CLPH-511 Cryopreserved Platelets Have Maximal Hemostatic Activity at Lower Platelet Counts than Apheresis Platelets Using In Vitro Hemostatic Assays (MHSRS-26-5037)

Maria Abreu-Blanco, Lisa Booth, Ana Swanson, Ben Kuhn and Mike Fitzpatrick
Cellphire Therapeutics, Inc., Rockville, MD, USA

MHSRS 2026 Military Health System Research Symposium

CELLPHIRE THERAPEUTICS

INTRODUCTION

CLPH-511, Frozen (Activated) Platelets, are an investigational pooled type O platelet product frozen in 6% DMSO, with an extended shelf life of 5 years compared to 5-7 days for room temperature apheresis platelets (RTP). In the US phase 2/3 CRYPTICS clinical trial (NCT04709705) investigating CLPH-511 for the treatment of acute hemorrhage, CLPH-511 was non-inferior to RTP by the pre-specified endpoint of a least squares mean difference in 24 hour chest tube drainage. The unit dose definition for CLPH-511 is ≥1.7x10^{11} platelets/unit, with the average dose of CLPH-511 administered during clinical trial being ~2.2x10^{11} platelets/dose. The *in vitro* assays selected for this study interrogated specific characteristics of CLPH-511 hemostatic activity critical for primary and secondary hemostasis, allowing evaluation and comparison of CLPH-511 and RTP potency and providing insights into product dosage and performance.

METHODS

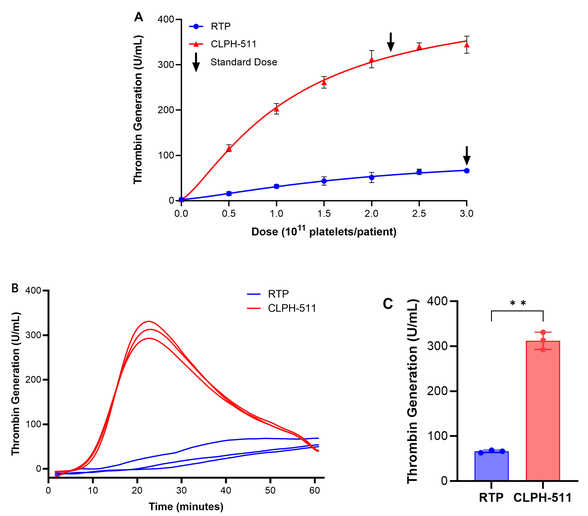
- CLPH-511 was prepared from licensed, irradiated apheresis platelets that were pooled, concentrated by centrifugation, formulated to 6% DMSO, and frozen at -80°C, per Cellphire's manufacturing method.
- Apheresis platelets were stored at room temperature (RTP) and tested on day 3-4. Packed Red Blood Cells (RBCs) were used to generated thrombocytopenic (TCP) blood samples. Blood from volunteers were collected and tested on the day of collection.
- Product concentrations were selected to assess hemostatic effect and correlate with the anticipated clinical doses (0.5-4x10^{11} platelets/patient, or 7,875-63,002 platelets/µL). The standard dose for RTP is 3x10^{11} platelets/patient (47,252 platelets/µL), and ~2.0x10^{11} platelets/patient (31,501 platelets/µL) for CLPH-511.
- CLPH-511 and RTP were evaluated *in vitro* for their ability to promote thrombin generation by thrombin generation assay (TGA), and the ability to assemble a clot triggered by calcium. Both assays were performed in detergent treated plasma (Octaplas).
- Hemostatic activity was evaluated by Total Thrombus Formation Analysis System (T-TAS) HD chip (collagen and tissue factor coated) and kaolin Thromboelastography (TEG) using a TCP blood model prepared with pRBCs, plasma and fresh platelets.
- CLPH-511, RTP, and cold stored platelets (CSP, day 10 stored at 4°C) ability to adhere to collagen was measured under sheer stress (30 dyn/cm^2), at 2.5x10^5 platelets/µL dose using Bioflux. Adhesion (Area of coverage) was estimated by collecting images under the FITC channel at 10x magnification every 30 second for a total of 7 min.
- For all assays, each dot corresponds to one of three independent units tested per product. Samples were tested as triplicates for TGA, clot assay and Bioflux, and as singlicate for T-TAS and kaolin TEG.

CONTACT INFORMATION

Presenting author: mfitzpatrick@cellphire.com. Additional information www.cellphire.com

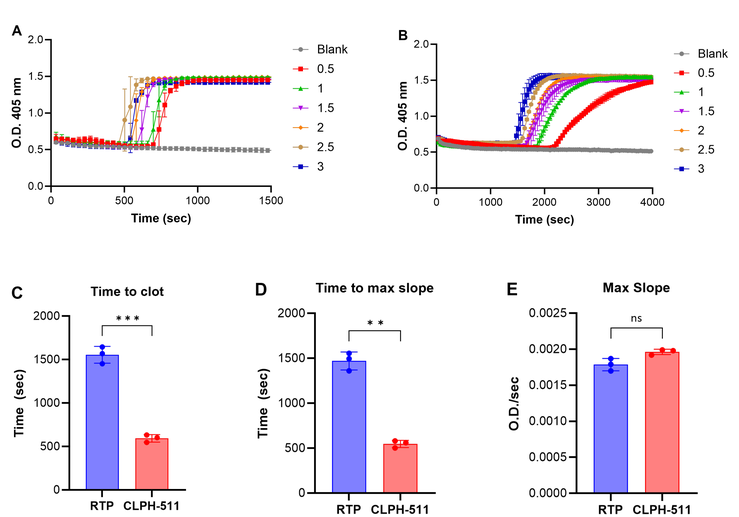
RESULTS

Thrombin Generation



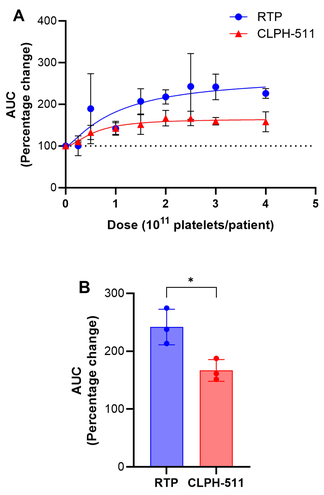
CLPH-511 has significantly higher activity than RTP in TGA, as shown by comparison of CLPH-511 and RTP thrombin generation by dose-response platelet count curves (A) and at the corresponding standard product dose traces (indicate by ↓ on panel A) (B) and thrombin concentration (C). TGA was performed in Octaplas. Error bars represent SD. Welch's unpaired t test was used for statistical analysis: ** p<0.01.

Clot Formation



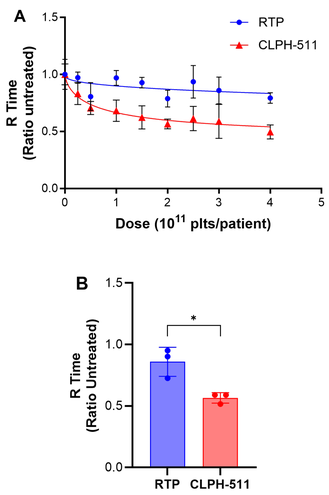
CLPH-511 accelerates clot initiation and formation. Clot formation dynamics were assessed using a microplate clot assay in plasma (Octaplas) triggered by calcium. Dose-response by platelet count curves showing clot formation for a representative batch of CLPH-511 (A) and RTP (B). Comparison of assay parameters at the standard dose for each product: time to clot (C), time to max slope (D), and max slope (E). Error bars represent SD. Welch's unpaired t test was used for statistical analysis: ns, not significant, **, p< 0.01, ***, p< 0.001.

Thrombus Formation



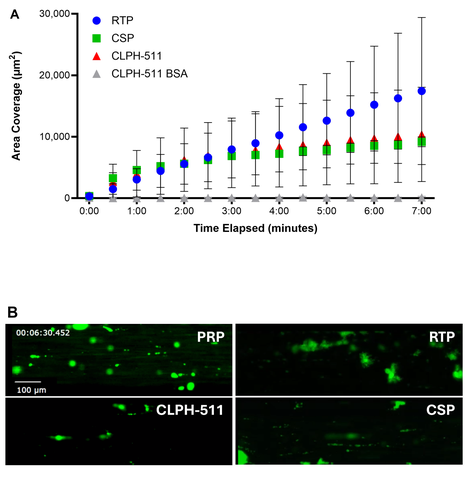
CLPH-511 and RTP recover thrombus formation in TCP blood samples with 25-30 x10^3 native platelets/µL, as shown by T-TAS HD chip assay. Data is shown as a dose response (A) and comparison at the standard product dose of the percentage change for area under the curve (AUC) (B). Error bars represent SD. Welch's unpaired t test was used for statistical analysis: *p≤ 0. 05.

Thromboelastography



CLPH-511 accelerate clot formation in TEG5000 kaolin assay, in a TCP blood model with 50x10^3 native platelets/µL. Data is shown as normalized R time as a dose response (A) and at the standard dose for each product (B). Error bars represent SD. Welch's unpaired t test was used for statistical significance: *p≤ 0. 05.

Adhesion to Collagen



CLPH-511 adheres to collagen as demonstrated by BioFlux assay. (A) XY plot showing CLPH-511, RTP and CSP ability to adhere to collagen as area of coverage. Error bars represent SD. (B) Representative images of collagen-adhered CLPH-511, RTP and CSP (green) at the ~6 minute 30 seconds of measurement. Products were labelled with Calcein AM and tested at 2.5x10^5 platelets/µL.

RESULTS SUMMARY

- CLPH-511 exhibited 4.7x higher thrombin generation activity than RTP (312.4±19.11 vs 66.13±2.87 U/mL) at the representative standard clinical unit dose (2.0x10^{11} and 3x10^{11} per administered unit). Dose saturation was observed at 2.5x10^{11} and 2.0x10^{11} platelets, respectively.
- CLPH-511 showed a correlation of platelet count and acceleration of clot initiation, reducing time to maximum slope and time to clot, with dose saturation observed at 2.0x10^{11} platelets. A correlation of platelet count and hemostatic effect was observed for RTP in the assay parameters but did not appear to saturate at the doses tested.
- CLPH-511 reduced clot initiation time >1.5 fold more compared to RTP, at each product's standard platelet count dose. CLPH-511 had the largest effect on R-time, shortening the R-time in a dose-dependent fashion, with dose saturation at 0.5-1 x 10^{11} platelets dose.
- RTP displayed higher thrombus formation activity, recovering the area under the curve by 2.4 fold, compared to 1.9 fold by CLP-511, at each product's standard platelet count dose. This assay is performed in the presence of heparin and is more responsive to primary hemostatic activity, perhaps explaining this difference.
- CLPH-511 adhered to collagen selectively and did not adhere to albumin. RTP adhesion on collagen was generally higher. CSP and CLPH-511 had similar rates of adhesion to collagen producing a final coverage area of 10,387.67 ± 4,677.86 and 9,170.33 ± 790.44 µm^2 respectively. RTP adhesion was generally higher, with an area of coverage of 17,448.33 ± 11,971.14 µm^2.

CONCLUSION

Our findings demonstrated CLPH-511 exhibits a correlation of platelet count dose to hemostatic effect in *in vitro* assays, generally achieving maximal hemostatic effect at lower platelet counts than RTP. CLPH-511 has an activated phenotype and higher secondary hemostasis activity than RTP, as shown by dose-response in TGA and clot formation assays. CLPH-511 can adhere directly and selectively to collagen (Type I), a key attribute for its mechanism of action. The results inform the mechanism of action, where CLPH-511 accelerates clot initiation and formation, while also promoting interactions with the endothelium. The results show that hemostatic effects are platelet count dependent, which can be measured *in vitro,* and support the potential for achieving hemostasis at a lower concentration than RTP. These findings support CLPH-511 as a potential hemostatic alternative to RTP, helping to address supply constraints and their operational consequences.

ACKNOWLEDGEMENTS

This work is supported by the Defense Health Agency under Contract No. W81XWH20C0030.