum inactivation in platelets from a donor with an asymptomatic infection. Blood Transfus 2012;10:536-41.
6. Jimenez-Marco T, Fisa R, Girona-Llobera E, et al. Transfusion-transmitted leishmaniasis: a practical review. Transfusion 2016;56:S45-51.
7. Jimenez-Marco T, Riera C, Girona-Llobera E, et al. Strategies for reducing the risk of transfusion-transmitted leishmaniasis in an area endemic for Leishmania infantum: a patient- and donor-targeted approach. Blood Transfus 2018;16:130-6.
8. Benjamin RJ, Stramer SL, Leiby DA, et al. Trypanosoma cruzi infection in North America and Spain: evidence in support of transfusion transmission. Transfusion 2012;52:1913-21.
9. Cancino-Faure B, Fisa R, Riera C, et al. Evidence of meaningful levels of Trypanosoma cruzi in platelet concentrates from seropositive blood donors. Transfusion 2015;55:1249-55.
10. Dodd RY, Foster GA, Stramer SL. Keeping Blood Transfusion Safe from West Nile Virus: American Red Cross Experience, 2003 to 2012. Transfus Med Rev 2015;29:153-61.
11. Vanlandingham DL, Keil SD, Horne KM, et al. Photochemical inactivation of chikungunya virus in plasma and platelets using the Mirasol pathogen reduction technology system. Transfusion 2013;53:284-90.
12. Musso D, Cao-Lormeau VM, Gubler DJ. Zika virus: following the path of dengue and chikungunya? Lancet 2015;386:243-4.
13. Cap AP, Pidcoke HF, Keil SD, et al. Treatment of blood with a pathogen reduction technology using ultraviolet light and riboflavin inactivates Ebola virus in vitro. Transfusion 2016;56:S6-15.
14. Rebulla P. The long and winding road to pathogen reduction of platelets, red blood cells and whole blood. Br J Haematol 2019;186:655-67.
15. Corash L, Lin L. Novel processes for inactivation of leukocytes to prevent transfusion- associated graft versus-host disease. Bone Marrow Transplant 2004;33:1-7.
16. Muench MO, Heitman JW, Inglis H, et al. Reduced alloimmunization in mice following repeated transfusion with pathogen-reduced platelets. Transfusion 2016;56:1419-29.
17. Norris PJ, Kaidarova Z, Maiorana E, et al. Ultraviolet light based pathogen inactivation and alloimmunization after platelet transfusion: results from a randomized trial. Transfusion 2018;58:1210-7.
18. Łętowska M, Przybylska Z, Piotrowski D, et al. Hemovigilance survey of pathogen-reduced blood components in the Warsaw Region in the 2009 to 2013 period. Transfusion 2016;56:S39-44.
19. Piotrowski D, Przybylska-Baluta Z, Jimenez-Marco T, et al. Passive haemovigilance of blood components treated with a riboflavin-based pathogen reduction technology. Blood Transfus 2018;16:348-51.
20. Girona-Llobera E, Jimenez-Marco T, Galmes-Trueba A, et al. Reducing the financial impact of pathogen inactivation technology for platelet components: our experience. Transfusion 2014;54:158-68.
21. Jimenez-Marco T, Garcia-Recio M, Girona-Llobera E. Our experience in riboflavin and ultraviolet light

pathogen reduction technology for platelets: from platelet production to patient care. Transfusion 2018;58:1881-9.
22. Jimenez-Marco T, Ruiz-Alderton D, Bautista-Gili AM, et al. Role of Riboflavin- and UV Light-Treated Plasma in Prevention of Transfusion-Related Acute Lung Injury. Transfus Med Hemother 2014;41:172-5.
23. Jimenez-Marco T, Garcia-Recio M, Girona-Llobera E. Use and safety of riboflavin and UV light-treated platelet transfusions in children over a five-year period: focusing on neonates. Transfusion 2019;59:3580-8.
24. Jimenez-Marco T, Cancino-Faure B, Girona-Llobera E, et al. The effectiveness of riboflavin and ultraviolet light pathogen reduction technology in eliminating Trypanosoma cruzi from leukoreduced whole blood. Transfusion 2017;57:1440-7.
25. Irsch J, Lin L. Pathogen Inactivation of Platelet and Plasma Blood Components for Transfusion Using the INTERCEPT Blood System™. Transfus Med Hemother 2011;38:19-31.
26. Irsch J, Seghatchian J. Update on pathogen inactivation treatment of plasma, with the INTERCEPT Blood System: Current position on methodological, clinical and regulatory aspects. Transfus Apher Sci 2015;52:240-4.
27. Goodrich RP, Doane S, Reddy HL. Design and development of a method for the reduction of infectious pathogen load and inactivation of white blood cells in whole blood products. Biologicals 2010;38:20-30.
28. Marschner S, Goodrich R. Pathogen Reduction Technology Treatment of Platelets, Plasma and Whole Blood Using Riboflavin and UV Light. Transfus Med Hemother 2011;38:8-18.
29. Mohr H, Steil L, Gravemann U, et al. A novel approach to pathogen reduction in platelet concentrates using short-wave ultraviolet light. Transfusion 2009;49:2612-24.
30. Seghatchian J, Tolksdorf F. Characteristics of the THERAFLEX UV-Platelets pathogen inactivation system - an update. Transfus Apher Sci 2012;46:221-9.
31. Gravemann U, Handke W, Müller TH, et al. Bacterial inactivation of platelet concentrates with the THERAFLEX UV-Platelets pathogen inactivation system. Transfusion 2019;59:1324-32.
32. Yonemura S, Doane S, Keil S, et al. Improving the safety of whole blood-derived transfusion products with a riboflavin-based pathogen reduction technology. Blood Transfus 2017;15:357-64.
33. Schubert P, Johnson L, Marks DC, et al. Ultraviolet-Based Pathogen Inactivation Systems: Untangling the Molecular Targets Activated in Platelets. Front Med (Lausanne) 2018;7;5:129.
34. Johnson L, Hyland R, Tan S, et al. In vitro Quality of Platelets with Low Plasma Carryover Treated with Ultraviolet C Light for Pathogen Inactivation. Transfus Med Hemother 2016;43:190-7.
35. Lozano M, Galan A, Mazzara R, et al. Leukoreduced buffy coat-derived platelet concentrates photochemically treated with amotosalen HCl and ultraviolet A light stored up to 7 days: assessment of hemostatic function under flow conditions. Transfusion 2007;47:666-71.
36. Perez-Pujol S, Tonda R, Lozano M, et al. Effects of a new pathogen-reduction technology (Mirasol PRT) on functional aspects of platelet concentrates. Transfusion 2005;45:911-9.
37. Ballester-Servera C, Jimenez-Marco T, Morell-Garcia D, et al. Haemostatic function measured by thromboelastography and metabolic activity of platelets treated with riboflavin and UV light. Blood Transfus 2020;18:280-289.
38. Prudent M, D'Alessandro A, Cazenave JP, et al. Proteome changes in platelets after pathogen inactivation--an interlaboratory consensus. Transfus Med Rev 2014;28:72-83.
39. Feys HB, Van Aelst B, Compernolle V. Biomolecular Consequences of Platelet Pathogen Inactivation Methods. Transfus Med Rev 2019;33:29-34.
40. Vostal JG. Efficacy evaluation of current and future platelet transfusion products. J Trauma 2006;60:S78-82.
41. Goodrich RP, Li J, Pieters H, et al. Correlation of in vitro platelet quality measurements with in vivo platelet viability in human subjects. Vox Sang 2006;90:279-85.
42. Mathai J, Resmi KR, Sulochana PV, et al. Suitability of measurement of swirling as a marker of platelet shape change in concentrates stored for transfusion. Platelets 2006;17:393-6.
43. Picker SM, Schneider V, Oustianskaia L, et al. Cell viability during platelet storage in correlation to cellular metabolism after different pathogen reduction technologies. Transfusion 2009;49:2311-8.
44. Murphy S, Snyder E, Cable R, et al. SPRINT Study Group. Platelet dose consistency and its effect on the number of platelet transfusions for support of thrombocytopenia: an analysis of the SPRINT trial of platelets photochemically treated with amotosalen HCl and ultraviolet A light. Transfusion 2006;46:24-33.
45. Apelseth TØ, Bruserud O, Wentzel-Larsen T, et al. In-vitro evaluation of metabolic changes and residual platelet responsiveness in photochemically-treated and gamma-

irradiated single donor platelet concentrates during long-term storage. Transfusion 2007;47:653-65.
46. Keuren JF, Cawenberghs S, Heeremans J, et al. Platelet ADP response deteriorates in synthetic storage media. Transfusion 2006;46:204-12.
47. Estcourt LJ, Malouf R, Hopewell S, et al. Pathogen-reduced platelets for the prevention of bleeding. Cochrane Database Syst Rev 2017;7:CD009072.
48. AuBuchon JP, Herschel L, Roger J, et al. Efficacy of apheresis platelets treated with riboflavin and ultraviolet light for pathogen reduction. Transfusion 2005;45:1335.
49. Bashir S, Cookson P, Wiltshire M, et al. Pathogen inactivation of platelets using ultraviolet C light: effect on in vitro function and recovery and survival of platelets. Transfusion 2013;53:990-1000.
50. Snyder E, Raife T, Lin L, et al. Recovery and life span of 111indium-radiolabeled platelets treated with pathogen inactivation with amotosalen HCl (S-59) and ultraviolet A light. Transfusion 2004;44:1732-40.
51. van Rhenen D, Gulliksson H, Cazenave JP, et al. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood 2003;101:2426-33.
52. McCullough J, Vesole DH, Benjamin RJ, et al. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial. Blood 2004;104:1534-41.
53. Janetzko K, Cazenave JP, Klüter H, et al. Therapeutic efficacy and safety of photochemically treated apheresis platelets processed with an optimized integrated set. Transfusion 2005;45:1443-52.
54. Snyder E, McCullough J, Slichter SJ, et al. Clinical safety of platelets photochemically treated with amotosalen HCl and ultraviolet A light for pathogen inactivation: the SPRINT trial. Transfusion 2005;45:1864-75.
55. Kerkhoffs JL, van Putten WL, Novotny VM, et al. Clinical effectiveness of leucoreduced, pooled donor platelet concentrates, stored in plasma or additive solution with and without pathogen reduction. Br J Haematol 2010;150:209-17.
56. Lozano M, Knutson F, Tardivel R, et al. A multi-centre study of therapeutic efficacy and safety of platelet components treated with amotosalen and ultraviolet A pathogen inactivation stored for 6 or 7 d prior to transfusion. Br J Haematol 2011;153:393-401.
57. Rebulla P, Vaglio S, Beccaria F, et al. Clinical effectiveness of platelets in additive solution treated with two commercial pathogen-reduction technologies. Transfusion 2017;57:1171-83.
58. Garban F, Guyard A, Labussière H, et al. Evaluation of the Efficacy of Platelets Treated With Pathogen Reduction Process (EFFIPAP) Study Group. Comparison of the Hemostatic Efficacy of Pathogen-Reduced Platelets vs Untreated Platelets in Patients With Thrombocytopenia and Malignant Hematologic Diseases: A Randomized Clinical Trial. JAMA Oncol 2018;4:468-75.
59. Mirasol Clinical Evaluation Study Group. A randomized controlled clinical trial evaluating the performance and safety of platelets treated with MIRASOL pathogen reduction technology. Transfusion 2010;50:2362-75.
60. van der Meer PF, Ypma PF, van Geloven N, et al. Hemostatic efficacy of pathogen-inactivated vs untreated platelets: a randomized controlled trial. Blood 2018;132:223-31.
61. Schulz WL, McPadden J, Gehrie EA, et al. Blood Utilization and Transfusion Reactions in Pediatric Patients Transfused with Conventional or Pathogen Reduced Platelets. J Pediatr 2019;209:220-5.
62. Trakhtman P, Karpova O, Balashov D, et al. Efficacy and safety of pathogen-reduced platelet concentrates in children with cancer: a retrospective cohort study. Transfusion 2016;56:S24-8.
63. Domanović D, Ushiro-Lumb I, Compernolle V, et al. Pathogen reduction of blood components during outbreaks of infectious diseases in the European Union: an expert opinion from the European Centre for Disease Prevention and Control consultation meeting. Blood Transfus 2019;17:433-48.
64. Benjamin RJ, Braschler T, Weingand T, et al. Hemovigilance monitoring of platelet septic reactions with effective bacterial protection systems. Transfusion 2017;57:2946-57.
65. Jutzi M, Mansouri Taleghani B, Rueesch M, et al. Nationwide Implementation of Pathogen Inactivation for All Platelet Concentrates in Switzerland. Transfus Med Hemother 2018;45:151-6.
66. Ciaravi V, McCullough T, Dayan AD. Pharmacokinetic and toxicology assessment of INTERCEPT (S-59 and UVA treated) platelets. Hum Exp Toxicol 2001;20:533-50.
67. Reddy HL, Dayan AD, Cavagnaro J, et al. Toxicity testing of a novel riboflavin based technology for pathogen reduction and white blood cell inactivation. Transfus Med Rev 2008;22:133-53.
68. Jacquot C, Delaney M. Pathogen-inactivated blood products for pediatric patients: Blood safety, patient safety,

or both? Transfusion 2018;58:2095-101.
69. INTERCEPT® Blood System for Platelets [approval date: December 18, 2014, accessed November 1st, 2020]. Available online: https://www.fda.gov/vaccines-blood-biologics/approved-blood-products/intercept-blood-system-platelets
70. A Open Label, Post Marketing Surveillance Study Following Transfusion of INTERCEPT Platelet Components (PIPER) [published September 15, 2015, accessed November 1st, 2020]. Available online: https://clinicaltrials.gov/ct2/show/NCT02549222
71. Efficacy of Mirasol-treated Apheresis Platelets in Patients With Hypoproliferative Thrombocytopenia (MIPLATE) [published November 16, 2016, accessed November 1st, 2020]. Available online: https://clinicaltrials.gov/ct2/show/NCT02964325
72. Phase III, randomized, double-blind, multicentre clinical trial on clinical efficacy and safety of platelet concentrates treated with the THERAFLEX UV-Platelets procedure in comparison to conventional platelet components (Capture). [published July 27, 2017, accessed November 1st, 2020]. Available online: https://www.clinicaltrialsregister.eu/ctr-search/trial/2015-001035-20/DE
73. Prowse CV. Component pathogen inactivation: a critical review. Vox Sang 2013;104:183-99.
74. Lozano M, Cid J. Pathogen inactivation: coming of age. Curr Opin Hematol 2013;20:540-5.
75. Klein HG, Glynn SA, Ness PM, et al. Research opportunities for pathogen reduction/inactivation of blood components: summary of an NHLBI workshop. Transfusion 2009;49:1262-8.
76. Drew VJ, Barro L, Seghatchian J, et al. Towards pathogen inactivation of red blood cells and whole blood targeting viral DNA/RNA: design, technologies, and future prospects for developing countries. Blood Transfus 2017;15:512-21.
77. Vermeulen M, Lelie N, Coleman C, et al. Assessment of HIV transfusion transmission risk in South Africa: a 10-year analysis following implementation of individual donation nucleic acid amplification technology testing and donor demographics eligibility changes. Transfusion 2019;59:267-76.
78. Seed CR, Styles CE, Hoad VC, et al. Effect of HIV pre-exposure prophylaxis (PrEP) on detection of early infection and its impact on the appropriate post-PrEP deferral period. Vox Sang 2021;116:379-87.
79. Hauser L, Roque-Afonso AM, Beylouné A, et al. Hepatitis E transmission by transfusion of Intercept blood system-treated plasma. Blood 2014;123:796-7.
80. Schmidt M, Hourfar MK, Sireis W, et al. Evaluation of the effectiveness of a pathogen inactivation technology against clinically relevant transfusion-transmitted bacterial strains. Transfusion 2015;55:2104-12.
81. Ragan I, Hartson L, Pidcoke H, et al. Pathogen reduction of SARS-CoV-2 virus in plasma and whole blood using riboflavin and UV light. PLoS One 2020;15:e0233947.
82. Eickmann M, Gravemann U, Handke W, et al. Inactivation of three emerging viruses - severe acute respiratory syndrome coronavirus, Crimean-Congo haemorrhagic fever virus and Nipah virus - in platelet concentrates by ultraviolet C light and in plasma by methylene blue plus visible light. Vox Sang 2020;115:146-51.
83. Hashem AM, Hassan AM, Tolah AM, et al. Amotosalen and ultraviolet A light efficiently inactivate MERS-coronavirus in human platelet concentrates. Transfus Med 2019;29:434-41.
84. Hindawi SI, Hashem AM, Damanhouri GA, et al. Inactivation of Middle East respiratory syndrome-coronavirus in human plasma using amotosalen and ultraviolet A light. Transfusion 2018;58:52-9.
85. Eickmann M, Gravemann U, Handke W, et al. Inactivation of Ebola virus and Middle East respiratory syndrome coronavirus in platelet concentrates and plasma by ultraviolet C light and methylene blue plus visible light, respectively. Transfusion 2018;58:2202-7.
86. Tonn T, Corman VM, Johnsen M, et al. Stability and neutralising capacity of SARS-CoV-2-specific antibodies in convalescent plasma. Lancet Microbe 2020;1:e63.

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Supplementary

Table S1 Study design, PLT characteristics, recruited patients, primary and secondary outcomes and results of randomized clinical trials of the three PRT methods, comparing PRT-treated PLTs with untreated control PLTs

Intercept 2002 CE mark	**euroSPRITE** 2003	**SPRINT** 2004	**Janetzko *et al.*** 2005	**HOVON** 2010	**TESSI** 2011	**IPTAS** 2017	**EFFIPAP** 2018	**PIPER** Study start date: December 2015
Study design	Controlled, randomized, double-blinded trial.	Controlled, randomized, double-blinded trial	Multicentre, controlled, randomized, double-blinded trial	Multicentre, open-label, randomized, non-inferiority trial	Multicentre prospective, randomized, controlled, double blinded, non-inferiority trial	Noninferiority, randomized, controlled trial	Noninferiority, randomized, 3-arm clinical trial	Prospective open-label, post-marketing surveillance study
PLT concentrates	PRT-treated and control PLTs derived from buffy coats diluted in PAS, and stored for up to 5 days	PRT-treated PLTs diluted in PAS and control PLTs diluted in 100% plasma, both collected by apheresis and stored for up to 5 days	PRT-treated PLTs diluted in PAS and control PLTs diluted in 100% plasma, both collected by apheresis. and stored for up to 5 days	PLTs derived from buffy coats; 3 arms: PRT-treated PLTs in PAS, *Control PLTs in PAS and **control. PLTs in 100% plasma, stored for up to 7 days	PRT-treated and control PLTs derived from buffy coats and apheresis diluted in PAS. and stored for 6 or 7 days	PRT-treated and control PLTs derived from buffy coats and apheresis diluted in PAS and stored for a maximum of 5 days	Apheresis or buffy coat PLTs; 3 arms: PRT-treated PLTs in PAS, *Control PLTs in PAS and **control PLTs in plasma	PRT-treated and control PLTs
Patients (T/C)	103 (52/51)	645 (318/327)	43 (22/21)	278 (85/94*/99**)	199 (101/98)	228 (113/115)	790 (263/265*/262**)	Estimated enrollment: 3,070 participants
Primary outcome	1-h CI and 1-h CCI	Proportion of patients with WHO grade 2 bleeding	1-hr CI and 1-hr CCI for the first 8 PLT transfusions	1-h CCI	1-h CCI with an acceptable inferiority of 30%	The proportion of patients with WHO grade 2 or higher bleeding	The proportion of patients with WHO grade 2 or higher bleeding	The proportion of patients requiring assisted mechanical ventilation in emergency
Secondary outcome	24-h CI and 24-h CCI; the number of PLT transfusions; the interval between PLT transfusions; clinical hemostasis, the number of RBC units; the proportion of patients with PLT refractoriness, and the proportion of patients with alloimmunization	Proportion of patients with WHO grade 3 or 4 bleeding; 1-hr and 24-hr CI and CCI; the number of days to next PLT transfusion.	1-h CI; 1-h CCI; 24-h CCI for all PLT transfusions; PLT transfusion frequency and interval; clinical hemostasis after PLT transfusion; number of RBC transfusions.	24-h CCI; bleeding; RBC and PLT transfusion requirement; PLT transfusion interval and adverse transfusion reactions.	1-h and 24-h CI; 24-h CCI; time to next PLT transfusion; RBC use; bleeding and adverse events.	Time to grade 2 or higher bleeding event; the number of days with grade 2 or higher bleeding; the number of transfused PLTs; proportion of patients with acute transfusion reactions; posttransfusion PLT CI; proportion of patients developing PLT transfusion refractoriness	Proportion of patients with grade 3 or 4 bleeding events; the number of days with grade 2 or higher bleeding; 24-h CCI; interval between the first and second transfusions; the number of PLT units and the total number of PLTs transfused per patient; PLT transfusion refractoriness	Time from first PLT transfusion to onset of treatment; emergency assisted mechanical ventilation; adverse events occurring within 24 h after the initiation of a PC transfusion
Results	Although 1-h CI was less for the PRT-treated than the control group, the differences were not significant. However, 24-h CI and 24-h CCI were less for the PRT-treated group. Clinical hemostasis, hemorrhagic adverse events, and overall adverse events did not differ between the treatment groups	The proportion of patients with grade 2, 3 or 4 bleeding was equivalent for both groups. 1-h CCI was less for the PRT-treated group and the number of days to the next PLT transfusion (1.9 PRT versus 2.4 control) and the number of PLT transfusions were significantly different between groups. PLT clinical refractoriness occurred in 21.4% of PRT-treated patients compared to 7.0% of the control group	1 and 24-h CI and 1 and 24-h CCI was lower in the PRT group than in the control. However, the mean difference was not significant. Number, frequency, and dose of PLT transfusions, acute transfusion reactions and adverse events were similar between the two groups	1-hr and 24hr-CCI were significantly lower for PRT-treated PLTs in PAS than for control PLTs in 100% Plasma. Bleeding was significantly higher in the group with PRT-treated PLTs in PAS	1-h CCI was not significantly different between groups. 24-h CCI was significantly lower for the PRT-treated PLT group than the control group. Post-transfusion bleeding and RBC use were not significantly different between groups, nor was the median time to the next PLT transfusion	Due to early termination of the study, non-inferiority of PR-treated PLTs was not proven. However, 24-h CCI, 1-h CI and 24-h CI were significant lower in PRT-treated PLTs versus the control. Refractoriness was significantly more frequent in recipients of PRT-treated PLTs versus the control	PRT-treated PLTs were non-inferior to control PLTs in PAS. Such non-inferiority was not achieved when comparing PRT-treated with control PLTs in plasma. The frequency of severe bleeding (grade 3 and 4) was not different among the treatment arms. 24-h CCI was significantly lower in PRT-treated PLTs compared with the other 2 arms. Patients in the PRT-treated PLTs group received significantly more transfusions	Study currently in progress

Mirasol 2007 CE mark	**MIRACLE** 2010	**IPTAS** 2017	**PREPAReS** 2018	**MIPLATE** Study start date May 2017 Study completion date June 2020
Study design	Noninferiority, randomized, controlled trial	Noninferiority, randomized, controlled trial	Multicentre, noninferiority randomized controlled trial	Randomized, parallel assignment, double non-blinded trial
PLT concentrates	PRT-treated and control PLTs derived from buffy coats and apheresis were diluted in plasma and stored for a maximum of 5 days.	PRT-treated and control PLTs derived from buffy coats and apheresis were diluted in PAS and stored for a maximum of 5 days	PRT-treated and control PLTs derived from buffy coats resuspended in plasma were stored for up to 5 or 7 days, depending on the blood center	PRT-treated and control PLTs obtained by apheresis in 100% plasma
Patients	110 (56/54)	195 (99/96)	556 (277/279)	330 participants
Primary outcome	1-h CCI	The proportion of patients with WHO grade 2 or higher bleeding	The proportion of transfusion-treatment periods in which the patient had grade 2 or higher bleeding	Number of days of WHO grade 2 or higher bleeding
Secondary outcome	24-h CCI; interval between transfusions; number of PLT and RBC transfusions; number of PLTs transfused normalized by body surface area and for the number of days in the treatment period; evidence of refractoriness	Time to a grade 2 or higher bleeding event; number of days with grade 2 or higher bleeding; number of transfused PLTs; proportion of patients with acute transfusion reactions; posttransfusion PLTs CI; proportion of patients developing PLT transfusion refractoriness	HLA antibody formation to determine whether PRT-PLTS are able to reduce alloimmunization in hemato-oncology patients	HLA alloimmunization; proportion of subjects with ≥ grade 2 bleeding; time to first ≥ grade 2 bleeding event; proportion of subjects with ≥ grade 3 bleeding; proportion of subjects with PLT transfusion refractoriness; immune PLT transfusion refractoriness
Results	PRT-treated PLT CCI was significantly lower than the control CCI. The study failed to demonstrate noninferiority for 1-h CCI and 24-h CCI. PLT and RBC utilization in the two groups was not significantly different	Due to the early termination of the study, non-inferiority of PRT-treated PLTs was not proven. However, 1-h, 24-h CCI, and 24-h CI were significantly lower in the PRT-treated PLT group versus the control. Refractoriness was significantly more frequent in recipients of PRT-treated PLTs versus the control group	The non-inferiority criterion for PRT-treated PLTs was met in the intention-to-treat analysis but not in the per-protocol analysis. All transfusion-increment parameters were significantly lower for PRT-treated PLTs. A higher number of PLT transfusions, and a shorter PLT transfusion interval was observed in PRT-treated PLTs. There was no difference in the proportion of patients developing HLA class I alloantibodies	Data analysis in progress

Theraflex 2009 CE mark	**CAPTURE** Study start date July 2015
Study design	Phase III, randomized, double-blind, multicenter clinical trial on clinical efficacy and safety of platelet concentrates treated with the THERAFLEX UV-Platelets procedure in comparison to conventional platelet components
PLT concentrates	PRT-treated and control PLTs diluted in PAS
Patients	N.R.
Primary outcome	1-h CCI of not more than 30% less than the control
Secondary outcome	24-h CCI, and 1-h and 24-h CI; PLT and RBC transfusion support; rate of bleeding ≥ WHO grade 3 and grade 4; rate of clinical refractoriness; rate of immunologic refractoriness; frequency of alloimmunization to neoantigens on PLTs; rate of PLT transfusion-related adverse events (AEs) and serious adverse events (SAE)
Results	Study currently in progress

PCs were prepared from five pooled whole-blood buffy-coats (BC). C, control; CI, count increment; CCI, corrected count increment; N.R, not reported; T (PRT-treatment); WHO, World Health Organization; RBC, Red Blood Cell; HLA, Human leukocyte antigen.

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