



BioPreservation Today[®]

 **BioLIFE SOLUTIONS[®]**
BIOPRESERVATION TOOLS FOR CELLS, TISSUES, AND ORGANS

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FEATURE ARTICLE:

DATA BEYOND TEMPERATURE: DIGITAL INTEGRATION OF THE SUPPLY CHAIN BOOSTS SUPPORT OF ADVANCED THERAPY PRODUCTS



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WEB RESOURCES

www.alliancerm.org | Alliance for Regenerative Medicine

www.aabb.org | American Association of Blood Banks

www.bestcollaborative.org | BEST Collaborative

www.ibclifesciences.com | ibc Life Sciences

www.celltherapysociety.org | International Society for Cellular Therapy

www.ishrs.org | International Society of Hair Restoration Surgery

www.phacilitate.co.uk | Phacilitate

www.regenerativemedicinefoundation.org | Regenerative Medicine Foundation

UPCOMING EVENTS

BEST Collaborative
Long Beach, CA
October 21-22

AABB Annual Meeting
Anaheim, CA
October 24-27, 2015

**CCRM's Cellular Therapies
Manufacturing and
Clinical Trials Workshop**
Toronto, Canada
November, 29-30, 2015

**Clinical Trail Supply
Southern California**
San Diego, CA
November 10-11, 2015

**9th World Congress
for Hair Research**
Miami, FL
November 18-21, 2015

**2015 World Stem
Cell Summit**
Atlanta, GA
December 10-13, 2015





EDITOR'S CORNER

Mike Rice, Chairman & CEO, BioLife Solutions, Inc.

Greetings from the IQPC 13th Cold Chain Global Forum,

Customers, suppliers, partners, and friends of BioLife, welcome back to Boston for what i'm sure will be another informative and productive cold chain forum organized by the team at IQPC. We're pleased and proud to support this key industry event in several ways. In our expanded corporate exhibit, we will discuss and demonstrate the evo™ Smart Shipper and biologistex™ cloud based cold chain management app. We're proud to have Kevin O'Donnell, our VP Cold Chain Standards, Practices, and Compliance acting as chair for the entire conference. Also, Aby J. Mathew, Ph.D., our Senior Vice President and Chief Technology Officer, will co-present with a colleague from NIST on *Biologics and Biopreservation – Optimizing Quality, Risk and Cost of Goods*.

We've been busy over the past few months validating and introducing new products and new and improved aseptic packaging designs for our biopreservation media products. Michael Weaver, Product Development Manager, Marketing, BioLife Solutions, Inc., provides an update on these innovations in an overview article.

One of the products profiled is BloodStor® 27 NaCl, a cryopreservation freeze media targeting applications including platelets. The Australian Red Cross is validating BloodStor 27 and contributed an informative article about clinical use of frozen therapeutic platelets.

Next up, Kevin O'Donnell provides an overview of best practices in cold chain management with a focus on the importance of data beyond temperature and digital integration of the supply chain.

Turning back to the evo Smart Shipper, we provide a recap of a prestigious design award bestowed on the evo and a summary of its key features and benefits.

Lastly, Aby Mathew provides an update on the expansion of our scientific and customer technical support team with profiles of two new team members.

Thank you for your continued interest in BioLife products. I hope you enjoy this issue of BioPreservation Today. We look forward to seeing you at our exhibit and during the conference.

Best regards,

Mike



NEW PRODUCT UPDATES

Michael Weaver, MSc., MBA., Product Development Manager, Sales & Marketing

Cell Thawing Media

Listening to customers and keeping abreast of news in the industry are important components of Product Development at BiLife. We were aware of the worldwide shortage of dextran solutions that are being used off-label following cryopreservation to transition frozen cells to room temperature. Current and prospective customers asked if we could assist them to find a solution. We assembled the Project Team and decided to respond by manufacturing these critical cell processing media products at BiLife, according to GMP, using the same quality system as our core CryoStor® cell freeze media and HypoThermosol® cell and tissue storage and shipping media, USP components and water for injection (WFI) quality water. Cell Thawing Media 10% Dextran 40 in 0.9% NaCl (Part Number 980203, 250mL/bag) and Cell Thawing Media 10% Dextran 40 in 5% Dextrose (Part Number 981203, 250mL/bag) are subject to release criteria including sterility per USP <71>, endotoxin per USP <85>, pH, osmolality, and appearance for no visible particulates. Cell Thawing Media products were launched in March, 2015.

BloodStor® 27 NaCl

BiLife has been monitoring clinical use of frozen platelets and has discussed the need for a stable supply of GMP manufactured, clinical grade freeze media for freezing therapeutic platelets with leading international clinical centers. BiLife is responding to the need for a clinical grade DMSO-based freeze media for platelet cryopreservation by manufacturing BloodStor® 27 NaCl, a pre-formulated

product with fill volumes of 80mL or 110mL packaged in sterile, single use DMSO-compatible bags with sterile weldable tubing and a separate luer connector. BloodStor® 27 NaCl (part number 327207) is quality tested against robust release criteria including sterility per USP <71>, endotoxin per USP <85>, DMSO content of 27% v/v, specific gravity, osmolality, and appearance for absence of visible particulates. BiLife expects inventory of BloodStor 27 NaCl to be available for customer orders early in the fourth quarter of 2015. A Master File for BloodStor® 27 NaCl will be submitted to the United States Food and Drug Administration for customers to cross reference in regulatory filings. Regulatory note: BloodStor® 27 NaCl is labeled For Research Use, For Human Cell and Tissue Preservation. Customers that incorporate the product into their frozen storage protocol for cells and tissues intended for human clinical uses are responsible for validation and fulfilling any required regulatory requirements related to the use of BloodStor® 27 NaCl.

CryoStor® CS5 and CS10 coming soon in 100ml bags

BiLife is responding to customer requests for CryoStor® CS5 and CryoStor® CS10 in a small volume, closed system format by manufacturing the products in 100mL sterile, single use DMSO compatible bags with sterile weldable tubing and a separate luer connector. The small volume format is analogous to CryoStor® CS10 in one liter DMSO-compatible bags (part number 210210) that features a spike port, sterile weldable tubing and a separate luer connector, launched Q4 2014. CryoStor® CS5 and CS10 in 100mL bags will be available Q4 2015.



OUR FAMILY OF BIOPRESERVATION PRODUCTS AND SERVICES IS GROWING



- biologistex™
- CryoStor®
- HypoThermosol®
- Cell Thawing Media
- BloodStor®
- New Packaging Solutions



 Intelligent • Informed • Precise
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DATA BEYOND TEMPERATURE: DIGITAL INTEGRATION OF THE SUPPLY CHAIN BOOSTS SUPPORT OF ADVANCED THERAPY PRODUCTS

by Kevin O'Donnell, Vice President, Cold Chain Standards, Practices & Compliance, Biolife Solutions, Inc.

The White House 2012 National Bioeconomy Blueprint forecasts that increased cell manufacturing research activities would lead not only to significant improvements in public health, but also provide tremendous economic benefits to the next generation of biotechnologies.¹ Predictably, groundbreaking techniques to elevate quality assurance, increase efficacy, enable production scale-up, and reduce manufacturing and product cost of requisite bioprocesses are needed to support the long-term growth of the regenerative medicine industry.

To this end, the Georgia Research Alliance, in conjunction with Georgia Institute of Technology and the National Institute of Standards and Technology (NIST), have formed the Cell Manufacturing Consortium (CMC). Member participants are leading the development of a regenerative medicine industry roadmap whose aim is to provide a pathway to overcome cell manufacturing, storage, and transportation challenges and capitalize on opportunities that will maintain the United States' position at the head of the rapidly expanding global regenerative medicine industry. Currently 52% of the world's leading companies investing in this technology are headquartered in the US. (Alliance for Regenerative Medicine).²

Investment in regenerative medicine continues to soar. In the 2nd Quarter of 2015 (the latest data available at time of publication), more than 580 leading worldwide companies raised USD \$4.9 billion, up 129% compared to the same period in 2014. Investment in gene and gene-modified cell therapy was up 200%, and cell therapy was up 111% over the same period the previous year. Corporate partnerships are leading the charge with deals valued at \$5.3 billion.²

Clinical trials in regenerative medicine are on a similar trajectory, climbing to 528 by the end of the 2nd Quarter of 2015, an increase of more than 30% since the summer of 2014. Commercialization and regulatory approval of cell therapies is methodically advancing, with 32% of all cell-based clinical trials in Phase I, 57.5% in Phase II, and 10.5% in Phase III. Today, 74 cell therapy products have been approved, up 85% since 2012.³

In the wake of such growth, the Cell Manufacturing Consortium has identified and prioritized a variety of research and development activities that the cell manufacturing industry must pursue to help maintain and advance the United States' lead in the global cell manufacturing industry. The impetus behind these activities is to increase cell manufacturing scale and speed while also improving cell quality and functionality, reducing cost, and ultimately improving the safety and efficacy of cell therapies.

The group has identified the following areas of focus:

- Automation and Closed- System Processing
- Critical Product Quality and Process Controls
- Production Scalability at Good Manufacturing Practice (GMP) Facilities
- Culture Platforms and Downstream Processing
- Cell Preservation, Distribution, and Handling
- Supply Chain Robustness and Management

It is the supply chain robustness and management category that has the greatest potential to change due to advanced technologies and next generation data sensors.

As the road to commercialization of cell therapies is paved, questions regarding safe and protective transport and ascertaining cell condition upon arrival prior to administration to the patient are beginning to emerge. Under the paradigm of current pharmaceutical and biological products, many require transportation using temperature-controlled systems to keep their therapeutic properties intact. Regulatory authorities require that the manufacturer ensures product quality - not only during storage or transport - but until it is used for patient treatment.⁴ It would be foolish to think that the commercialization of cell therapies would not undergo the same regulatory scrutiny or expectations.



When talking about sensitive products one usually thinks about those that are sensitive to temperature, but other environmental conditions should also be considered, including humidity, light, oxygen, shocks, pressure, vibrations and X-rays encountered during shipment by truck, train, boat, or plane. Such sensitivities can impact cell stability, survival, viability, recovery, function and yield. Near UV light, for example, has been shown to be responsible for many unique photosensitizing effects on certain human cell cultures, including a decrease in the ability of DNA to transform bacterial cells. Furthermore, UV radiation destroys photoreactivating enzymes, causing unexpected biological effects. Biomechanical shear forces and shock from impact can also influence cell behavior of cell therapies or tissue engineered products used in bone regeneration.



It is therefore no surprise that cell preservation, distribution and handling has been identified as one of the six primary areas of focus critical to commercialization according to the CMC's list above. A revealing comment regarding these complexities is attributed to Phil Vanek, Ph.D., General Manager, Cell Therapy Technologies at GE Healthcare. Dr. Vanek stated, "Confidence in and throughout the sector has really grown – there's increasing sophistication in supply chain and production plans, shaping the infrastructure needed to support advanced therapy products. We expect to see significant movement in the next few years regarding the physical integration of manufacturing workflow unit operations, along with increased digital integration. This digitization will enable huge amounts of data to be mined, which will further inform everything from process optimization to patient stratification, improving systems and outcomes."

A holistic digital integration of processes related to the transportation supply chain carries with it significant clinical and economic benefit beyond the traditional scope of manufacturing. This extends to autologous and allogeneic cell therapy applications and in the execution of clinical trials. It conforms to the execution of current Good Manufacturing Practices (cGMP)

through its processes, tools, and data that can be used to monitor and manage the movement of biologic materials across time and space. The use of sophisticated and reliable multiple-use packaging systems with multi-function environmental monitoring and GPS tracking allows for far greater visibility of previously unknown or "uncontrollable" transportation environments for every individual temperature-sensitive and time-critical cell therapy shipment.

Leading edge solutions such as the biologistex™ cloud-based cold chain management service from BioLife Solutions, featuring the evo™ Smart Shipper, empower key stakeholders with a full suite of such features to provide maximum control over the integrity of shipments, revolutionizing the management of critical biologic materials.

BioLife's biologistex cloud-based cold chain management service is a new integrated logistics and track & trace web app used by shippers of time and temperature-sensitive biologic materials. The evo Smart Shipper is a state-of-the-art precision thermal shipping container with embedded payload monitoring, GPS location tracking, and cellular communication electronics that transmit critical shipment information to the cloud. This SaaS app enables users to monitor high value shipments during transit and configure actionable alerts for downstream recipients for location, approaching destination, delivery, package open, and remaining shelf life or stability via the patent pending StableAlert™ countdown timer.



Solutions such as biologistex provide this critical information in real time, elevating the development of best practices for shipment of precious temperature-controlled biologics to ensure these vital cargoes arrive at their destination with the highest viability and life-saving functionality. Integrated logistics and analytics, location, geo-fencing, chain-of-custody, countdown timers and actionable interventions within the transportation environment are therefore now a reality, significantly raising the bar when it comes to incorporating continuous improvement within a quality management system.

The adoption of such integrated data management systems within the supply chain will bring greater awareness and understanding of the perils within the transportation environment and will provide the tools necessary to make informed, data-driven decisions on the quality of biologic shipments in real-time, rather than post shipment. It will identify process gaps, negligent logistics practices and other risks in transportation that pose a serious threat to maintaining the quality of advanced therapy products and it will put a necessary end to the practice of periodic monitoring and the pervasive validate-and-assume approach currently employed as best practice.



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MEET THE BIOLIFE SOLUTIONS SCIENTIFIC TEAM

Aby J, Mathew, PhD Biolife Solutions, Inc.

Brian J. Hawkins, Ph.D.



Dr. Brian Hawkins received his Ph.D. in Molecular Cell Biology and Biotechnology from Virginia Tech in 2003 and completed his postdoctoral training at the University of Pennsylvania in Philadelphia. After a brief research faculty position at Temple University, Dr. Hawkins

was recruited as an Assistant Professor and founding member of the Mitochondria and Metabolism Center at the University of Washington in Seattle. For the past 15 years as both a trainee and faculty member, Dr. Hawkins has concentrated his efforts on decoding how alterations in metabolism influence the development of human disease and ultimately death.

Dr. Hawkins has an extensive publication record and received numerous grants to pursue his research, including a prestigious Pulmonary Hypertension Association Fellowship and a National Institutes of Health K99/ROO Pathway to Independence Award. While at the University of Washington, Dr. Hawkins became intrigued at the notion that metabolism could be manipulated in order to better preserve organs for transplant. He advanced his ideas by actively partnering with organ transplant physicians and professionals to translate his basic laboratory research into clinical realities. In doing so, he realized the vital importance of commercializing scientific discoveries in order to positively impact human health.

Dr. Hawkins joined Biolife Solutions, Inc. in 2014 as Senior Application Scientist, where he utilizes his extensive expertise in the metabolic basis of cell and organ dysfunction to advance the science of biopreservation. Dr. Hawkins continues to engage in graduate education and to support collaborative

academic research into the mechanisms of metabolic dysfunction and cell death as an affiliate Assistant Professor at the University of Washington. He is a member of the American Heart Association, the International Society for Cell Therapy (ISCT), AABB (formerly the American Association of Blood Banks), BEST (the Biomedical Excellence for Safer Transfusion collaborative), and the Alliance for Regenerative Medicine (ARM), and is the Treasurer and founding member of the Board of Directors of the Complex Biological Systems Alliance.

Alireza Abazari, M.Sc., Ph.D.

Dr. Alireza Abazari received his Ph.D. in Chemical Engineering from University of Alberta in Canada in 2010, and began his postdoctoral training in bioengineering in 2011 with Dr. Mehmet Toner at the Center for Engineering in Medicine at Harvard Medical School and Massachusetts General Hospital. In



2012, Dr. Abazari was the recipient of the Canadian Natural Sciences and Engineering Research Council of Canada (NSERC) Post-doctoral Fellowship award. The interdisciplinary nature of his training has enabled Dr. Abazari to tackle obstacles in biopreservation of cells and tissues using tools such as mathematical modeling, Magnetic Resonance Imaging and Raman spectroscopy.

Dr. Abazari has extensive experience with methods for banking of human tissue and organs, and has studied novel and radical methods for cryogenic, hypothermic and ambient temperature preservation of biological cells for applications in tissue engineering, cellular therapies and bioprocessing. In his most recent publication, Dr.

Abazari introduced an innovative technique for delivering intracellular trehalose, a disaccharide impermeant to mammalian cells, to offer freezing and desiccation tolerance.

In January 2015, Dr. Abazari joined BioLife Solutions, Inc. as Senior Application Scientist. In his new role, Dr. Abazari is keen on improving the yield, and maximizing the impact, of delivered doses to patients through the application of biopreservation principles, and by consulting, teaching and researching innovative solutions for the dynamic landscape of bioprocessing and cellular therapies. Dr. Abazari is a member of the International Society for Cell Therapy (ISCT), AABB (formerly the American Association of Blood Banks), BEST (the Biomedical Excellence for Safer Transfusion collaborative), the Society for Cryobiology (SfC) and the Biomedical Engineering Society (BMES). He is also a former member of the Biophysical Society and the Materials Research Society.

Aby J. Mathew, Ph.D

Dr. Hawkins and Dr. Abazari join longtime BioLife scientific team member, Dr. Aby J. Mathew. Dr. Mathew was part of the founding team of BioLife Solutions, Inc., and is a co-developer of BioLife's biopreservation media solutions. He is a co-inventor on multiple issued and pending patents related to methods, devices, and formulations for the preservation of cells, tissues, and organs. He holds a Ph.D. in Biological Sciences within the Biochemistry, Cell and Molecular Biology Program from Binghamton University and a B.S. in Microbiology from Cornell University. Dr. Mathew has been researching low temperature biopreservation since 1994, and his studies contributed to the development of BioLife's current commercial HypoThermosol® and CryoStor® product platforms and intellectual property foundation.

Dr. Mathew was part of a scientific team that linked cell death via apoptosis (programmed cell death) to exposure to hypothermic and/or freezing temperatures. These discoveries were integral to the development of BioLife's improved intracellular-like biopreservation solutions, and also contributed to improvements in cryosurgical ablation of cancer. Dr. Mathew was BioLife's first Director of Manufacturing, established BioLife's initial Quality system, and is currently Senior Vice President & Chief Technology Officer for BioLife Solutions, Inc. Dr. Mathew is currently active within, or previously a member of, the International Society for Cell Therapy (ISCT), AABB (formerly the American Association of Blood Banks), BEST (the Biomedical Excellence for Safer Transfusion collaborative), the Alliance for Regenerative Medicine (ARM), Tissue Engineering & Regenerative Medicine International Society (TERMIS), Society for Cryobiology (SfC), IBC Life Sciences, Centre for Commercialization of Regenerative Medicine (CCRM), International Society for Biological and Environmental Repositories (ISBER), American Society for Cell Biology, and the Society for In Vitro Biology. Dr. Mathew is a member of the Advisory Panel of the Parent's Guide to Cord Blood Foundation, the Scientific Advisory Board of HemaCare Corporation, and the founding Board of Directors of the Cord Blood Association.

The BioLife Solutions scientific team welcomes discussions regarding potential scientific collaborations, answering scientific questions about our technology, providing consultation for biopreservation process development, feedback regarding customer applications, and sharing our scientific experience with our academic, clinical, and industry partners. If you have interest in discussions with the BioLife scientific team, please email Dr. Mathew at amathew@biolifesolutions.com. Further review of articles, posters, data, and supporting scientific information related to BioLife's technology is available on our website BiolifeSolutions.com.



ARE CRYOPRESERVED PLATELETS JUST AROUND THE CORNER?

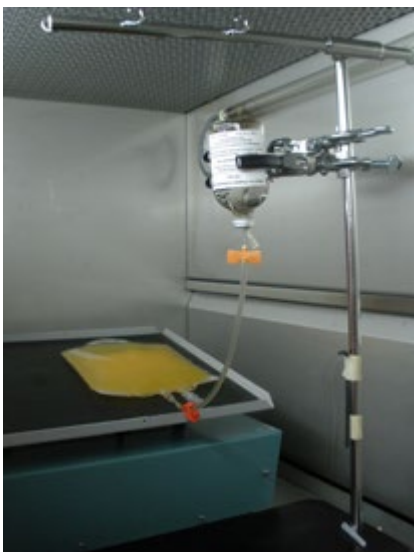
by Dr. Denese Marks and Dr. Lacey Johnson

The greatest limitation with the storage of platelets is their short shelf-life, which is typically between 5 and 7 days when stored at room temperature. Attempts to extend their shelf-life by improving platelet additive solutions have so far yielded few benefits, and no new platelet additive solutions have been commercialised recently. An alternative approach is to cryopreserve platelets at $-80\text{ }^{\circ}\text{C}$, which can extend their shelf-life to 2-4 years.

Methods for platelet cryopreservation have been investigated for the past 30 years, with the greatest attention from the military for use in austere environments, where a supply of fresh platelets is limited or non-existent. The Netherlands military have been using frozen platelets to support their overseas deployments in Bosnia, Afghanistan and Liberia since 2001.¹ In Afghanistan during the period from 2006-2011, the Dutch military transfused over 1000 platelet units without any reported transfusion reactions.² During deployment to Afghanistan, Australian Defence Force surgical teams embedded into the Netherlands health facility used frozen blood products to treat Australian soldiers with clinical success and no reported side effects.³ Since 2012, the Australian Red Cross Blood Service has been working with the Australian Defence Force to develop cryopreserved platelets, and other frozen blood components, suitable for use in military deployments.^{4,5}

Military and civilian use of cryopreserved platelets

The methods of platelet cryopreservation used by the Dutch military and in Australia are based on that of Valeri and colleagues⁶, which involves the addition of DMSO (27% DMSO/0.9% saline) as a cryoprotectant, to a final concentration of 5-6%. The platelet units are then centrifuged to concentrate the platelets and to remove almost all of the DMSO, leaving the platelets concentrated in a small volume (20-30 mL). The platelets are then frozen at $-80\text{ }^{\circ}\text{C}$. Upon thawing, the platelets are reconstituted in one unit of plasma.⁷ The major limitation with this method is the time required to thaw a unit of plasma, which takes approximately 30 minutes if it has been stored at $-80\text{ }^{\circ}\text{C}$. Alternative reconstitution solutions have been investigated, and from in vitro data, it appears that platelet additive solutions, particularly those containing glucose, could be considered as suitable alternatives to plasma.⁸ Again, the major limitation with this approach is the lack of any commercially available platelet additives solutions containing glucose. Once thawed, the platelet has a shelf-life of only 6 hours, as DMSO is currently added using an 'open' system. If a suitable closed system for DMSO addition was available, the post-thaw shelf-life could potentially be extended.⁸ For the civilian community, the greatest advantage of using cryopreserved platelets



would be improved availability in remote and rural regions, and for supplementing a platelet inventory when blood collections are reduced, such as over a long weekend or holiday period. Cryopreservation could also facilitate establishment of a bank of phenotyped platelets, similar to the inventory of frozen red cells with rare phenotypes held by many blood services. To date, however, cryopreserved platelets are only approved for military use, and this is limited to very few countries.⁹

Cryopreserved platelets are more haemostatically active *in vitro*

It is clear from *in vitro* data that cryopreserved platelets are phenotypically different from conventional blood bank platelets, and exhibit defects in agonist-induced aggregation.^{7, 10-13} However, *in vitro* functional assays suggest that cryopreserved platelets may be more haemostatically active than fresh liquid-stored platelets.^{14, 15} They contain more procoagulant microparticles than fresh platelets, with a faster time to clot formation using thromboelastography (TEG) and a FXa-based procoagulant phospholipid assay (ProCoag-PPL), as well as increased thrombin generation potential using a calibrated automated Thrombogram (CAT).^{14, 16} Recent data suggests that the microparticles play an important role in mediating the increased procoagulant activity, due to the high levels of phosphatidylserine on their surface.¹⁴

Clinical trials using cryopreserved platelets

There are published reports of *in vivo* use of cryopreserved platelets dating back to the 1970s.¹⁷ Although *in vivo* recovery is lower, the survival of cryopreserved platelets is similar to stored blood bank platelets.¹⁸ However, only one randomised controlled trial comparing cryopreserved platelets to fresh liquid platelets has been published.¹⁹

Although the sample size was small, cryopreserved platelets were significantly more effective than fresh platelets in controlling bleeding, with fewer transfusions needed in the group receiving cryopreserved platelets. A similar randomised control trial, known as the CLIP trial (Cryopreserved versus liquid platelets; Australian New Zealand Clinical Trials Registry ACTRN12612001261808) is currently recruiting patients in Australia, comparing cryopreserved apheresis platelets with fresh platelets for controlling bleeding in post-surgical cardiac patients.²⁰ In the US, a Phase I dose escalation study in patients with thrombocytopenia and uncontrolled bleeding (ClinicalTrials.gov Identifier NCT02078284) is also currently recruiting patients. Provision of further clinical evidence regarding the efficacy and safety of cryopreserved platelets may pave the way for their registration for civilian use.

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