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Cold-Stored Amotosalen-UVA Pathogen-Reduced Platelet Concentrates Display Distinct Platelet Subpopulations With Specific Functional Properties

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BACKGROUND: Current platelet transfusion requirements are evolving rapidly, with a significant increase in the need for therapeutic platelet transfusions compared to prophylactic. In this context, cold-stored platelets have gained interest owing to their potentially advantageous characteristics, including the possible emergence of specific platelet subpopulations with particular functional properties.

AIMS: To assess the emergence of various platelet subpopulations and related functional properties in buffy-coat (BC) platelet concentrates (PCs) treated with amotosalen-UVA pathogen reduction and stored at 22°C or 4°C for up to 21 days.

METHODS: A pool-and-split strategy was used to obtain double-dose BC-PCs collected into PAS-III/plasma (55/45%) treated with amotosalen-UVA and stored at 22°C with constant agitation or at 4°C without agitation. Various platelet subpopulations were identified using a combination of platelet activation markers (P-selectin and phosphatidylserine exposure, mitochondrial transmembrane potential, PAC-1 binding for activated GPIIbIIIa) analyzed by multicolor flow cytometry. Platelet aggregation was measured by light transmission aggregometry, and real time thrombin generation by calibrated automated thrombography (CAT). Platelet contribution to clot formation was evaluated in reconstituted whole blood (Ht 40%; platelet count 300 G/L) using the Quantra QPlus Hemostasis analyzer (HemoSonics LLC, Stago). Thrombus formation was measured on a collagen-coated surface under flow conditions (1500 s-1).

RESULTS: At 22°C, flow cytometry analysis indicated that the subpopulation of resting platelets dominated up to day 7. In contrast, at 4°C, resting platelets were progressively replaced by procoagulant platelets accounting for 35% of PC from days 7 to 21, and by apoptotic platelets rising to half of PC by day 21. Cold-stored platelets displayed enhanced thrombin generation capacity by day 7, as shown by increased peak thrombin concentration and increased endogenous thrombin potential compared to room temperature-stored PCs, in agreement with the increased proportion of procoagulant platelets. Aggregation to various agonists (collagen, PAR-1 agonist peptide or arachidonic acid) decreased progressively during storage, with better retention of aggregation at 4°C than at 22°C, when compared on the same day. In contrast, when compared with day 7 at 22°C, cold-stored platelets showed reduced aggregation responses at day 14 and 21, consistent with presence of a majority of procoagulant platelets, which are characterised by the loss of aggregation capacity. The ability of cold-stored platelets to form thrombi gradually diminished over time up to day 14 and disappeared at day 21. Cold-stored platelets displayed faster clot formation as compared to platelets stored at 22°C, in agreement with CAT measurements. Interestingly, cold-stored platelets displayed lower clot stiffness consistent with the presence of a predominant proportion of procoagulant platelets, lacking the ability to aggregate, contract and contribute to clot strengthening.

SUMMARY/CONCLUSIONS: Cold-stored platelets have an activated profile consisting of procoagulant and apoptotic platelet subpopulations gradually replacing resting platelets. Accordingly, cold-stored platelets exhibit increased thrombin generation, reduced aggregation capacity and reduced clot firmness. Whether these properties affect their efficacy in the therapy of bleeding patients, is currently under clinical investigation.

Reduced Hemoglobin Use with Amustaline/Glutathione Pathogen-Reduced Red Cells in Complex Cardiac Surgery: Results of a Randomized, Controlled Phase III Trial

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BACKGROUND: The Red Cell Pathogen Inactivation (ReCePI) trial was a Phase III, double-blinded, randomized noninferiority study comparing amustaline/glutathione pathogen-reduced (PR) and conventional red blood cells (RBCs) for the support of acute anemia in complex cardiac surgery.

AIMS: Blood utilization was compared between the study arms.

METHODS: Complex cardiac or thoracic-aorta surgery patients were randomized to receive conventional (Control) or PR RBCs (Test) during and for 7 days after surgery. Patient hemoglobin (Hb) levels were assessed at baseline, daily for 7 days and at 28 days after surgery. Surgical and post-operative blood loss were estimated from medical records. The primary endpoint was the incidence of acute kidney injury (AKI) measured as change in serum creatinine from baseline within 48 hours of surgery, a strong predictor of adverse postoperative outcomes. Blood utilization (number of components and total red cell Hb transfused [measured as volume x Hb concentration of each RBC]) was prospectively assessed as one indicator of efficacy.

RESULTS: Five-hundred and eighty-one subjects were randomized in 18 US hospitals and 321 (55%) (159 Test and 162 Control) RBC transfused recipients constituted the modified intent-to-treat (mITT) population. Test and Control subjects had similar medical histories and baseline characteristics, underwent similar types of surgery with similar median [IQR] total blood loss (Test 1500 [940-2475] mL, Control 1733 [1060-2880] mL, p=0.310) over seven days and had comparable hemoglobin levels at baseline, immediately post-surgery (median (IQR] 9.8 [8.9-10.9] g/dL Test, 9.6 [8.6-10.6] g/dL Control, p=0.157) and for 28 days after surgery. PR RBC units contained ~5% less mean total Hb (median [IQ] Test 58.0 (53.0-62.0) g Hb vs. Control 61.0 (57.0-66.0) g Hb, p<0.001). Test RBC were slightly older at the time of transfusion (median [IQR] Test 23.8 (16.9-29.4) days vs. Control 21.8 (15.0-28.3) days, p<0.001). The PR RBC arm was transfused with ~10% less total red cell Hb, measured as total study plus non-study Hb transfused over 7-days (median [IQR] Test 169.0 [102.0-240.0] g Hb vs. Control 188.0 [126.0-295.0] g Hb, p=0.008). Both groups received a median of three (3) RBC components. However, 48/162 (29.6%) Control and 32/159 Test (20.1%) subjects required 5 or more RBC units within 7 days (p=0.05), and more Control subjects required non-study RBC transfusions after exceeding the available supply of study RBCs [Test 35/159 (22.0%) subjects vs. Control 45/162 (27.8%) subjects]. Plasma utilization was significantly less in the Test group (median [IQR] Test 2 (1-3) units vs. Control 2 (2-4) units, P=0.021) while platelet and cryoprecipitate use were not different. The incidence of AKI was 29.3% (46/157) for Test and 28.0% (45/161) for Control subjects in the modified intention-to treat group. Non-inferiority for the incidence of AKI was achieved in both the mITT (p<0.001) and the per protocol analyses (P=0.03). Adverse events, serious adverse events and deaths on study were not different.

SUMMARY/CONCLUSIONS: PR RBCs and conventional RBCs demonstrated equivalent support for acute bleeding patients undergoing cardiac or thoracic-aorta surgery while using fewer plasma units and ~10% less transfused total red cell Hb. The incidence of AKI, the primary efficacy endpoint, in patients transfused with PR RBCs was non-inferior to that observed with conventional RBCs.

The INTERCEPT Red Blood Cell System is in late stage clinical development in Europe, and in the US.

Surface-bound Acridine as a Novel Marker to Track Entire Units of Transfused Pathogen-Reduced Red Cells in Sickle Cell Patients

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BACKGROUND: Amustaline/glutathione pathogen reduction (PR) of red blood cells (RBCs) is an investigational process to reduce the risk of transfusion-transmitted infections and replace irradiation. RBC-surface acridine, a degradation product of amustaline, can be detected on circulating PR-RBCs by flow cytometry using an acridine-specific monoclonal (2S197-2M1) antibody.

AIMS: We used biotin and acridine labelling to track allogeneic RBCs and PR-RBCs transfused in patients with sickle cell disease (SCD) receiving chronic transfusion therapy.

METHODS: Patients received 1 unit of PR-RBCs and 1 unit of conventional RBCs. Aliquots were removed from each unit and labeled with different biotin densities. Pre-PR and PR-RBCs derived from 1 donor RBC unit were labeled at 2μg or 6μg biotin; the conventional RBC unit at 18μg. The PR-RBC unit (no biotin) and biotin-labeled Pre-PR RBC and PR-RBC aliquots were transfused followed by the conventional RBC unit and biotin-labeled conventional RBC aliquot. Flow cytometry analysis of acridine- and biotin-labeled RBCs was performed on samples drawn at 10 time points over 16 weeks during which patients received monthly conventional RBC transfusions. Acridine was detected using 2S197-2M1 followed by goat-anti-mouse IgG-phycoerythrin (PE) and biotin with streptavidin-allophycocyanin (BD Biosciences). Acridine surface density was quantitated using calibrated PE beads (BD). RBC survival was expressed as the proportion of red cell count (RCC) drawn 15 minutes post-transfusion for each biotinylated aliquot and for the non-biotinylated acridine-labeled PR-RBC unit. The study was funded by the DHHS Biomedical Advanced Research and Development Authority and the National Institutes of Health (clinicaltrials.gov NCT04426591).

RESULTS: Acridine-labeled PR RBCs (peak concentration 7.5-18.7% circulating RBCs) showed near identical survival kinetics to biotin-labeled PR-RBCs (peak concentration 0.6-1.4% of circulating RBCs). Acridine-labeled populations remained uniform and discrete over 16 weeks; however, acridine RBC-surface density declined 83% from a mean of 5,062 PE molecules/RBC at 1-hour post transfusion to 1,260 PE molecules/RBC by 7 days, decreasing further but remaining detectable with 110 PE molecules/RBC at 16 weeks. Biotin and acridine RBCs exhibited non-linear RBC survival curves with interpatient and inter-RBC component variation in lifespan and kinetics. Peak circulating RBC concentrations occurred 1-14 days after transfusion for many PR and non-PR components, suggesting initial *in vivo* sequestration of transfused RBCs with subsequent release into the circulation, followed by clearance over time. PR RBCs showed similar survival kinetics to the biotin labeled Pre-PR RBCs for the initial ~60 days post-transfusion, with a 9.2% relative decline in terminal lifespan. The volume of distribution of the acridine-labeled PR-RBC unit was used to estimate subjects' total blood volume, which was consistently lower than estimates based on sex, height and weight (Nadler).

SUMMARY/CONCLUSIONS: Survival of PR RBC units up to 16-weeks post transfusion can be tracked *in vivo* by flow cytometry measurement of RBC surface acridine with similar sensitivity as biotin labeling without extra processing or radiolabeling. PR-RBCs survival was comparable to Pre-PR RBCs from the same donation for the first ~60 days after transfusion. RBC survival, with or without PR, is non-linear and highly variable in SCD patients. **The INTERCEPT Red Blood Cell System is in late stage clinical development in Europe, and in the US.**

Four Step Optimization of a Six-Buffy-Coat Pooling Protocol for Pathogen Inactivated Double Dose Platelet Concentrates

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BACKGROUND: Globally, pooled platelet (PP) production methods vary, with the buffy coat (BC) method predominating in Europe. This approach pools 4–8 BCs to generate single or double-unit platelet concentrates. By systematically optimizing four key manufacturing steps, we achieved a significant improvement in product yield.

AIMS: We aimed to reliably produce double pathogen-inactivated platelet concentrates using a standardized pool of six BCs, aligning with EDQM guidelines for efficient and compliant platelet production.

METHODS: To optimize the pooling process for platelet concentrates, we systematically analyzed our current process, focusing on improving platelet volume and count. This approach served as the baseline, where we produced pooled platelets (PP) from 5 buffy coat (BC) pools (n=107), representing the standard product from our blood bank. In the first step, we aimed to increase platelet counts by switching the BCs pool from 5 to 6 (n=110). In the second step, we implemented a new hematology analyzer equipped with a so-called blood bank mode to enhance the accuracy of platelet measurements (n=107). In the third step, we adjusted the BC production program on our blood cell separators to yield a higher volume in both BCs and the resulting platelet concentrates (n=107). Finally, in the fourth step, we refined the softspin settings in our centrifugation protocol to further optimize platelet recovery and concentration (n=197). The efficacy of these adjustments was evaluated against a cutoff yield of ≥ 4.4×10^{11} platelets per unit, which defines the threshold for dividing a concentrate into multiple units.

RESULTS: Comparing BC5 vs BC6 the yield increased from 2.83 (SD: 0.39) to 3.12 (0.38). Comparing group BC6 vs. BC6+analyzer (BC6a) the yield per product increased from 3.12 (0.38) to 4.03 (0.49). After adapting the separation program the BC volume in mL increased from 47.78 (5.09) vs 55.59 (5.11). By comparing BC6a vs BC6+analyzer+separation the volume increased from 301.0 (IQR: 293.2-308.0) to 343.0 (332.0-352.0) and the yield from 4.03 (SD: 0.49) to 4.30 (0.53). After adapting the centrifugation protocol, volume increased from 343.0 (IQR: 332.0 – 352.0) to 358.0 (349.0 – 368.0) and yield increased from 4.30 (0.53) to 4.81 (0.58).

SUMMARY/CONCLUSIONS: In summary, changing the pooling process by our 4-step optimization significantly improved platelet yields. As a result, 76% of the products achieved a divisible unit threshold (≥ 4.4×10^{11} platelets), enabling efficient production of pathogen-inactivated double-unit platelet concentrates. These improvements reduce waste of blood products while maintaining high product quality and being compliant with regulatory and operational standards.

In Vitro Evaluation of Cold Stored and Room Temperature Double Dose Platelets Treated With Amotosalen and UVA Light

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BACKGROUND: Platelets (PLT) are normally transfused either prophylactically or therapeutically. Historically, PTL have been stored at room-temperature with constant agitation and up to 5 days, or up to 7 days if bacterial detection or a pathogen reduction treatment is performed. However, recent research suggests that the cold storage of PLT preserves their hemostatic function, which may be best for actively bleeding patients.

AIMS: To assess the biochemical and functional properties of RT and cold-stored Double Dose PLT Concentrates (DDPC) treated with Amotosalen and UVA light during storage up to 21 days. Volume and PLT content of PC, pH, swirling, pO_2, pCO_2, HCO_3^-, LDH, glucose, have been analyzed to determine PLT quality. In addition, the expression of CD62P has been also measured by flow cytometry as a marker of PLT activation.

METHODS: DDPC are prepared from 8 ABO-matched whole blood-derived (WB) buffy-coats (BC) and pooled with 280mL of platelet additive solution (PAS, Intersol Fresenius Kabi) using manual pooling method to obtain a DDPC. For each replicate, two ABO-identical DDPC are gently mixed and divided into 4 equal PCs (pool and split design). Two of these are subjected to pathogen inactivation treatment (PI – PC) with amotosalen and UVA (Intercept, Cerus Corporation), the other two are left untreated (CTR – PC). One CTR-PC and one PI-PC are stored at RT with continuous agitation, while the other two are stored at 4°C without agitation. All PCs are stored for 21 days in PLT storage containers from INTERCEPT DS kit. Samples are taken on Days 2, 5, 7, 14 and 21 for evaluation of *in vitro* PLT function and metabolism.

RESULTS: For the 12 PC replicates prepared PLT yield and PLT count are preserved well until Day 14 with a marked decrease on Day 21. The pH remained > 6.4 for all sample points except for two PCs which showed a value slightly below 6.4. MPV was evaluated in a subset of replicates (N=6) with a steady increase up to day 21. Swirling (score scale 0 – 2) was well maintained up to Day 14 in PCs stored at RT but was lower (1 or 0) in PCs stored at 4°C. The table below compares results for RT storage to cold storage on Days 2 (baseline), and Days 5, 7, 14 and 21 for the rest of biochemical and functional parameters.

SUMMARY/CONCLUSIONS: *In vitro* studies on cold-stored PLT can help understanding how storage at low temperatures affects PLT function, viability, and other characteristics crucial for their role in hemostasis. Clinical trials are being conducted to prove the efficacy compared to room-temperature-stored PLT and may demonstrate the advantages in transfusion therapy, particularly in trauma and surgical patients who require rapid hemostatic responses.

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Metabolic Parameters (N=6)	CTR-PC 22°C	CTR-PC 4°C	PI-PC 22°C	PI-PC 4°C
Glucose mg/dL				
D2	148±3	148±3	145±2	145±3
D5	92±5	119±5	95±4	119±4
D7	48±8	98±6	52±5	98±6
D14	<1	33±13	<1	33±13
D21	<1	<1	<1	<1
pO_2 mm Hg				
D2	43±22	60±21	80±34	61±21
D5	57±21	63±22	74±20	78±17
D7	74±35	67±30	82±26	93±19
D14	112±24	103±15	140±20	125±18
D21	168±8	142±11	179±6	167±6
pCO_2 mm Hg				
D2	36.2±5	36.5±5	38.7±3	38.8±2
D5	20.5±1	27.4±3	19.4±1	26.3±2
D7	17.8±1	23.5±1	17.3±1	22±2
D14	<12	13.5±1	<12	13.9±0
D21	<12	<12	<12	<12
HCO_3-mm Hg				
D2	9.1±0.3	9.1±0.2	7.8±0.3	7.8±0.3
D5	5.8±0.3	6.4±0.2	4.8±0.3	5.2±0.4
D7	3.9±0.5	4.8±0.3	3.2±0.4	3.8±0.5
D14	<0.5	1.5±0.3	<0.5	1.1±0.2
D21	<0.5	<0.5	<0.5	<0.5
LDH U/L				
D2	109±14	113±12	113±10	113±10
D5	120±20	113±11	123±14	118±12
D7	148±29	125±12	135±9	140±24
D14	227±77	143±66	200±56	169±37
D21	271±57	208±12	282±45	220±17
CD62P (MFI-PE)				
D2	517±236	561±321	358±127	368±123
D5	746±102	1786±188	814±116	1876±421
D7	1064±287	2952±1213	1239±445	3064±1050
D14	1786±302	3933±925	1869±295	5173±1575
D21	1342±403	3296±1397	1220±915	4245±1170

Amotosalen or Photo-Induced By-Products In Pathogen-Reduced Blood Products Do Not Induce Non-Specific *In-Vitro* Activation or Degranulation of Basophils From Healthy Volunteers

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BACKGROUND: Platelet concentrates (PCs) and fresh frozen plasmas (FFPs) are the leading cause of hypersensitivity transfusion reactions (HTRs). In several countries, PCs and FFPs are treated with amotosalen and UV-A (INTERCEPT™ Blood System – IBS, Cerus Corporation, Concord, CA) for pathogen reduction. The imputability of amotosalen or byproducts in HTRs remains elusive but not supported by epidemiology.

AIMS: To assess the *in-vitro* effect of free amotosalen or photo-induced by-products potentially generated in IBS-treated PCs/FFPs, on non-specific *in-vitro* activation of basophils from healthy volunteers using a basophil activation test (BAT).

METHODS: Free amotosalen (0.0003 to 30 µM) or supernatants derived from IBS-FFPs and IBS-PCs (1:10 and 1:20) were added to citrated whole blood of healthy volunteers for 30 min at 37°C. Samples were analysed by flow cytometry to identify the basophil population (IgE+/CD203c+). Their activation status was assessed by measuring the percentage of CD63 positive cells (correlated to histamine release) and the stimulation index (SI) based on CD203c upregulation calculated as mean fluorescence intensities (MFI) of CD203c on stimulated basophils divided by the MFI of CD203c on resting basophils with a threshold at 1.6. Positive controls were obtained by stimulation of blood with a mouse anti-human IgE monoclonal antibody (clone G7-18) and fMLP (an IgE-independent activating peptide). Results were expressed as mean ± SEM (n=5), with $p<0.05$ considered significant.

RESULTS: Free amotosalen had no effect on CD203c-SI or CD63 exposure at any concentrations (3 µM represents the transfusion of 10 FFPs or 2-3 PCs). FFPs (1:10 represents 10 FFPs) were tested before and after treatment with amotosalen + UV-A, then after CAD (Compound Adsorption Device) and 48 hours after storage liquid at +4°C. When PCs were stored at +22°C, CD63 exposure remained at basal levels, while CD203c-SI tended to increase up to day 3 only at 1:10 (equivalent to 2-3 PCs) without reaching statistical significance and remained stable thereafter; a higher dilution (1:20, 1 PC) had no effect on both markers. Interestingly, untreated (non-IBS) PC supernatants also displayed a nonsignificant increase in CD203c-SI over time at 1:10, suggesting that amotosalen or photo-induced by-products are not responsible for this effect. When IBS-PCs were stored at +4°C ("cold-stored platelets") up to day 21, the same tendency was observed at 1:10 for CD203c-SI as early as day 3 and then remained stable until day 21, while CD63 was not exposed. Of note, PCs stored up to day 21 at +22°C exhibited a similar tendency but reaching statistical significance only at day 21 for low dilution (1:10). These data suggest storage lesions as the main mechanism for non-specific basophil activation (without CD63 exposure) potentially involving a change in platelet subpopulations, extracellular vesicles (EVs) and/or soluble mediator release, which remains to be investigated.

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CONCLUSION: These results indicate that a wide range of concentrations of free amotosalen is unable to activate blood basophils from healthy volunteers *in-vitro*. Moreover, PC supernatants and FFPs, including residual free amotosalen and photo-induced by-products, are unable to induce non-specific basophil activation or granule release. We are currently undertaking a clinical study in patients with HTR after IBS-PC transfusion.

Hemostatic Functions of Platelets and Their Subpopulations According to Platelet Concentrate Storage Conditions, Assessed by Viscoelastic Platform

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BACKGROUND: Platelets form a homogenous population in buffy-coat platelet concentrates (BC-PCs) stored at 22°C up to 7 days, which is not the case with cold storage. Using multicolor flow cytometry analysis of platelet activation markers, we recently highlighted the emergence of procoagulant and apoptotic platelet subpopulations that progressively supplant native platelets during cold storage of PCs up to 21 days. The contribution of these various subpopulations to clot formation is not known. The Quantra Hemostasis Analyzer (HemoSonics LLC, a Stago Group company) is a novel point-of-care device based on SEER (Sonic Estimation of Elasticity via Resonance) sonorheometry to measure changes in viscoelastic properties of whole blood during clot formation. The Quantra provides a direct quantification of the Platelet Contribution to Clot Stiffness (PCS), derived from direct measurements of the clot shear modulus, which accounts for both platelet count and their ability to aggregate, contract and contribute to clot strengthening. It can thus provide a more comprehensive understanding of how platelets contribute to the mechanics of clot formation.

AIMS: To evaluate the effects of storage conditions on the hemostatic functions of PCs based on the Quantra viscoelastic analyzer.

METHODS: A pool-and-split strategy was used to obtain double-dose BC-PCs collected into PAS-III/plasma (55%/45%) treated with amotosalen-UVA (INTERCEPT™ Blood System) and stored (i) at 22-24°C with constant agitation or (ii) at 4°C without agitation up to 21 days. Platelet samples were reconstituted with washed red blood cells and thawed fresh frozen plasma (Ht 40%; platelet count 300 G/L) as a model of whole blood. The Quantra analyzer and associated QPlus cartridge-measured parameters included Clot Time (CT), Clot Stiffness (CS), Fibrinogen Contribution to clot Stiffness (FCS), Platelet Contribution to clot Stiffness (PCS). Platelet aggregation was measured by light transmission aggregometry (LTA).

RESULTS: When stored at 22°C, CT and CS of BC-PCs remained stable from D1 to D7. In contrast, CT of cold-stored BCPCs was faster at D7 (189±4 s) compared to D1 (277±10 s, p=0.005), while CS was reduced (7.6±0.8 vs. 19.0±4.0 hPa, p=0.0004). FCS remained stable, highlighting a reduced contribution of platelets to clot stiffness during cold storage, evidenced by reduced PCS at D7 compared to D1 (5.9±0.6 vs. 16.8±3.9 hPa, p=0.0003). Interestingly, the procoagulant platelet subpopulation correlated (inversely) with CT, and only in cold-stored PCs. At 22°C, the platelet count, the proportion of native platelets and the ability of platelets to aggregate on LTA highly correlated with PCS, while the proportion of apoptotic platelets showed strong inverse correlation with PCS. At 4°C, only the proportion of native platelets remained highly correlated with PCS, while procoagulant platelets in addition to apoptotic were strongly inversely correlated with PCS, in line with the loss of their aggregation properties on LTA.

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SUMMARY / CONCLUSIONS: At 4°C, platelets display faster clot formation but lower clot stiffness as compared to platelets stored at 22°C, as evaluated with the Quantra viscoelastic analyzer. These results are consistent with the decline in platelet aggregation capacity and the emergence of procoagulant and apoptotic platelet subpopulations during cold storage.

Amotosalen/UVA Treatment of Buffy Coat Platelet Concentrates in SSP+ to Inactivate Bacterial Strains Involved in Clinical TTBI Cases

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BACKGROUND: The INTERCEPT® Blood System for Platelets is intended to inactivate pathogens in donor platelets to reduce the risk of transfusion-transmitted infections (TTIs). The INTERCEPT system uses amotosalen and ultraviolet A (UVA) light to inactivate a broad spectrum of pathogens in platelet concentrates. The maximum shelf-life of platelets is typically 5 days prior to transfusion, but in combination with pathogen reduction, platelets can be stored up to 7 days in many geographies.

AIMS: The aim of this study was to evaluate the inactivation of bacterial strains involved in clinical TTBI cases in buffy coat platelet concentrates (BCPC) suspended in 47% plasma/53% SSP+ after amotosalen/UVA treatment and at Day 7 post-treatment.

METHODS: At least two units were combined to obtain a final pool volume of 420 mL with a platelet dose of 7.1 to 8.0 × 10^{11} . A minimum of four replicates were performed for each bacterial strain. The BCPC units were spiked with bacteria and treated with amotosalen/UVA. Samples were taken pre-UVA illumination (2 mL), post-UVA illumination (55 mL), post-CAD (55 mL), Day 3 post-treatment (55 mL), Day 5 post-treatment (55 mL) and Day 7 post-treatment (55 mL) and were analyzed for bacterial titer by plating on appropriate media (100 μL – 5 mL/plate) and incubated for at least 24 hours.

RESULTS: **Table 1** shows the inactivation of bacterial strains in BCPC out to Day 7 post-treatment.

Table 1: Bacterial Inactivation Using Amotosalen/UVA Treatment of Buffy Coat Platelet Concentrates in SSP+

Bacteria	Log cfu/mL	
	Pre-UVA (OJ)[a]	LRF (Log Reduction Factor)
Cutibacterium acnes CDHS 00A-6608	6.9 ± 0.1	6.9 ± 0.1
Klebsiella pneumoniae CDHS 92A-2214	2.9 ± 0.1	2.9 ± 0.1
Pseudomonas aeruginosa CDHS 91A-5818	5.4 ± 0.2	5.4 ± 0.2
Serratia marcescens CDHS 2574-3-79	4.6 ± 0.2	4.6 ± 0.2
Staphylococcus aureus CDHS 89A-3667	7.7 ± 0.2	7.7 ± 0.2
Staphylococcus epidermidis CDHS 85A-2560	6.8 ± 0.0	6.8 ± 0.0
a. Input titers assessed were determined previously to be the limit of inactivation with no detectable bacteria posttreatment.		

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SUMMARY/CONCLUSIONS: Inactivation of clinical bacterial strains in BCPC using amotosalen/UVA treatment was evaluated out to Day 7 post-treatment. After photochemical treatment with amotosalen and UVA light, 6.9 ± 0.1 log cfu/mL, 2.9 ± 0.1 log cfu/mL, 5.4 ± 0.2 log cfu/mL, 4.6 ± 0.2 log cfu/mL, 7.7 ± 0.2 log cfu/mL, and 6.8 ± 0.0 log cfu/mL of *C. acnes, K. pneumoniae, P. aeruginosa, S. marcescens, S. aureus*, and *S. epidermidis* were inactivated, respectively. While the limit of inactivation may differ depending on the bacterial strain, for each strain tested, no viable bacteria were detected at the end of platelet shelf-life.

Controlled Side-By-Side Comparison of the Impact of 2 Pathogen Inactivation Technologies on Platelet *In Vitro* Quality and Clot Formation for 7 Days of Storage

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BACKGROUND: Two different commercially available pathogen inactivation technologies (PIT) for platelets, with different modes of action, are currently in use. Metabolic *in vitro* comparison studies have been conducted previously, however, not always controlled. A significant impact of *in vitro* metabolic parameters and activation markers of certain PIT was recognized previously, as well on the clinical efficacy of platelets with respect to component use and hemostasis. Comparative data regarding clot formation, linking *in vitro* and clinical data, is still missing.

AIMS: A controlled side-by-side comparison study to assess the impact of PIT on platelet *in vitro* quality and clot formation with identical platelet units. For our knowledge, that is the first PIT side-by-side comparison study with thromboelastometry.

METHODS: Platelet units in 65% SSP+ (Macopharma) and 35% plasma were prepared from 6 pooled buffy coats using a TACSI system (Terumo BCT). For each experiment, 3 ABO identical platelet units were pooled and split into 3 identical adult transfusion units. One unit was treated with Riboflavin/UVB PIT (Mirasol PRT, Terumo BCT) (R), one unit was treated with amotosalen/UVA PIT (INTERCEPT Blood System, Cerus) (A) and one unit was kept as untreated control (C). The units were kept together until day 7 under continuous agitation at 20-22°C. Samples for further analysis were taken simultaneously at day 2, 5 and 7. Glucose and lactate were assessed with a Cobas 400 plus analyzer (Roche). HSR was assessed with an Ultrospec 2000 Photospectrometer (Pharmacia). Flow cytometry analyses were conducted with a FACSCanto II flow cytometer (Becton-Dickinson). Thromboelatometry analyses were conducted with a ROTEM delta analyzer (Werfen). Results are shown in mean ± SD. The p-value was calculated with the two-sample t-test.

RESULTS: 12 experiments were conducted. The results are indicated in table 1. The swirling score was not affected in the control and AS arm, in the RB arm it dropped 25% at day 5 and 75% at day 7. The average pH was 7.2 ± 0.1 (C), 7.1 ± 0.1 (A) and 6.7 ± 0.1 (R) at day 5; 7.1 ± 0.1 (C), 7.0 ± 0 (A) and 6.7 ± 0.1 (R) at day 7.

SUMMARY/CONCLUSIONS: Method-dependent impact of PIT on platelet *in vitro* quality and clot formation, increasing during the course of storage, should be considered when planning extension of shelf-life to 7 days. A careful validation of 7-day storage eligibility pre-implementation may be appropriate.

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(n=12)	PIT	Day 2	Day 5	Day 7
glucose (mmol/L)	C	7.16 ± 0.36	4.96 ± 0.50	2.75 ± 0.81
	A	6.84 ± 0.34	5.02 ± 0.38	3.25 ± 0.57
	R	6.04 ± 0.35	0.72 ± 0.64*	0 ± 0*
lactate (mmol/L)	C	9.62 ± 0.71	14.12 ± 1.34	19.11 ± 2.21
	A	8.98 ± 0.69	12.24 ± 0.89*	15.60 ± 1.73*
	R	9.65 ± 0.70	21.31 ± 1.40*	22.75 ± 0.86*
HSR (%)	C	76.3 ± 7.3	72.4 ± 4.7	74.1 ± 6.2
	A	82.8 ± 5.3	78.0 ± 4.6*	76.9 ± 5.3
	R	75.3 ± 5.5	58.8 ± 10.0*	9.3 ± 5.7*
annexin V (%)	C	1.1 ± 0.4	2.1 ± 0.9	3.1 ± 1.3
	A	1.5 ± 0.5	2.7 ± 0.8	5.2 ± 3.1*
	R	1.8 ± 0.6	4.5 ± 1.1*	17.3 ± 5.9*
P-Selectin (%)	C	5.9 ± 3.2	11.9 ± 3.4	21.2 ± 5.8
	A	6.8 ± 2.9	15.3 ± 3.0*	23.8 ± 3.7
	R	20.6 ± 4.6	38.6 ± 7.6*	47.6 ± 5.0*
CFT INTEM (s)	C	39.7 ± 5.3	39.2 ± 4.0	42.1 ± 6.5
	A	41.8 ± 5.9	42.1 ± 5.1	44.0 ± 5.2
	R	47.3 ± 9.6	52.0 ± 7.3*	90.7 ± 24.5*
MCF INTEM (mm)	C	74.0 ± 2.3	74.5 ± 1.8	74.8 ± 2.0
	A	72.4 ± 2.3	73.8 ± 1.6	73.6 ± 2.0
	R	72.4 ± 3.1	70.0 ± 3.4*	50.5 ± 7.6*
CFT EXTEM (s)	C	58.3 ± 17.0	55.0 ± 9.3	57.2 ± 11.0
	A	63.8 ± 11.6	66.5 ± 9.3*	70.4 ± 9.8*
	R	49.8 ± 9.0	57.3 ± 7.6	146.7 ± 63.8*§
MCF EXTEM (mm)	C	52.9 ± 9.1	47.6 ± 3.8	41.9 ± 3.8
	A	46.2 ± 4.8	41.4 ± 3.2*	37.6 ± 3.1*
	R	40.2 ± 3.2	34.4 ± 3.6*	22.8 ± 4.4*

*p-value compared to control <0.05 §n=10

The Impact of Platelet Pathogen Inactivation on Component Use: Results of a Meta-Analysis of Randomized Controlled Trials Versus Real-World Data

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BACKGROUND: The preservation of clinical efficacy of pathogen-reduced platelet concentrates (PCs) was historically assessed with CCI as an endpoint, despite the lack of any correlation between CCI and bleeding prevention. Bleeding or platelet consumption may be more meaningful clinical endpoints. The impact of pathogen inactivation (PI) treatment of PCs on platelet consumption has been assessed in multiple randomized clinical trials (RCTs) as well as in prospective observational or retrospective registry studies (real world data, RWD). Since RCTs use more stringent transfusion criteria compared to RWD studies, and in RCTs there is often a protocol-specific difference in platelet dose between test and control arm, we were interested in potential differences in the outcome focusing on the amotosalen/UVA PI technology.

AIMS: Comparative assessment of the impact of PI treatment on PC consumption, PC transfusion interval and RBC consumption in RCTs versus RWD studies.

METHODS: A meta-analysis stratified by study design (RCT vs RWD) was conducted (amotosalen/UVA (INTERCEPT Blood System, Cerus) treated PCs). Data was extracted from all publications (search terms “amotosalen” AND “platelet” on PubMed) for the following clinically meaningful endpoints, which have been evaluated in both RCTs and RWD studies: number of platelet transfusions per patient, duration of intervals between platelet transfusions and number of RBCs transfused per patient. Results are shown in mean difference (MD) and 95% CI between the test arm (T, pathogen reduced PCs) and the control arm (C, conventional PCs).

RESULTS: For the number of PC transfusions per patient endpoint, 5 RCTs (590 T/608 C transfusions) and 10 RWD studies (8065 T/6998 C transfusions) were included in the analysis. The MD is 1.48 (0.72; 2.24) for RCTs and 0.26 (-0.07; 0.59) for RWD studies. For the PC transfusion interval endpoint, 7 RCTs (613 T/628 C transfusions) and 4 RWD studies (3377 T/2997 C transfusions) were included in the analysis. The MD is -0.37 (-0.60; -0.14) for RCTs and -0.16 (-0.32; -0.01) for RWD studies. For the number of RBC transfusions per patient endpoint, 6 RCTs (695 T/714 C transfusions) and 4 RWD-studies (2587 T/2696 C transfusions) were included in the analysis. The MD is -0.05 (-0.58; 0.68) for RCTs and -0.59 (-1.33; 0.15) for RWD studies. The meta-analysis is limited by the small number of studies included and the methodological heterogeneity of the studies.

SUMMARY/CONCLUSIONS: The results indicate an increased amotosalen/UVA PI-PC consumption in RCTs only. In both RCTs and RWD studies, slightly shorter transfusion intervals were found for PI-PCs. RBC consumption did not differ between PI-PCs and PCs in both RCTs and RWD studies. Despite the methodological shortcoming of the meta-analysis, the results nevertheless point towards a potential impact of RCT protocols on the study outcome.

The Impact of Pathogen-Reduction Treatment With Two Different Technologies on Apheresis Platelet Concentrates In Platelet Additive Solution for 7 Days of Storage

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BACKGROUND: Increased shelf-life of human platelet concentrates (PCs) allows reduction of outdating rates and increased PC availability. The shelf-life of PCs can be extended to 7 days, in combination with pathogen inactivation (PI) to mitigate the risk of bacterial contamination. We have currently two PI technologies for PCs available in Russia (amotosalen/UVA and Riboflavin/UVB) for prolongation of shelf-life, which we assessed for their impact on PC *in vitro* quality.

AIMS: Assessment of the impact of PI-treatment methods on PC *in vitro* quality until end of shelf life to expand the PC shelf-life to 7 days.

METHODS: PCs in 65% SSP+ (Macopharma) were collected by apheresis with a Trima Accel device (Terumo BCT). Units were either treated with amotosalen/UVA (INTERCEPT Blood System, Cerus) in the A-arm, riboflavin/UVB (Mirasol PRT, Terumo BCT) in the R-arm or untreated in the C-arm. Post-treatment, units were stored until day 7 at 20-24°C under continuous agitation. Samples were collected 2 h post platelet collection, post-treatment and at day 3, 5 and 7 of storage for analyses. Platelet count was assessed with a Drew D3 analyzer (Drew Scientific), glucose, lactate and pH with an ABL-800 FLEX analyzer (Radiometer). Statistical analysis was conducted with the two-sample t-test. Significance was defined as $p<0.05$. Results are reported as mean ± standard deviation.

RESULTS: Twenty PCs were collected in each study arm. The donor characteristics were not significantly different between the study arms with respect to sex (female: A (3), R (5), C (5)), body weight (kg: A (85.5 ± 5.1), R (82.9 ± 3.9), C (87.3 ± 5.3)) and pre-donation platelet count ($x10^9$/L: A (231.7 ± 12.8), R (236.8 ± 11.5), C (235.3 ± 10.6)). The platelet count was not significantly different between the A- and C-arms during the course of storage. In the R-arm, it dropped significantly versus the C-arm at day 5 ($x10^{11}$/U: R (4.2 ± 0.6), C (5.6 ± 0.6)) and at day 7 (R (2.2 ± 0.5), C (3.7 ± 0.6)). pH values were comparable in all arms for all time points tested during storage. The glucose concentration was significantly lower in the A-arm compared to the C-arm at day 1 (mM/L: A (6.2 ± 0.5), C (7.0 ± 0.3)), but not at the other days of storage. The glucose concentration in R-arm was significantly lower compared to C at day 1, 3 and 5, reaching glucose use-up at day 5 (R (0.4 ± 0.4), C (3.2 ± 0.7)), while the glucose concentration was mainained comparable between the A- and C-arms at day 5 (A (3.1 ± 0.9), C (3.2 ± 0.7)). As a consequence, the lactate concentration was significantly higher in the R-arm compared to the C-arm at day 3 (mM/L: R (8.1 ± 1.0), C (4.5 ± 0.8)) and day 5 (R (13.6 ± 0.6), C (9.5 ± 1.5)), but not between the A- and C-arms. At day 7, glucose use-up and maximal lactate concentrations (mMol/L: 13.5 ± 1.3 to 16.2 ± 0.8) were reached in all study arms.

SUMMARY/CONCLUSIONS: Different PI-technologies have a different impact on PC *in vitro* quality, increasing with duration of storage. In the riboflavin/UVB arm we recognized a significant drop of platelet count together with an increased glucose consumption, pointing towards platelet loss due to glucose absence between day 5 and 7. The PI method suitable for 7-day extension of PCs should be carefully selected.

Sorting Buffy Coats By Whole Blood Platelet Counts from Donors Significantly Enhances the Proportion of Divisible Pathogen-Inactivated Platelet Units

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BACKGROUND: The buffy coat (BC) method is predominantly used in Europe when manufacturing pooled platelet products (PP). In addition to our methodological optimizations (see Poster Amato *et. al.*), we additionally achieved a marked improvement in platelet (PLT) yield when sorting BCs by PLT counts of whole blood (WB) samples from donors, using an in house-developed program.

AIMS: We aimed to further enhance yields of PP and in parallel increase the proportion of divisible PP (threshold yield of ≥ 4.4 × 10^{11} PLTs).

METHODS: To date, in our current setting, six BCs were pooled together randomly to a PP. Now, we use a hematology analyzer and measure whole blood samples from donors to get the PLT count of each donor, and then sort six BCs together appropriately. We therefore developed a program aiming to calculate the maximum possible yield for all manufactured PP per day by sorting them suitably together. 100% of the products were pathogen-inactivated using INTERCEPT (Cerus).

RESULTS: First, PP were produced by random sorting of BCs (n=61) resulting in a yield of 4.6 (interquartile range (IQR) 4.3- 4.8) × 10^{11} per PLT unit. Second, PP were produced by sorting BCs according to the report of our in-house developed program (n=57), achieving a yield of 4.8 (IQR 4.5-5.1) × 10^{11} per PLT unit. This increase was statistically significant (P=0.0191). In line, PLT (*103/μL) measured by the hematology analyzer in PP increased from 1247.0 (IQR 1207.0- 1349.0) to 1307.0 (IQR 1237.0-1381.0) when comparing randomly assigned BCs to sorted BCs. Volume of the PP did not change significantly for both approaches 366 mL (356.0-374.0) vs. 367 mL (360.0-373.0). Further, the percentage of divisible PP (yield of ≥ 4.4 × 10^{11} PLTs per unit) increased from 72.1% to 89.5% – an increase of 17.4% when the BCs were sorted according to our program rather than through random selection. When considering a threshold yield of ≥ 4.8 × 10^{11} PLTs per unit, the percentages increased from 32.8% to 57.9% for randomly assigned vs sorted BCs.

SUMMARY/CONCLUSIONS: Optimizing the pooling process by sorting BCs based on donor PLT counts using an in-house developed program increased the proportion of divisible units from 72.1% to 89.5%. This approach, using a hematology analyzer and targeted BC pooling, significantly improved yields of PP, therefore enabling the production of more PP with the same amounts of BCs (higher divisibility rate). Additionally, this optimization enables the production of high-quality pathogen-inactivated PLT concentrates with greater consistency.

Inactivation of California Encephalitis Virus in Platelet Concentrates Using Amotosalen and UVA Treatment

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BACKGROUND: California encephalitis virus (CEV) is a negative-sense, single-stranded RNA virus of the Orthobunyavirus genus, a group of viruses within the family Peribunyaviridae. CEV, like other members of the genus, is transmitted to humans through the bite of an infected mosquito. CEV infections in humans are relatively rare, however, a recent outbreak of a related virus, Oropouche (OROV) in South America and the Caribbean has highlighted the risk of mosquito borne viruses to the blood supply, particularly as the virus' reach extends to other parts of the world via travel-associated cases. Although OROV and CEV are in separate serogroups, they are both members of the same genus, highlighting their similarities in genome and virion structure. CEV was used as a representative to determine the efficacy of the INTERCEPT Blood System pathogen reduction technology to inactivate orthobunyaviruses and to indicate whether this technology may be used to mitigate risks associated with the OROV outbreak.

AIMS: The aim of this study is to evaluate the inactivation of CEV in platelet concentrates using the INTERCEPT Blood System for Platelets.

METHODS: Apheresis platelet concentrates prepared in both 100% plasma and in 35% plasma/65% platelet additive solution (PAS) were adjusted to achieve a volume of approximately 285 mL. Four independent replicates were performed for each matrix (100% plasma and 35% plasma/65% PAS), with each replicate contaminated, at a 1:100 dilution, with a stock CEV virus of approximately 6 log pfu/mL (resulting in a target of approximately 4 log pfu/mL in the contaminated unit). Each contaminated unit was dosed with amotosalen and a control sample (pre-UVA) was taken prior to UVA illumination. Each unit was then illuminated, and a post-treatment control (post-UVA) sample was taken. The pre-UVA and post-UVA viral titers were determined by plaque assay on Vero76 cells and levels of inactivation were calculated based on the difference between the log pfu/mL values in the pre- and post-treatment samples.

RESULTS: The table shows the average log reduction factor for CEV achieved in both platelets prepared in 100% plasma and in 35% plasma/65% PAS using the INTERCEPT Blood System for Platelets.

SUMMARY/CONCLUSIONS: Treatment with the INTERCEPT Blood System for Platelets inactivated 3.9 log pfu/mL of CEV in platelets prepared in 100% plasma. Comparatively, treatment inactivated ≥ 4.5 log pfu/mL of CEV in platelets prepared in 35% plasma/65% PAS. These results indicate that INTERCEPT treatment effectively inactivates CEV in platelet components and, thus, may also be an effective mitigating factor to reduce the risk of OROV transfusion transmitted infections.

Blood Component	Pre-UVA Control (Log pfu/mL)	Post-UVA Treatment (Log Reduction Factor [Log pfu/mL])
Platelets in 100% Plasma	4.3	3.9
Platelets in 35% Plasma/65% PAS	4.5	≥ 4.5

Quality Assessment of Cryoprecipitate Routinely Produced from Pathogen-Reduced Plasma

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BACKGROUND: In 2014 the Kuwait Central Blood Bank implemented the production of cryoprecipitate from pathogenreduced (amotosalen/UVA-treated), whole blood derived plasma (PRC) to mitigate the risk of transfusion transmitted infections. In 2017, an alternative automated method to produce plasma was introduced, and the PRC production protocol was revised to align with this new process.

AIMS: Assessment of the locally implemented PRC production process compliance with local and international guidelines.

METHODS: Whole blood was collected from voluntary donors, then plasma (FFP) was produced within 8 hours post collection using an automated Reveos device (Terumo BCT), or produced by a manual process. A pool of two ABO identical plasma units (≥450 mL) was treated with amotosalen/UVA pathogen reduction (INTERCEPT Blood System for Plasma, Cerus) and split into two storage containers (≥200 mL each). Each storage container was connected to a second storage container of the same size with a sterile welding device (TSCDII, Terumo BCT) followed by transfer to a shock freezer (MABAG) for 45 min (-42°C top and -44°C bottom). After overnight incubation at 4°C, the plasma containers were centrifuged for 15 min at 3000 rpm (HR-1200 rotor) at 4°C to pellet the cryoprecipitate. The cryo-poor plasma was removed with a manual extractor into the connected storage container, subsequently PRC and cry-poor plasma were shock-frozen and stored at -18°C for one year of shelf-life. Fibrinogen and FVIII content were assessed with an ACL TOP 350 analyzer (Werfen). Results are reported as mean ± standard deviation.

RESULTS: QC data of PRCs manufactured in 2023 and 2024 was included in the analysis. In 2023, 9240 PRCs were produced, 96 QC samples were analyzed during the year (1% of the units). The average volume was 28.6 ± 2.0 mL (22.5- 34.0). The average fibrinogen content was 310.7 ± 92.0 mg/unit (152.0-727.2), the average FVIII content was 74.3 ± 20.3 IU/unit (50.2-149.5). In 2024, 12903 PRCs were produced, 96 QC samples were analyzed during the year (0.75% of the units). The average volume was 28.6 ± 1.7 mL (25.0-32.0). The average fibrinogen content was 314.4 ± 82.6 mg/unit (161.0-593.3), the average FVIII content was 82.3 ± 23.9 IU/unit (51.6-188.0). All QC-tested units in 2023 and 2024 met local (≥150 mg/unit) and EDQM (≥140 mg/unit) requirements for fibrinogen content as well as local and EDQM FVIII requirements (≥50 IU/unit).

SUMMARY/CONCLUSIONS: Single-donor equivalent cryoprecipitate units produced from whole-blood-derived, amotosalen/UVA pathogen-reduced plasma, meets local and EDQM requirements.

Evaluation of Whole Blood Plasma Pool Stability After Treatment for Pathogen Reduction With Amotosalen/A Prototype LED Illuminator and 2 Years of Storage

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BACKGROUND: A new illuminator with a light-emitting diode (LED) ultraviolet A (UVA) light source has been developed for the pathogen reduction of platelets and plasma (INTERCEPT™ Blood System, Cerus). EFS participated to the validation studies for regulatory approval. The ergonomics, the software and the user interface have been updated.

AIMS: The purpose of this study was to evaluate the quality of fresh frozen whole blood-derived pooled plasma pathogen reduced by amotosalen and UVA LED light (FPP A-UVA), using a pre-production LED illuminator. A focus on the 2-year stability of this plasma at -25°C is provided.

METHODS: 19 pools of 5 units of plasma derived from leukocyte depleted whole blood were prepared, then divided into 2 parts of close to 650 mL, each connected to an INT31 plasma processing set and treated with amotosalen and UVA (LED light), before excess compound adsorption (through a CAD) and freezing to ≤-25°C within 9 ± 1 hours. The results of a selection of parameters out of the 30 tested before treatment (T2), then after treatment & CAD before freezing (T3), and after 3 months (T4: not shown), 1 year (T5: not shown) and 2 years(T6) at ≤-25°C, are presented.

RESULTS: The results of the analyses are shown in **Table 1**. FFP A-UVA treated with the LED illuminator complies with the French regulatory requirements (Official Journal of 4 June 2020) of at least 70 % of the units with Factor VIII:c ≥ 0.50 IU/mL and Fibrinogen concentration ≥2.0 g/L, with 95 % and 100% compliance, respectively, after 2-year storage. All tested parameters at T6 are within at least 90% of T3 baseline values, except Factor VIII (85.5%), TAT (76.9%) and Protein S (84.6%).

SUMMARY/CONCLUSIONS: The process of preparing pathogen reduced plasma with amotosalen and UVA generated by a LED illuminator delivers products that meet the expectations with regard to the quality of therapeutic plasma after frozen storage of at least two years. The LED illuminator brings advances in ergonomics and user interface.

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Table 1: Results of a Selection of Parameters Tested at Different Time Periods in FFP A-UVA Treated with an LED Illuminator

N=19	T2 Pre-treatment pool	T3 FFP A-UVA after CAD	T6* FFP A-UVA 2 year at ≤-25°C	T6/T3
Total Proteins (g/L)	60.08 ± 1.45	57.79 ± 1.45	57.44 ± 1.23 [s]	99.4%
Fibrinogen (g/L)	2.68 ± 0.16	2.43 ± 0.17	2.52 ± 0.16 [s]	103.7%
Factor V (IU/mL)	0.88 ± 0.07	0.86 ± 0.07	0.81 ± 0.06 [s]	94.2%
Factor VII (IU/mL)	0.99 ± 0.13	0.86 ± 0.09	0.78 ± 0.08 [s]	90.7%
Factor VIII (IU/mL)	1.15 ± 0.25	0.83 ± 0.18	0.71 ± 0.15 [s]	85.5%
Willebrand activity (Rco %)	85 ± 16.5	82 ± 16.5	91 ± 19.0 [s]	111.0%
Protein S (% activity)	100 ± 8.4	91 ± 7.0	77 ± 5.1 [s]	84.6%
TAT complexes (µg/L)	43 ± 24.7	39 ± 22.2	30 ± 17.2 [s]	76.9%
ADAMTS 13 (% activity)	101 ± 16.8	96 ± 10.6	98 ± 14.5 [ns]	102.1%
*Paired t-test T6 versus T3 – « s » if significant difference p<0.05				

Pathogen Reduced Cryoprecipitated Fibrinogen Complex Supports Collagen-Mediated Platelet Adhesion and Thrombus Formation of Pathogen-Reduced Platelet Components Stored Up to Seven Days

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BACKGROUND: Extended storage of platelet components (PC) increases supplies, but platelet hemostatic function decreases with extended storage. Platelets are transfused concurrently with fibrinogen supplements for traumatic hemorrhage resuscitation. Different types of fibrinogen supplements may differ in constituents that impact platelet function. Plasma cryoprecipitates contain fibrinogen, von Willebrand factor (vWF), FVIII, FXIII, and fibronectin compared to commercial fibrinogen concentrates (CFC) that contain primarily only fibrinogen. Previous studies demonstrated CFC contained limited functional vWF activity. The interaction of different types of fibrinogen supplements with platelets may impact microvascular hemostasis.

AIMS: To compare *in vitro* platelet thrombus formation using U.S. FDA approved amotosalen-UVA Pathogen Reduced (PR) Cryoprecipitated Fibrinogen complex or IFC (INTERCEPT™ Fibrinogen Complex, Cerus, Concord, CA) and cryoprecipitate anti-hemophilic factor (CRYO-AHF) with CFC (Fibryga, Octapharma) used concurrently with PRPC (Cerus).

METHODS: Whole blood-derived buffy coat PRPC suspended in PAS-III/plasma (55/45%) were prepared by validated methods (*Transfusion* 2013; 53:1187), stored at 22°C with constant agitation, and sampled on days 2, 5 and 7 to assess platelet hemostatic function (n=4). IFC was prepared from PR plasma using validated methods (https://intercept-usa.com). CRYO-AHF was obtained from FDA licensed U.S. blood centers. Hemostatic function assessed by thrombus formation was measured in capillary microfluidic chambers coated with type I fibrillary collagen. Hirudin anticoagulated suspensions of washed red blood cells (40% Hct) containing washed PRPC (1.0×10^5 platelets/μL) fluorescently labelled with $DIOC_6$, and CRYO-AHF, IFC or CFC with an adjusted fibrinogen level (3 mg/mL); or a buffer control without fibrinogen were perfused at 1500 s^{-1} and thrombus area was measured over 10 min. Data were analyzed by two-way analysis of variance, a p value of <0.05 was considered to be statistically significant.

RESULTS: Perfusion of reconstituted blood containing washed PRPC stored for 2 days supplemented with CRYO-AHF or IFC perfused through collagen-coated microfluidic chambers resulted in the formation of thrombi of increasing area over 10 minutes ranging from 1.0×10^4 to 1.5×10^4 μm^2 that were not significantly different between CRYO-AHF and IFC. In contrast, reconstituted blood containing washed PRPC stored for 2 days supplemented with CFC or buffer resulted in small thrombi < 5×10^3 μm^2. PRPC stored for 5 days supplemented with CRYO-AHF or IFC demonstrated retained thrombus formation compared to 2-day-old PRPC; however, PRPC supplemented with CFC or buffer demonstrated decreased adhesion and thrombus area. PRPC stored for 7 days supplemented with either CRYO-AHF or IFC retained slightly less thrombus formation capacity than 2-day stored PRPC, but resulted in substantially larger thrombi than 7-day stored PRPC supplemented with CFC or buffer, which showed minimal adhesion or thrombus area compared to IFC and supplements.

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SUMMARY/CONCLUSIONS: CRYO-AHF and IFC supported *in vitro* platelet thrombus formation of PRPC stored up to 7 days. In contrast, CFC resulted in lower levels of thrombus formation with 2-day-old PRPC, which decreased to negligible levels with 7-day-old PRPC. These studies indicate that PR IFC supplements containing fibrinogen, FVIII, vWF, FXIII, and fibronectin supported collagen-induced thrombus formation of extended storage PRPC more effectively than CFC.

Pathogen Reduced Cryoprecipitated Fibrinogen Complex (INTERCEPT® Fibrinogen Complex) manufactured using the INTERCEPT Blood System for Cryoprecipitation is only approved in the United States. INTERCEPT Fibrinogen Complex may be stored at room temperature for five days, post-thaw. In the US, cryoprecipitated AHF manufactured from INTERCEPT® Plasma must be transfused within six hours of thaw.

The INTERCEPT Blood System for 7 day storage of platelets is not approved in the US.

Investigations into the Clinical Significance of Treatment-Emergent Antibodies to Amustaline/Glutathione Pathogen-Reduced Red Blood Cells

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BACKGROUND: Subjects exposed to Amustaline/Glutathione Pathogen-Reduced Red Blood Cells (PR-RBCs) in the ReCePI Phase III randomized, controlled clinical trial developed treatment-emergent antibodies specific for PR-RBCs.

AIMS: We investigated the clinical significance of the antibodies.

METHODS: ReCePI study subjects received Test or Control study RBCs during and for 7 days after complex cardiac or thoracic aorta surgery and were screened using an indirect antiglobulin test (IAT) for PR-RBC-specific antibodies at baseline, whenever a routine IAT test was performed, and at 28- and 75-days post-surgery. Subjects with positive antibody screens were followed at ~2-week intervals and investigated for clinical evidence of hemolysis. Serological confirmation was performed at Versiti Blood Center (Milwaukee, WI) and monocyte monolayer assays (MMA) at American Red Cross (Pomona, CA). Circulating PR-RBCs were characterized by flow cytometry for surface-bound immunoglobulin and for acridine, a residual component of amustaline treatment, using a specific mouse monoclonal antibody (2S197-2M1). Antigen density was determined using a commercial calibrated Phycoerythrin (PE) bead panel (QuantiBrite PE, BD Biosciences CA) (analyses were funded by the Biomedical Advanced Research and Development Authority, DHHS; ClinicalTrials.gov, NCT03459287).

RESULTS: Five of 159 Test (3.1%,) and 0 of 162 Control subjects (P=0.015) developed IgG PR-RBC specific antibodies, first detected on Days 26 to 80 after surgery after receipt of 1 to 3 study RBC units. Antibody titers were low (titer ≤8) and decreased over time. A MMA for clinically relevant antibodies was either non-reactive (3/5 subjects) or indeterminate (2/5 subjects) and the DAT was negative in 4 of 5 subjects. One subject (011-011) had a weak positive DAT (IgG +, C3-) with an eluate specific for PR-RBCs. Three of the four antibodies tested were inhibited by free acridine (S-300, a breakdown component of amustaline) and one was not inhibited, suggesting two epitope specificities. No clinical or laboratory evidence of hemolysis was observed. Flow cytometry of frozen patient blood samples revealed circulating acridinepositive PR-RBCs in all cases at levels consistent with the expected post transfusion proportions (**Table**) considering RBC dose, hemorrhagic loss, RBC survival and dilution by hematopoietic recovery after surgery. RBC surface human IgG was detected at low levels in 3 of 5 cases. RBC acridine surface density on transfused PR-RBCs *in vivo* was substantially lower (200-300 acridine/cell) than on PR-RBCs before transfusion (~7,500 acridine/cell) and decreased over time, suggesting *in vivo* acridine loss from the RBC surface.

SUMMARY/CONCLUSIONS: PR-RBC-specific antibodies occurred in 3.1% of Test subjects. Circulating PR-RBCs were detected by flow cytometry in all cases with a progressive loss of RBC surface acridine expression. Antibodies were IgG isotype and low titer. There was no clinical evidence of hemolysis or any *in vitro* properties indicative of clinical significance.

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Flow Cytometry Results for the First Timepoint after Discovery of PR-RBC-specific Antibodies

Subject	Days since Surgery	% Acridine+ RBCs[a]	% Human IgG+ RBCs[a]	Predicted % PR-RBCs
Negative Control	-	0.0	0.0	-
Positive Control	-	100	100	-
011-011	39	9.7	10.2	10.1
016-012	91	0.2	0.7	0.3
002-029	32	2.6	0.0	4.3
010-049	39	3.0	1.6	6.3
008-014	55	1.3	0.5	2.1
a. Acridine and human IgG percentages represent the mean of three tests minus the background reactivity				

The INTERCEPT Red Blood Cell System is in late stage clinical development in Europe, and in the US.

INTERCEPT REGULATORY APPROVALS

Canada (Health Canada)
2016 (plasma), 2018 (platelets)

Brazil (ANVISA)
2015 (platelets and plasma)

United States (FDA)
2014 (platelets and plasma)

Mexico (COFEPRIS)
2014 (platelets and plasma)

Singapore (HSA)
2014 (platelets)

Switzerland (Swissmedic)
2009 (platelets), 2010 (plasma)

Germany (PEI)
2007* (platelets), 2011* (plasma)

France (ANSM)
2003 (platelets), 2006 (plasma)

CE Mark, Class III
2002 (platelets), 2006 (plasma)

*** First blood center marketing authorization approved.**

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Use of INTERCEPT Plasma or Platelets is contraindicated in patients with a history of allergic response to amotosalen or psoralens.
Consult instructions for use for indications, contraindications, warnings, and precautions.