

**Blood Products Advisory Committee
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Bethesda Marriott Hotel
5151 Pooks Hill Road, Bethesda, MD 20814**

Issue Summary

Topic II: Study Designs (Phases 3 and 4) for Product Development of Human Platelets Using the Cerus INTERCEPT Blood System for Pathogen Inactivation

Issue:

The Cerus SPRINT study of pathogen inactivated (S59) platelets identified potential safety and efficacy concerns. FDA and Cerus have discussed the design of future studies aimed at addressing these issues. FDA seeks the advice of the Committee regarding the design of a new Phase 3 study and the adequacy of the proposed product development program which includes a Phase 4 study and staged rollout of the product.

Background:

One of the approaches to providing safe blood products in the US is to screen donors and blood products for various pathogens. This approach has worked well and has dramatically decreased the incidence of transfusion transmitted diseases (TTD).

Occasionally pathogens are not detected by screening tests because organisms are present at levels that are below the assay's level of detection. It is estimated that the residual risk of TTD from tested transfusion products is in the order of 1/277,000 for viral pathogens, 1/70,000-118,000 for bacterial agents and 1/1,000,000 for protozoa (1).

In addition, the potential exists for the emergence of a new pathogen for which specific screening tests are not available. In the past this occurred with the emergence of HIV-1 and West Nile virus prior to the availability of screening assays. The risk of emergence of a new pathogen that could be transmitted by blood components is difficult to estimate.

Platelets products have an additional disadvantage with respect to bacterial pathogens because platelets, unlike red blood cells, are stored at room temperature. Even very low levels of contaminating bacteria at collection can proliferate to very high bacterial concentrations over the course of platelet storage. In the absence of uniformly adopted, rapid bacterial detection tests for use as release tests prior to transfusion, transfusion of bacterially contaminated products will remain a concern, especially for immunosuppressed individuals in whom the use of such product may have devastating consequences.

An alternative to the detection of pathogens in transfusion products would be to apply a process that reduces or inactivates contaminating pathogens, while not damaging the transfusion products or harming the patients receiving the treated products. Potential advantages of this approach are that 1) pathogens may be inactivated 2) inactivation may

be effective against emerging pathogens for which tests are not available 3) it may be cost effective when compared to the use of multiple screening tests and other blood bank processing such as gamma irradiation. This alternate approach is referred to as pathogen reduction methodology or PR.

Several PR processes have been developed and these take advantage of the fact that most known pathogens proliferate through nucleic acid replication (prions are an exception). Red cells and platelets are cells that lack nuclei and do not divide and thus processes that interfere with nucleic acid replication should not affect their function. However current PR methodologies are only partially specific for nucleic acids and may react with proteins, lipids and carbohydrates, and thus could potentially damage red cells and platelets. In addition, the PR chemical compounds may also require activation energy in the form of ultraviolet light (UV), which may be harmful to cells.

The Cerus PR process for platelets utilizes a psoralen compound (S59 or Amatosalen) which is added to a unit of platelets and irradiated with UV light. The compound can intercalate into nucleic acids and when activated by UV light will crosslink adjacent nucleic acids and prevent replication of a variety of pathogens. Efficacy of this process in reducing pathogens has been demonstrated in vitro with units of platelets intentionally contaminated with pathogens. Such studies have demonstrated that this method is effective in reducing the pathogen load by 5-6 logs or more for viruses, bacteria and protozoa. It is effective in vitro against extracellular and intracellular organisms but is less effective against spores. FDA has accepted in vitro testing as a demonstration of pathogen reduction efficacy since clinical trials to demonstrate reduction of transfusion transmitted diseases (TTD) would be very large, given the current low levels of TTDs.

Pre-clinical evaluation of the safety of Cerus S59 process

Ideal PR methodologies should be safe and effective. They should not 1) reduce the efficacy of the transfusion product, 2) be acutely or chronically toxic to patients receiving PR treated products or 3) be toxic to healthcare workers preparing the treated products. The sponsor conducted a broad evaluation of the toxicity of the parent compound, of the derivatives formed after UV activation and of the treated platelets.

Direct toxicity

The S59 compound and the treated platelets were tested in standard toxicological protocols with acute and chronic administration to mice, dogs and in limited numbers to primates. A potential area of concern was the development of cardiac arrhythmias in dogs and primates. Repeated testing with primates did not identify an arrhythmogenic effect for the treated platelet product.

Mutagenicity and carcinogenicity

Since the PR chemicals modify nucleic acids there is a possibility that such compounds could be mutagenic, carcinogenic and interfere with reproduction. In standard mutagenic assays the S59 was found to be mutagenic. To evaluate the carcinogenic potential, the S59 was evaluated in a p53 knock-out mouse model. This study was found

to be negative. Effects on reproductive health were tested in rat multigenerational studies and found to be negative.

Phase 2 study - Effects of PR on efficacy of platelet products.

Platelets are transfused because of the increased risk of bleeding in patients with low platelets counts(2). Allogeneic platelets are transfused to thrombocytopenic patients when their own platelet production fails to keep the level of circulating platelets above 10,000 platelets/ul. In most cases this is considered as the “trigger” to transfuse platelets. In healthy individuals, platelet counts range from 200,000-400,000/ul. Platelets function together with clotting factors, endothelium and arterioles to provide effective hemostasis. At low platelet counts, hemostasis can be compromised particularly in patients who also have additional limitations such as a decrease in clotting factors.

The FDA evaluates the function of new platelet products by initially examining platelet in vitro responses. Early clinical studies evaluate platelet intravascular kinetics in healthy human volunteers. These clinical studies involve a donation of a unit of platelets by volunteers, treatment of the unit by the new methodology, storage of the unit to the limit of its shelf life and re-infusion into the donor of a small portion of the platelet unit that has been radioactively labeled. If the platelets are damaged by the PR process they will be rapidly cleared from the circulation and have a reduced initial recovery. The remaining portion of platelets in circulation may also have a shortened half life. When the Cerus platelets were tested, their performance was compared to that of an untreated platelet product. The results of a standard radiolabeling study showed that Cerus S59 platelets had decreased initial recovery and platelet survival compared to control platelets (see Table 1). These results indicated that the S59 treated platelets were damaged by the Cerus PR process and suggested that such products would need to be transfused more often to maintain hemostasis because of their reduced in vivo recovery and survival.

In vivo radiolabeling studies	Cerus S59 platelets	Control platelets	Percent of control platelets*	p value
Recovery (%)	42.5 ± 8.7	50.3 ± 7.7	84.5%	P < 0.01
Survival (days)	4.8 ± 1.3	6.0 ± 1.2	80.0%	P < 0.01

Table 1. Data reproduced and calculated* from reference (3)

Phase 3 study - Evaluation of hemostatic efficacy (SPRINT trial)

The role of platelets in vivo is to prevent and reduce bleeding. Consequently, the efficacy of PR platelet products is evaluated in Phase 3 clinical trials with bleeding as the primary endpoint.

The Cerus Corporation conducted a randomized, double blind, Phase 3 non-inferiority study (SPRINT trial, 2001) of S59 treated platelets compared to conventional apheresis platelets prepared on the Amicus platelet apheresis instrument (Fenwal). The study had 318 thrombocytopenic patients in the test arm (supported by S59 platelets) and 327 patients in the control arm (supported by conventional apheresis platelets). Eighty percent (80%) of the patient population consisted of stem cell transplant recipients. The patients received platelet transfusion support for up to 28 days followed by a 7 day observation period. The primary objective of the study was to evaluate safety and hemostatic efficacy of the S59 platelets. Efficacy was monitored by daily observations of the patients for evidence of bleeding by blinded observers. Bleeding events were categorized according to a WHO bleeding scale (Grade 1 = mucocutaneous bleeding, Grade 2= mucocutaneous bleeding of greater intensity including hematuria, vaginal bleeding and hemoptysis, Grade 3 = bleeding that requires RBC transfusion, CNS bleeding, with no clinical consequence on CT, Grade 4 = fatal bleeding, CNS bleeding with clinical consequence, retinal bleeding with impairment of vision). The primary efficacy endpoint was the proportion of patients with Grade 2 bleeding.

Statistical Plan

The study was designed as a non-inferiority trial. Differences between treatment groups for the primary endpoint (the proportion of patients with Grade 2 bleeding) and one secondary endpoint (the proportion of patients with Grade 3 or 4 bleeding) were analyzed using one-sided tests of non-inferiority with pre-specified non-inferiority margins of 12.5% and 7%, respectively. All other secondary endpoints were analyzed for differences between treatment groups. For the primary endpoint, the test statistic was $(P_T - P_R - 0.125) / (\text{Var}[P_T - P_R])^{1/2}$, where P_T is the observed proportion of patients with Grade 2 bleeding in the PCT group, P_R is the observed proportion of patients with Grade 2 bleeding in the control group, and $\text{Var}(P_T - P_R)$ is the variance estimated by the maximum likelihood estimate. The one-sided 95% confidence interval for the treatment difference in the proportion used the same estimated variance. Analysis-of-variance with treatment and study site in the model was used for continuous variables. Fisher's exact test was used for comparison of adverse events. Time to Grade 2 bleeding was compared using the log rank test. Longitudinal regression analysis was used to adjust platelet count increment and transfusion interval for platelet dose. Except for the tests of non-inferiority, all other statistical tests were two-sided with a significance level of 0.05 (4).

Evaluation of Safety

In the SPRINT trial, adverse events were recorded by blinded study personnel according to an NCI toxicity scale (Grade 1-4) and were coded by blinded Cerus personnel according to MedDRA medical definitions. In the final analysis, 898 adverse event terms were compared for both groups of patients (6).

Study Results

The target dose for issued platelets was 3×10^{11} for both control and treated platelets. Mean dose per transfusion was lower in the treatment arm vs. the control arm (3.7×10^{11} vs 4.0×10^{11} , respectively). More treatment arm patients received platelet doses of less than 3.0×10^{11} (the standard dose recommended by AABB and FDA) than control arm

patients (190 vs 110, $p < 0.001$, respectively). When protocol platelets were not available the patients received “off protocol” conventional platelets: 8.5% of platelet transfusions in the treatment arm were off protocol compared to 4.8% for control (5).

Efficacy endpoints:

The study met the primary endpoint, i.e., the proportion of patients with Grade 2 bleeding in the treatment arm did not exceed that of the control arm. This would appear to indicate the non-inferiority of S59 platelets to conventional platelets. However, a number of secondary endpoints that reflect platelet function suggested decreased efficacy of the test product:

Increased mean days of Grade 2 bleeding in the treatment arm;
 Shorter average interval in days between platelet transfusions;
 Lower average CCI at 1 and 24 hours after platelet transfusion;
 Higher average number of platelet transfusions;
 Higher proportion of subjects who develop refractoriness to platelet transfusions.

Endpoints	S59 platelets	Conventional platelets	P value
Primary Endpoint			
Proportion of patients with Grade 2 bleeding	58.5	57.5	$< 0.001^*$
Secondary Endpoints			
Total number of platelet transfusions	2678	2041	Not reported
Mean number of days of Grade 2 bleeding	3.2	2.5	0.023
Mean number of plt units transfused per patient	8.4	6.2	< 0.001
Days between transfusion	1.9	2.4	< 0.001
Average CCI at 1 hour	11.1	16.0	< 0.001
Average CCI at 24 hours	6.7	10.1	< 0.001
Mean RBC transfusion per patient	4.8	4.3	0.13
Clinical platelet refractoriness	21.4%	7.0%	< 0.001

Table 2. * non-inferiority study, $p < 0.025$ indicates non inferiority, prespecified non-inferiority margin of 12.5%, CCI = Corrected count increment

Based on the results of the SPRINT study FDA concluded that mean days of Grade 2 bleeding was a more sensitive measure of hemostasis than proportion of patients with Grade 2 bleeding and recommended the former be used as a primary endpoint in future studies.

Adverse Events (AE)

Of the 898 MEDRA AE terms that were reported by blinded observers, 11 AE terms showed statistical difference between the test and control. All 11 differences were against the treated platelets. Four of these were judged to be Grade 3 and 4 events. They included hypocalcemia, syncope, pneumonitis (not otherwise specified), and acute respiratory distress syndrome (ARDS). The increased incidence of ARDS in the treatment arm (5 vs. 0, $p = 0.03$) raised the most concern since ARDS can be associated with a high morbidity and mortality.

TABLE 5. Summary of AEs by MedDRA preferred term significantly different between treatment groups			
Preferred term	Treatment group*		P value†
	PCT (n = 318)	Control (n = 327)	
All grades combined			
Petechiae	124 (39)	94 (29)	<0.01
Fecal occult blood positive	106 (33)	83 (25)	0.03
Dermatitis NOS	99 (31)	67 (21)	<0.01
Rash maculopapular	15 (4.7)	6 (1.8)	0.06
Pleuritic pain	12 (3.8)	3 (0.9)	0.03
Muscle cramps	10 (3.1)	2 (0.6)	0.02
Pneumonitis NOS	7 (2.2)	0 (0)	<0.01
Mucosal hemorrhage NOS	5 (1.6)	0 (0)	0.03
ARDS	5 (1.6)	0 (0)	0.03
Grade 3 or 4			
Hypocalcemia	21 (6.6)	8 (2.4)	0.01
Syncope	6 (1.9)	0 (0)	0.01
Pneumonitis NOS	5 (1.6)	0 (0)	0.03
ARDS	5 (1.6)	0 (0)	0.03

* Data are reported as number (%).

† Based on Fisher's exact test.

Table 3. Summary of AEs including Grade 3 and 4 AEs from the SPRINT study. Table copied from (6)

Several of the adverse events were hemostasis related: petechiae, mucosal hemorrhages, and fecal occult blood. These differences in hemostasis could be attributed either to low platelet counts or to platelets that do not function adequately. Since the patients in the treatment arm had lower CCIs and needed to be transfused more frequently, it is likely that they had lower platelet counts. However, it is also possible that the function of the treated platelets was impaired.

The sponsor noted that the study was not designed to identify acute lung injury and ARDS. To re-examine the data, Cerus consulted with a panel of 3 experts. The experts restricted their review to medical charts of patients who had only pulmonary events. There were 148 patients such patients, 78 in the treatment group, and 70 in the control. The expert panel was blinded to the treatment and analyzed the pulmonary events using predetermined criteria for ALI and ARDS.

	S59 platelets	Control Platelets	p value
Total ALI	19 (6.0%)	16 (4.9 %)	0.60
ARDS	12 (3.8%)	5 (1.5%)	0.09
ALI	7 (2.2%)	11 (3.4%)	0.48

Table 4. Summary of re-analysis of SPRINT cases of pulmonary AEs. Table reproduced from (6)

Additional cases of ALI and ARDS were detected for both test and control. This analysis showed no statistically significant difference in ARDS and ALI between the test and control arms although the number of patients with ARDS remained higher in the treatment arm compared to the control arm. (12 vs. 5).

Conclusions from the Phase 3 clinical trial

The FDA has concerns about efficacy (bleeding events) and safety (imbalance of adverse events). Even though the study met the primary endpoint, secondary endpoints did not support the study conclusion that the PR platelets were non-inferior to untreated platelets. More platelets and more frequent transfusions were needed. Mean days of Grade 2 bleeding were higher in the treatment arm ($p = 0.023$). Additionally, hemostatic adverse events were more frequently observed in the test arm. The data did not establish whether the reduced hemostatic efficacy was attributable to lower platelet numbers or impaired platelet function.

The major differences in Grade 3 and 4 clinical adverse events were of concern to the FDA (Table 3). The increased ARDS in the treatment arm compared to the control is particularly noteworthy since, ARDS is a clinical condition with a relatively high mortality (40%). If this result is true, relatively minor improvements in transfusion transmitted diseases safety (reduction in 1/118,000 incidence of sepsis) would be potentially offset by a 1.6% (5/318 vs. 0/327) or 2.2% (12/318 vs. 5/327) increase in incidence of serious pulmonary events compared to reference platelets.

The expert pulmonary panel that re-analyzed patient charts identified additional cases of ALI and ARDS. According to this analysis the differences between the treatment and control arm for ALI and ARDS were not statistically significant, but the number of cases of ARDS found in the test arm was more than 2 fold higher than the control arm (12 vs. 5, Table 4). FDA regards this difference as a safety concern even though the post-hoc analysis did not achieve statistical significance. A larger study and/or a study with more careful pulmonary monitoring may have yielded a more definitive statistical outcome.

Potential Pathophysiology of Pulmonary Adverse events

The association of ARDS with S59 platelets in the SPRINT trial was unexpected but in retrospect may be attributable to activated platelets. This would be consistent with new research that implicates activated platelets in the pathophysiology of acute lung injury (8). Consistent with this research, it is possible that the reports of ARDS associated with S59 platelets may have been cases fitting the definition of Transfusion Related Acute Lung Injury (TRALI).

TRALI and ARDS have similar clinical presentations. Like ARDS, TRALI presents as respiratory distress. It occurs within 6 hours of a transfusion and is accompanied, as in ARDS, by hypoxemia, pulmonary dysfunction, hypotension and fever (7). The chest x-ray in TRALI and ARDS may look similar and patients may require mechanical ventilation in both conditions.

The pathophysiology of TRALI appears to involve at least two mechanisms (7). One is the presence of anti-leukocyte alloantibodies in the transfused product, while the other is the presence of non-antibody mediators (lipids, platelet antigens) present in stored blood products. Transfusion of products that have these characteristics may lead to TRALI if the recipient has an additional co-morbidity or a “hit” that makes them susceptible. Lipopolysaccharide (LPS) has been found to be a permissive “hit” in animals. Animals primed with LPS are more likely to develop acute respiratory distress when transfused with blood products containing antibodies or non-antibody mediators. Recent studies with animal models have shown that activated platelets contribute to neutrophil accumulation in the lung (8). Activated platelets bind to pulmonary endothelial cells and, through P-selectin (a platelet activation marker), can act as a tether to neutrophils. Accumulation of neutrophils in the lung can mediate local tissue damage.

Platelets treated with S59 are damaged as evidenced by reduced recovery and circulation in vivo (3). Infusion of these cells to patients, who may have underlying sepsis or otherwise inflamed endothelium, may lead to TRALI similar to what has been shown in animal models.

European Clinical Data

In 2000, Cerus conducted a controlled, randomized, double blinded clinical trial in Europe comparing buffy coat whole blood derived platelets with or without S59 pathogen reduction (EuroSprite) (9). The primary endpoint was Corrected Count Increment (CCI) which reflects the initial recovery of transfused platelets in the circulation but does not measure the hemostatic function of the platelets. The study had 103 patients, 52 in the test arm and 51 in the control arm with 35% of the patients receiving peripheral stem cell therapy and 63% receiving chemotherapy. Although the numbers were higher in the control arm, the 1 hour CCI counts were not statistically different between the two arms of the study. The difference for 24 hour CCI counts for the test and control platelets were statistically different with the test platelets having lower CCIs (t test on up to 8 initial platelet transfusions, Table 5).

CCI	Test	Control	p value
1 hour	13,100 \pm 5400	14,900 \pm 6200	0.11
24 hour	7,400 \pm 5500	10,600 \pm 7100	0.02

Table 5. Results of CCI endpoints for EuroSprite trial.

The total number of hemostatic adverse events did not differ statistically between the two arms of the study. However there were some clear differences in hemostatic adverse events between the two arms as described in Table 6.

Hemorrhagic adverse events by system organ class in cycle 1

	Test (n=52), no. (%)		Reference (n=51), no.(%)	
System organ class	Total	Severe	Total	Severe
No. of patients with hemorrhage	41 (79)	3 (6)	38 (79)	3 (6)
Eye disorders	7 (13)	1 (2)	0 (0)	0 (0)*
Eye hemorrhage	3 (6)	0 (0)	0 (0)	0 (0)*
Retinal hemorrhage	3 (6)	1 (2)	0 (0)	0 (0)*
Gastrointestinal disorders	18 (35)	0 (0)	14 (27)	1 (2)*
Gingival bleeding	9 (17)	0 (0)	6 (12)	0 (0)*
Rectal bleeding	4 (8)	0 (0)	1 (2)	0 (0)*
Gastrointestinal hemorrhage	3 (6)	0 (0)	5 (10)	1 (2)
General disorders and administration site conditions	11 (21)	0 (0)	3 (6)	0 (0)*
Injection site hemorrhage	9 (17)	0 (0)	3 (6)	0 (0)*
Investigations	9 (17)	0 (0)	7 (14)	0 (0)*
Hematuria	7 (13)	0 (0)	6 (12)	0 (0)*
Respiratory, thoracic, and mediastinal disorders	24 (46)	1 (2)	21 (41)	0 (0)*
Epistaxis	22 (42)	1 (2)	21 (41)	0 (0)*
Hemoptysis	6 (12)	0 (0)	2 (4)	0 (0)*
Skin and subcutaneous tissue disorders	19 (37)	0 (0)	15 (29)	0 (0)*
Purpura	8 (15)	0 (0)	7 (14)	0 (0)*
Petechiae	7 (13)	0 (0)	8 (16)	0 (0)
Ecchymosis	3 (6)	0 (0)	0 (0)	0 (0)*
Surgical and medical procedure	3 (6)	0 (0)	1 (2)	0 (0)*
Postoperative hemorrhage	3 (6)	0 (0)	0 (0)	0 (0)*

Vascular disorders	7 (13)	0 (0)	4 (8)	0 (0)*
Hematoma	7 (13)	0 (0)	4 (8)	0 (0)*

Table 6. This table was reproduced from Table 6 of reference (9): Examples of hemorrhagic AEs from the EuroSprite trial that occurred in more than 5% of patients.

* indicates that the frequency of this particular AE was higher in the test platelets.

Of the 22 types of system/organ hemorrhagic AEs reported, 20 were more frequent in the test platelets. None of the AEs listed reached a statistically significant difference between test and control.

Current Clinical Use in Europe

Several European countries have approved the use of Cerus S59 platelets and approximately 300,000 platelet transfusions have taken place (Cerus communication). The adverse events associated with S59 platelets have been reported to hemovigilance networks in some of these countries (10, 11). The safety data collected in these studies does not help resolve the issue of serious adverse events associated with transfusion of S59 platelets observed in the prospective, randomized and blinded clinical trial (SPRINT) because the patient population is different, the rates of adverse event reporting through hemovigilance networks are much lower than in a prospective, blinded clinical study, and there is no control group against which the treatment group is compared.

As can be seen in the Table below, the adverse event rate per patient is much higher in the SPRINT study as compared to rates published in two European Hemovigilance reports. This is true for all reactions (99.7 vs. 6.4 or 3.2), platelet related reactions (26 vs. 4.9 or 2.8), and serious platelet reactions (27 vs. 0.15 or 0), when comparing to those observed in the two European Hemovigilance studies. Hemovigilance data on 7,437 platelet transfusions included no serious adverse events attributable to the platelet transfusions. It is important to note that the patient population in Europe consisted of many fewer HSCT recipients (7.2 or 8.6 vs. 78). The outcomes of these data do not resolve FDA's concern regarding pulmonary adverse events because the patients who received PR platelets under the hemovigilance program were different and were monitored differently.

Comparison of SPRINT and published European Hemovigilance Adverse Event Data

	SPRINT Phase 3 US study		Osselar et al. Transfusion 2008 Cerus plts 2005-2007 Hemovigilance		Osselar et al. Vox Sang 2008 Cerus plts 2003-2005 Hemovigilance	
	Per transfusion	Per patient	Per transfusion	Per patient	Per transfusion	Per patient
N	2678	318	5106	651	7437	1400
% stem cell transplant patients		78		7.2		8.6
% Any reaction		99.7	1.1	6.4	0.9	3.2
% Plt related reaction	3.0	26.0	0.8	4.9	0.7	2.8
% Serious plt related reaction		27.0	0.1	0.15	0	0

Table 7. Reporting rates of adverse events associated with S59 platelets. Comparison of adverse event reports from a blinded prospective, randomized trial (SPRINT) and non-blinded Hemovigilance reporting in Europe.

Some countries have carried out their own evaluation of S59 platelets independent of the manufacturer (Cerus). In 2007, in the Netherlands, a randomized, controlled, multicenter, 3 arm trial was conducted in which patients received buffy coat whole blood derived platelets stored in plasma, additive solution (Intersol PAS III) or treated with S59 PR process (12). The storage time of the platelets was up to 7 days. The primary endpoint was a 1 hour corrected count increment (CCI). The secondary endpoints were 24 hour CCI, incidence of Grade 2 bleeding and other transfusion parameters. An interim analysis was conducted after enrollment of 199 patients and a report has been released as an abstract for the 2009 AABB annual meeting. The results released in the abstract are summarized in the table below and only include the plasma stored platelets vs. the S59 treated platelets. Note that FDA has not reviewed a complete study report.

According to the abstract, there were statistically significant differences indicating that the S59 platelets had reduced 1 hour and 24 hour CCI and increased frequency of bleeding compared to untreated platelets.

	Plasma Platelets	S59 Platelets	P value
No. patients	68	67	
No. transfusions	212	252	
1 hour CCI	17.5 \pm 7.1	11.4 \pm 5.4	< 0.0001
24 hour CCI	13.0 \pm 7.9	8.0 \pm 5.6	< 0.0001
No. patients with \geq Grade 2 bleeding	14	24	0.045

Table 8. Results of 2007 clinical trial conducted in The Netherlands with buffy coat platelets stored in plasma or treated with S59 pathogen reduction process. From 2009 AABB Annual meeting abstract (12)

After reviewing the preliminary data, the Data Safety Monitoring Board halted further enrollment in the S59 treated platelets arm of the study and the authors of the study concluded that the available data strongly suggest that the S59 platelets were inferior to the control arm. .

Discussion:

FDA's assessment of the available clinical data

The SPRINT study, a blinded, randomized, prospective clinical trial of S59 platelets compared to conventional platelets raised both safety and efficacy concerns. Although the study met its primary endpoint of equal proportion of patients with Grade 2 bleeding the trial also demonstrated that patients in the treatment arm had a lower numbers of circulating platelets, required more platelet transfusions, and experienced more bleeding complications (mean number of days of Grade 2 bleeding was statistically higher in the PR arm) which may have been due to platelet losses in processing (5), reduced recovery and survival or dysfunction of treated platelets. These results indicate that the S59 platelets are not as effective as conventional platelets and may be damaged during PR processing.

The safety of S59 platelets was also brought into question by this study. The statistically significant differences in the rates of 10 of 11 adverse event categories were all against the S59 platelets. These adverse events included 4 Grade 3-4 AEs , namely, hypocalcemia, pneumonitis, syncope and ARDS. FDA has focused mainly on ARDS because of the associated morbidity and mortality.

Cerus's experts performed a post hoc analyses of the SPRINT data. These analyses focused on pulmonary adverse event and are summarized as follows:

- ARDS rate comparing PR and reference platelets was 5 vs. 0 (p = 0.03) in the initial study analysis and 12 vs. 5 (p = 0.09,) as reported by blinded observers in a post-hoc analysis
- ALI rate was similar in the two arms (19 vs. 16, p=0.60)

- Mortality was similar in the two arms

Cerus proposed that these findings should resolve the safety concerns regarding pulmonary AEs. However, FDA remains concerned because of the higher number of ARDS cases found in the treatment arm compared with the control arm of the study.

Cerus has expressed the view that hemovigilance data obtained in Europe demonstrated the safety and efficacy of PR platelets.

FDA questions whether European hemovigilance data resolve the issue of pulmonary AEs for the following reasons:

- Different and probably sicker patients (fewer HSCT)
- Different source of platelets (buffy coat and apheresis)
- Insufficient monitoring and/or sicker patients as indicated by fewer adverse events in recipients of PR and untreated platelets compared to SPRINT

FDA's Proposed Path Forward

Cerus and FDA have agreed to resolve the safety and efficacy issues based on the following drug development plan:

- A premarketing study will be performed as a new prospective randomized, controlled, and blinded Phase 3 clinical trial focused on hemostatic efficacy and product safety. All AEs should be captured but the study should have enhanced monitoring to focus on detection of pulmonary adverse events.
 - Primary efficacy endpoint
 - Mean days of Grade 2 bleeding (shown to be a more sensitive endpoint than proportion of patients with bleeding)
 - Primary safety endpoint
 - Based on an ALI rate of 5% (SPRINT study) or higher in the control arm, the safety endpoint will be less than 5% more in the PR arm. If the control ALI rate is less than 5% then the PR arm should be less than double that rate.
- A Phase 4 study to confirm efficacy and safety, to exclude a 1% excess of ALI in treated vs. control arms.
- Staged roll-out of the product so that until the Phase 4 study is completed successfully the product is only used by centers participating in the Phase 4 trial.

The proposed premarketing protocol is described in detail in the Appendix. FDA is in general agreement with the protocol

Questions for the committee:

1. Is the Phase 3 study design adequate to provide information to resolve the safety and efficacy concerns raised by the outcomes of the SPRINT study?
 - a. Is ALI (inclusive of ARDS) a relevant safety endpoint?
If yes, is it appropriate to have two co-primary hypotheses that have to be met, namely, non-inferiority of the treatment arm compared to the control arm regarding rate of ALI, to exclude increases in the treatment arm of:
 - (i) 5% or more, and
 - (ii) two fold or more?
 - b. Is mean days of Grade 2 bleeding a relevant efficacy endpoint?
2. Please comment on the overall drug development plan that includes
 - a. a premarket study (see above)
 - b. a post marketing study that is initiated prior to licensure to exclude a 1% increase in ALI compared to the control group
 - c. a staged roll-out of the product.

References

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Appendix 1. Design for Proposed Phase 3 Clinical Trial of Photochemically Treated Platelet Concentrates [S-59 (Psoralen), PAS III (Platelet additive solution), UV Illumination System]

Title: A Phase 3 prospective, randomized, double blinded, multicenter clinical trial to determine effectiveness and safety of routine use of platelets prepared by the photochemical treatment (PCT) INTERCEPT system compared to platelet concentrates prepared by conventional processes (Reference)

Objectives:

- To demonstrate the hemostatic efficacy of PCT platelets compared to Reference (conventional) platelets
 - Primary Efficacy Endpoint: Days with Grade 2 bleeding
 - Secondary Efficacy Endpoints
 - Proportion of Patients with Grade 2 Bleeding During the Period of Platelet Support
 - Proportion of Patients with Grade 3 and Greater Bleeding During the Period of Platelet Support
 - Number of RBC Units Transfused Per Total Days of Platelet Support
 - Total Dose of Platelets Transfused Per Total Days of Platelet Support
 - Time (in days) to Onset of Grade 2 Bleeding
- To confirm the safety of PCT platelets compared to Reference (conventional) platelets
 - Primary Safety Endpoint: acute lung injury (ALI)

Inclusion:

- Patients with thrombocytopenia who require platelet transfusion (or who are expected to develop thrombocytopenia that requires platelet transfusion) and for whom the investigator agrees to manage with an initial platelet transfusion threshold of $10 \times 10^9/L$ or an appropriate transfusion threshold per clinical indications.
- Age ≥ 6 years
- Has one of the following disorders: acute leukemia (ALL or ANLL), chronic leukemia (CML, CLL, CMML, or hairy cell leukemia), lymphoma, myelodysplasia, other hematopoietic or non-hematopoietic malignancy, or therapy (chemotherapy or radiation) induced or idiopathic bone marrow aplasia or hypoplasia (e.g., aplastic anemia).

- A substantial proportion (>50%) of study patients will be recipients of autologous or allogeneic bone marrow or stem cell transplants (including cord blood) transplants.

Exclusion:

- Any medical, surgical, or psychiatric condition that makes the subject a poor candidate for this trial for conduct of hemostatic assessments.
- Prior history of clinical refractoriness to platelet transfusion as defined by two successive platelet transfusions with a 1 hour CCI <5000. Patients with a strong clinical history of refractoriness without successive CCIs <5000, or a positive lymphocytotoxic antibody test (>20% panel reactive cells) are also excluded.
- History of immune thrombocytopenia (ITP), thrombotic thrombocytopenic purpura (TTP), or hemolytic uremic syndrome (HUS), or has received PUVA (psoralen UVA) treatment in the 30 days prior to screening.
- Current participation in a clinical trial involving the use of another device or drug for pathogen inactivation of platelet concentrates, the use of platelet substitutes, the use of platelet growth factors, or the use of pharmacologic agents to alter platelet hemostatic function.
- History of acute promyelocytic leukemia (FAB subgroup M3). Use or expected use of IL-11 (Neumega) or other platelet production growth or differentiation factor during the period of platelet support.
- Diagnosis of ALI at study entry.

Withdrawal of Patients from the Study

- Voluntarily withdrawal
- Investigator also may withdraw a patient from the study for reasons of safety, non-compliance, or inability to conduct the required study assessments
- Reasons for withdrawal must be documented by the Investigator on the appropriate CRF
- Withdrawn patients should be monitored for adverse events for seven days after the last study transfusion. If a withdrawn patient refuses to participate in post-transfusion adverse event monitoring, the inability to monitor during this period must be documented on the withdrawal CRF
- Any patient who has received a granulocyte transfusion during the active transfusion period will be withdrawn from the study. The patient will complete the active transfusion period after the last platelet transfusion prior to receiving the granulocyte transfusion and will be followed for 7 days of active surveillance. All hemostatic assessments will be completed per the protocol. Granulocyte transfusions will be recorded on the CRF.

Dosing:

- Eligible patients will be randomized to either the Test or Reference study group as close to the first study platelet transfusion as possible. Randomization must take

- place within 30 days of enrollment. If a patient is not randomized within 30 days of enrollment, they must be re-screened for eligibility.
- Patients who do not require platelet transfusion after enrollment due to a change in clinical condition will not be randomized and will be recorded as enrolled in the Screening Log, but will not be randomized for transfusion.
 - Randomization will be stratified by site and blocked using variable block sizes.
 - At randomization patients will be assigned to receive all platelet transfusions, either Test or Reference, until independence from platelet transfusion support or for up to four weeks of platelet support after the first study platelet transfusion.
 - Test and Reference platelet concentrates will be prepared according to protocol to attain a targeted minimum transfusion dose of 3.0×10^{11} platelets.
 - In order to stay on protocol, products of any dose will be transfused as required when products are in limited supply.
 - Study concentrates may be stored for up to five days prior to transfusion.
 - Prophylactic platelet transfusions will be ordered by primary care medical staff using the daily platelet count and the institutional threshold for platelet transfusion as the guideline for ordering transfusion. The suggested minimum threshold for transfusion in this study is 10,000 platelets per μL . This threshold may be adjusted by the primary care physician for individual patient requirements.
 - Platelet transfusions to treat active bleeding or prepare patients for invasive procedures, defined as therapeutic transfusions in contrast to prophylactic transfusions, will be ordered as required by primary medical care staff.
 - The platelet content of each study platelet concentrate (Test and Reference) will be measured at time of issue for transfusion.
 - Samples will be retained from all issued study platelet concentrates for bacterial cultures pending specific patient assessments.
 - Study subjects will have all medications, diagnostic procedures, and therapeutic procedures ordered by primary care medical staff.

Collection and Preparation of Study Platelet Concentrates

- Test and Reference concentrates will be collected using US licensed apheresis platforms.
- All donor concentrates will be tested in accordance with US standards.
- Reference concentrates may be gamma-irradiated for patients in the study at the discretion of the treating physician
- Test concentrates do not need to be gamma irradiated.
- Test and Reference platelets may be HLA typed or similarly selected at the discretion of the treating physician
- Platelet components will be process leukoreduced and will not need filtration except for those collected on Haemonetics MC+. Off-study platelet transfusions should be leukoreduced.

- Two samples will be collected from Test and Reference concentrates before they are issued for transfusion.
 - One sample will be used for measurement of platelet count and
 - the other will be held in reserve for bacterial culture in the event of suspected post transfusion sepsis.
- PCT platelet concentrates will not be gamma irradiated for T cell inactivation.
- Platelet concentrates prepared for this study will have the following parameters recorded, and samples taken from both Test and Reference concentrates issued for each transfusion:
 - ABO and Rh group of the platelet concentrate
 - Unit identification number
 - Gamma irradiation status
 - Date and time of collection of each transfusion product (starting from the time of initiation of collection)
 - Platelet content of each concentrate as determined by platelet count ($\times 10^9/L$) and total volume determined by weight of each concentrate at time of issue after all samples are obtained before transfusion. (Samples will be taken before transfusion. If the transfusion container is returned to the blood bank, the volume will be determined by net weight.)
 - an approximately 2 mL aliquot of each platelet concentrate is withdrawn for possible bacterial culture

Monitoring:

	Baseline	day - 4	prior to each plt trx	6 hours prior to each plt trx	6 hours after each plt trx	24 hours after each plt trx	Daily after 1st plt trx	3 days after last plt trx	day 28
Demographics	√								
Pregnancy Status	√								
ALI Status	√								
Lymphocytotoxicity Analysis	√								√
Albumin		√							
Creatinine		√							
Sgpt (Alt)		√							
Alkaline Phosphatase		√							
Ldh		√							
Total Protein		√							
Bilirubin, Total		√							
Potassium		√							
Uric Acid		√							
Bun		√							
Sgot (Ast)		√							

Calcium/Total And Free.		√							
Hemoglobin		√							
Pt		√	√			√			
Hematocrit		√							
Aptt		√	√			√			
WBC Count		√							
Fibrinogen		√	√						
Platelet Count		√							
Urinalysis		√	√			√	√		
Immunohematology Tests		√							
ABO Group And Rh Type		√							
Splenomegaly Status		√		√					
Red Cell Transfusions, Numbers Of Units Transfused			√			√			
Temperature				√	√	√			
Infection Status				√					
Vital Signs (Blood Pressure, Heart Rate, Respiratory Rate)				√	√				
Hemostatic Assessment							√	√	
Record Additional Platelet Transfusions						√			
Adverse Events					√	√			
Concomitant Medications						√			

The number of red cell units transfused in the 24 hours prior to the first study platelet transfusion and all subsequent red cell transfusions during the active transfusion period and active surveillance period must be recorded. After the first study platelet transfusion all subsequent platelet transfusions will be study transfusions for the four week transfusion period. For exceptions see the discussion of off-protocol transfusions.

Conduct of the Daily Hemostatic Assessment

- A study nurse will examine each study patient at least once in every 24 hour period.
 - The study nurse will review the daily nursing care notes, medical progress notes, and care orders for reports of bleeding.

- The daily period is defined as the interval from 00:00 to 23:59 of each calendar day.
- A urine macroscopic test for blood will be done in each 24 hour period following a study platelet transfusion.
- If these tests were conducted for routine care, then the chart data may be used. If the tests were not done for routine care then they will be done as part of the study.
- The daily hemostatic assessments will be conducted using the WHO Bleeding Criteria as outlined in the following section. Each patient will be assigned a daily hemostatic score for 8 potential organ system and sites of bleeding, and the maximal score in any one organ system or site will be the daily bleeding assessment score. The organ systems and sites for bleeding assessments are as follows:
 1. Mucocutaneous: skin, upper airway, and oropharynx
 2. Gastrointestinal: upper and lower
 3. Genitourinary
 4. Broncho-pulmonary
 5. Musculoskeletal and soft tissue
 6. Body cavity: pleural, peritoneal, pericardial, retroperitoneal
 7. Central nervous system including retinal
 8. Invasive sites: intravenous and intra-arterial catheters, urinary catheters, endotracheal tubes, nasogastric tubes, surgical sites, phlebotomy sites.

The WHO Bleeding Criteria are as follows:

- Grade 1:** rare petechiae, oropharyngeal bleeding and epistaxis < one hour in duration, purpura less than 1 || in diameter. Stool occult blood test scored as trace and slight or up to 1+. Urine hemoglobin scored as trace and slight or up to 1+. Retinal hemorrhage without visual impairment. Abnormal vaginal bleeding with spotting < two pads per day.
- Grade 2:** melena, hematemesis, hemoptysis, hematuria, hematochezia and abnormal vaginal bleeding **not requiring red cell transfusion within 24 hours of onset and without hemodynamic instability** (change in systolic or diastolic BP > 30 mm Hg). Epistaxis or oropharyngeal bleeding > one hour in duration. Stool occult blood test scored as moderate or 2+ and greater. Urine hemoglobin scored as moderate or 2+ and greater. Bleeding from invasive sites, musculoskeletal bleeding, or soft tissue bleeding not requiring red cell transfusion support. Red cells in body cavity fluids on microscopic examination.
- Grade 3:** melena, hematemesis, hemoptysis, hematuria - including intermittent gross bleeding without clots, abnormal vaginal bleeding, hematochezia, epistaxis, and oropharyngeal bleeding requiring red cell transfusion specifically for support of bleeding within 24 hours of onset and without hemodynamic instability. Bleeding from invasive sites, musculoskeletal

bleeding, or soft tissue bleeding requiring red cell transfusion specifically for support of bleeding within 24 hours of onset. Body cavity fluids reported as grossly bloody in laboratory, nursing, or medical progress notes. CNS bleeding noted on CT (computerized tomography) without clinical consequences. (Note red cell transfusion must be specifically related to treatment of bleeding within 24 hours of onset for bleeding Grade 3).

Grade 4: debilitating bleeding including retinal bleeding with visual impairment, non-fatal CNS bleeding with neurologic signs and symptoms, bleeding associated with hemodynamic instability (hypotension, > 30 mm Hg change in systolic or diastolic BP), and fatal bleeding from any source. (Visual impairment is defined as a field deficit and patients with suspected visual impairment require an ophthalmologic consult for documentation).

Acute Transfusion Reactions

- For the six hours after the platelet transfusion, data will be collected for evidence of related symptoms (acute transfusion reactions).
- Patients will be evaluated during and post-transfusion for common transfusion-related reactions, including
 - fever (rise in temperature of at least 2°C),
 - chills,
 - nausea,
 - skin rash,
 - urticaria,
 - bronchospasm,
 - tachycardia or bradycardia (change in heart rate by > 25 bpm),
 - hypotension or hypertension (fall or rise in systolic or diastolic BP > 30 mm Hg respectively),
 - hemoglobinuria,
 - hemolysis, and
 - general well-being.
- Such reactions should also be recorded as adverse events.

Evaluation of Post-Transfusion Sepsis (Up to 24 hours Post-Transfusion)

- If a patient develops an increase in temperature > 2°C above the pre-transfusion temperature, or > 1°C above the pre-transfusion temperature with shaking chills (rigors) within 24 hours of platelet transfusion,
 - blood cultures from the patient will be obtained as part of this study.
 - The reserved aliquot of the transfused study platelet concentrate also will be cultured.
 - The technical methods of culturing the patient blood samples may be that of the investigational site. However, the technique of culturing the platelet aliquots should follow guidelines of the microbiology technical guidance

in the study. The Technical guidance also reviews criteria for determining if isolates are false positives.

- If bacteria are isolated from both the patient blood and the platelet concentrate aliquot, the isolated strain(s) will be sent to a reference laboratory and typed using either serologic or molecular methods to confirm that the platelet concentrate was the source of bacteremia.
- Bacterial isolates from cultured platelet aliquots, without blood culture isolates, also will be sent to the reference laboratory for identification.
- If a patient has a blood culture for other reasons as part of their medical care within 24 hours after a study transfusion, the study platelet aliquot must be cultured as well.
- All positive blood cultures identified during the study period will be recorded in the CRF.

Active Surveillance after each Transfusion Period

- Following each transfusion period, subjects will be followed for seven days of Active Surveillance.
 - Surveillance will consist of chart review (by the Investigator or designated person) and contact with the primary care physician and/or patient.
 - The post-transfusion surveillance can be made by telephone contact if the patient has been discharged.
- Forty-nine days after the first study transfusion, the mortality status of each patient will be determined as well as follow up on any serious and unexpected adverse event observed during the 28 day study period.

Stopping Rules

- The study will be monitored by a Data and Safety Monitoring Board (DSMB).
 - The DSMB will review data monthly during the trial.
 - The primary focus of the DSMB review is patient safety.
 - The DSMB may recommend to the Sponsor that the trial be stopped for safety-related concerns.

STATISTICAL METHODS

Primary Efficacy Endpoint: Mean Days with Grade 2 bleeding

Simulation studies to determine sample size and power were performed using R (Ihaka and Gentleman 1996). The Test mean was assumed to be 20% larger than the Reference mean (as observed in the SPRINT study when patients were administered the targeted transfusion dose). A non-inferiority margin of 1 day relative to the Reference arm was selected due to the discrete nature of the random variable and the evaluation of Grade 2 bleeding representing a summary of the hemostatic assessments carried out each day. A sample size of 475 patients per group provided power of 0.904 to reject inferiority if there was less than 1 day difference (relative to the Reference arm) in Grade 2 bleeding between the groups.

Mean days with Grade 2 bleeding (λ) is described by the following negative binomial regression model, i.e.,

$$\log(\lambda) = \mu + \tau X$$

0, Reference group

where $X = \begin{cases} 1, & \text{Test group} \end{cases}$

As a consequence of the model formulation, the log mean days with Grade 2 bleeding for the Reference group is $\log(\lambda_R) = \mu$ and for the Test group $\log(\lambda_T) = \mu + \tau$. Therefore, τ represents the difference in the log mean Grade 2 bleeding days between the Test and Reference groups. The non-inferiority hypothesis that is of interest is:

$$H_0: \tau \geq \exp(0.40) \text{ vs. } H_A: \tau < \exp(0.40)$$

or equivalently

$$H_0: \lambda_T / \lambda_R \geq \exp(0.40) \text{ vs. } H_A: \lambda_T / \lambda_R < \exp(0.40)$$

The null hypothesis (H_0) is rejected when

$$\exp[\text{UCB}(\tau)] < 1.49,$$

where $\text{UCB}(\tau)$ denotes the upper confidence bound of the 95% Wald confidence interval for the treatment effect model parameter (τ).

Secondary Efficacy Endpoints

Proportion of Patients with Grade 2 Bleeding During the Period of Platelet Support

Fisher's exact test will be used to evaluate the proportion of patients with Grade 2 bleeding during the period of platelet support. Prior data (from SPRINT trial) indicate

that the proportion of Reference patients with Grade 2 bleeding is 57.5%. The proposed study with 484 patients per arm (and assuming the Reference from SPRINT represents the Reference population) will have the ability to detect true Grade 2 bleeding rates of less than 48.3% or greater than 66.4% in tests patients with probability (power) 0.80. The Type I error probability (significance level) associated with this test of the null hypothesis that the proportion of patients with Grade 2 bleeding for Test and Reference patients are equal is 0.05.

Proportion of Patients with Grade 3 and Greater Bleeding During the Period of Platelet Support

Fisher's exact test will be used to evaluate the proportion of patients with Grade 3 and greater bleeding during the period of platelet support. SPRINT trial data indicate that the proportion of Reference patients with Grade 3 and greater bleeding is 6.1%. The study will have the ability to detect true Grade 3 and greater bleeding rates of less than 2.3% or greater than 11.4% in Test patients with probability (power) 0.80. The Type I error probability (significance level) associated with this test of the null hypothesis that the proportion of patients with Grade 3 and greater bleeding for Test and Reference patients are equal is 0.05.

Number of RBC Units Transfused Per Total Days of Platelet Support

The null hypothesis of interest is that the distribution of number of RBC units, or the volume of RBC, transfused per total days of platelet support in the Reference population is the same as that in the Test population. The Wilcoxon rank-sum test detects location shifts between the Reference and Test distributions. However, there may be significant site variability with regard to how RBC units are administered. Van Elteren's test (stratified Wilcoxon rank-sum test) is a nonparametric test that compares treatments (Reference and Test) in the presence of blocking (site). By accounting for site variability, van Elteren's test is better able to detect differences attributable to treatment that do not include treatment variability between sites. Van Elteren's test (two-sided) will be used to evaluate the potential differences in the number of RBC units transfused per total days of platelet support between Reference and Test arms at the 0.05 significance level.

Total Dose of Platelets Transfused Per Total Days of Platelet Support

The null hypothesis of interest is that the total dose of platelets transfused per total days of platelet support distribution in the Reference population is the same as that in the Test population. From the SPRINT statistical analyses, platelet transfusion exposure data stratified by treatment group and study site indicate differences between sites. Van Elteren's test is a nonparametric test that compares treatments (Reference and Test) in the presence of blocking (site). Van Elteren's test (two-sided) will be used to evaluate the potential differences in the total dose of platelets transfused per total days of platelet support between Reference and Test arms at the 0.05 significance level.

Time (in days) to Onset of Grade 2 Bleeding

Time to event (failure time data analysis) methods will be used to analyze the time to onset of Grade 2 bleeding. The Kaplan-Meier method will be used to analyze the time to onset of Grade 2 bleeding. Failure time distributions will be compared using a two-sided log rank test at the 0.05 significance level. The log rank test is used to test the null hypothesis that there is no difference between the reference and test failure time distributions (i.e., the probability of the onset of Grade 2 bleeding is the same for each treatment group). Patients that do not incur Grade 2 bleeding will be censored based upon the time (in days) they were enrolled in the study.

Plan for Data Analysis

Analysis will be by intent-to-treat. All patients who have at least one Test or Reference transfusion will be analyzed.

Primary Safety Analysis.

- 1) Powered for analysis of the adverse event of acute lung injury (ALI) by non-inferiority analysis.
 - a) In the SPRINT trial, the incidence of ALI differed across the categories of indication for platelet transfusion.
 - i) Chemotherapy only patients (those patients that did not have HSCT) had an incidence of ALI of 5.6% (8/143);
 - ii) Patients that had undergone autologous hematopoietic stem cell transplantation had an ALI incidence of 0.90% (3/325);
 - iii) The highest incidence of ALI was observed in patients that had undergone allogeneic hematopoietic stem cell transplantation with an incidence of 13.5% (24/177).
 - b) An estimate of ALI may be calculated by multiplying the incidence by the percentage of patients within each category. The resulting combined (Test and Reference) incidence of ALI is 5.62% from the SPRINT study, rounded to 6%, for use as the estimate for the Reference group for the current trial.
 - i) This ALI estimate will be used as a baseline in calculating the study sample size. The following co-primary hypotheses for the safety endpoint will be evaluated:

$$\begin{array}{ccc}
 H_{0A}: \pi_T - \pi_C \geq 0.05 & \text{versus} & H_{A1}: \pi_T - \pi_C < 0.05 \\
 H_{0B}: \frac{\pi_T}{\pi_C} \geq 2 & \text{versus} & H_{A2}: \frac{\pi_T}{\pi_C} < 2
 \end{array}$$

- ii) The test arm is not inferior to reference if non-inferiority is declared for both the additive and multiplicative scales.

- iii) Furthermore, the hypothesis tests carried out must maintain an overall significance level of 0.025.
- (1) If each hypothesis test is carried out at the 0.025 significance level and both hypotheses must be rejected to declare non-inferiority then the overall significance level is maintained at 0.025.
 - (2) The overall significance level is preserved when both hypotheses must be rejected to declare non-inferiority rather than one or the other.
- c) The rationale for using risk ratios versus risk differences when the Reference response rate is small was examined by Welleck (2005).
- i) Welleck (2005) noted that when using risk differences it becomes easier to demonstrate noninferiority as the control response rate approaches 0.
 - (1) Hence, the implementation of co-primary hypotheses provides assurance of non-inferiority if both null hypotheses are rejected at the 0.025 significance level.
 - d) Table 1 displays sample size estimates for unconditional exact tests of non-inferiority based upon a binomial ratio and a binomial difference (corresponding to the hypotheses stated above).
 - i) Assuming
 - (1) a Reference ALI rate of 6% (significance level of 0.025 and
 - (2) power equal to 0.80),
 - ii) a sample size of 1024 total patients is required to be able to reject both co-primary hypotheses (this is a result of taking the larger of the two sample size estimates).

Table 1: Exact Sample Sizes (Chan, 2003) Per Arm of Noninferiority Tests for ALI Rates Assuming a Fixed Length Trial

Category	Study hypotheses	Under H_A	π_c (control)	n (total) (80% power)
ALI rate of the control $\leq 5\%$	$H_0: \pi_T/\pi_C \geq 2$ $H_A: \pi_T/\pi_C < 2$ (no more than a doubling)	$\pi_T/\pi_C = 1$	0.01	6470
			0.02	3202
			0.03	2114
			0.04	1570
			0.05	1242
			0.06	1024
			0.07	870
			0.08	752
ALI rate of the control $>5\%$	$H_0: \pi_T - \pi_C \geq 5\%$ $H_A: \pi_T - \pi_C < 5\%$	$\pi_T - \pi_C = 0$	0.09	662
			0.01	212
			0.02	246
			0.03	368
			0.04	486
			0.05	596
			0.06	698

	(Noninferiority margin: 5%)	0.07	816
		0.08	924
		0.09	1024

2) Sample size determination.

- a) The noninferiority margins specified for sample size calculations do not need to be accurate estimates, but should reflect clinical relevance.
- b) In contrast, the reference rate of ALI (a nuisance parameter) in sample size calculations needs to be estimated accurately to determine the appropriate sample size.
 - i) As noted, there is limited knowledge of the reference rate of ALI.
 - (1) If the reference rate of ALI in the SPRINT trial analysis is not representative of current medical diagnostic criteria, there is either a significant risk of ending up with an inadequately powered study or a sample size that is considerably larger than necessary.
 - (2) To insure a properly designed and efficient study, a sample size re-estimation (SSR) design is proposed.
 - (a) Based upon review of interim analysis results, this adaptive design allows for sample size re-estimation.
 - (b) Sample size re-estimation procedures will be based on unblinded data review.
 - (c) When the data are unblinded, the Reference and Test rate of ALI will be directly estimated from the data which further permits the estimation of the effect size and its variability.
 - (d) To protect the overall Type-I error rate, adjustment of the critical value at the end of the study will be based upon conditional power.
 - (e) Cerus is currently determining the best approach in designing the SSR procedure and is taking into consideration Type-I error control, and futility.
 - (i) The statistical operating characteristics of this primary safety endpoint will be evaluated via Monte Carlo simulations.

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3. SPRINT study.

Secondary Safety Analyses

Fisher's exact test will be used to evaluate all of the safety endpoints that are summarized as frequencies or proportions.

DATA AND SAFETY MONITORING BOARD

The study will be monitored by a Data and Safety Monitoring Board (DSMB) composed of three transfusion medicine experts and one biostatistician assembled by the Sponsor. The DSMB will review data monthly during the trial. The DSMB will be composed of members who are independent of the Sponsor. The primary charter of the DSMB is to ensure patient safety. Based on the analyses, the DSMB may make recommendations to the Sponsor.

ASSESSMENT OF SAFETY

CRFs for pulmonary assessments will be submitted to an expert panel composed of three pulmonary experts. The pulmonary experts will determine the diagnosis of ALI according to the AECC criteria. The sponsor will be responsible for monitoring CRF in relation to the primary medical record.

Pulmonary Event Assessment

Systematic and consistent assessment of all patients for respiratory status will be performed at baseline and throughout the study (Appendix B). Respiratory assessments will be conducted by trained study personnel. Peripheral oxygen saturation and clinical respiratory status will be assessed for all patients during each day of study participation. Chest radiograph (CXR), oxygen saturation, FiO₂, and arterial blood gas (ABG) will be obtained and recorded from primary medical records for all patients who have adverse pulmonary signs and symptoms, as described in Appendix B.

Patients with signs and symptoms of acute lung injury (ALI) will also be evaluated for evidence of left atrial hypertension. Diagnosis from the treating physician will be recorded in the CRF and the final assessment for the safety endpoint will be made by a panel of physicians with expertise in pulmonary medicine who will evaluate blinded data gathered from patients who have adverse pulmonary signs and symptoms to determine the diagnosis of ALI and ARDS according to established American-European Consensus Conference (AECC) criteria.

Study investigators, research associates, and clinical monitors will be trained in the use of AECC criteria for the diagnosis of ALI/ARDS. Adverse event case report forms for pulmonary AE with severity ≥ 2 will be monitored against the primary medical record to insure that descriptions of pulmonary AE conform with the AECC criteria for diagnosis of ALI/ARDS.

Pulmonary Assessment Algorithm

B1 Assessment of Respiratory System Status of Each Patient at the Time of Study Entry (Performed By Trained Research Study Personnel).

1. A medical history will be obtained and recorded for each study patient, including:
 - a. Cigarette smoking history including number of pack years and whether current or prior smoker
 - b. Prior respiratory disease – Asthma, COPD, interstitial pulmonary fibrosis, recurrent pneumonias.
2. If the patient is receiving supplemental oxygen or requires mechanical assisted ventilation at the time of study entry, then specific information will be collected regarding:
 - a. Arterial blood gas measurements and/or pulse oximetry measurement of oxygen saturation with clear notation of the degree of oxygen support (i.e., such as 2 liters nasal oxygen or 40% oxygen face mask or 70% oxygen rebreather mask). If ventilated, then the exact FiO₂ to be recorded.
 - b. Pulmonary support, including
 - i. Supplemental O₂
 1. FiO₂
 2. Method of oxygen delivery (e.g., mask, nasal cannula, helmet, intubation, etc.) and oxygen flow rate
 - ii. Mechanical assisted ventilation (method and settings, tidal volume, respiratory rate, FiO₂, level of positive end expiratory pressure, mean airway pressure).
3. Physical exam (PE) and vital signs
 - a. A PE will be performed within 24 hours of study entry and results will be used to complete study CRF data fields
 - b. Study site personnel will not perform this physical exam but will record vital signs from the medical record (heart rate, respiratory rate, blood pressure, temperature).
 - c. Abnormalities of respiratory system and cardiovascular status will be specifically noted
4. Chest radiograph (CXR)
 - a. A CXR is required at the time of study entry, unless a CXR was already performed within 7 days prior to study entry and the patient did not subsequently have clinically relevant change in pulmonary status.
5. Pulse oximetry measurement of oxygen saturation within 24 hours of study entry. If the patient is already being monitored by pulse oximetry, then study personnel will record result in study records.
6. Assessments of left atrial hypertension, pulmonary edema or fluid overload. Relevant information (e.g., results of echocardiogram, pulmonary exam, fluid balance etc.) will be gathered from available medical records at the time of study entry.

B2 Detection and evaluation of changes in pulmonary status for each subject during the clinical study (Performed By Trained Research Study Personnel)

1. For each study day, subject records will be reviewed and data collected regarding changes in pulmonary status, including
 - a. New or worsening signs or symptoms of pulmonary disease
 - b. Requirement for supplement oxygen or mechanical assisted ventilatory support
 - i. FiO₂ (including the type of support, e.g., nasal cannula or face mask)
 - ii. Type of mechanical assisted ventilation (with the FiO₂).
2. Results (if performed) of chest radiograph, echocardiogram, CVP (central venous pressure), PCWP (pulmonary capillary wedge pressure), bronchoscopy (including gram stain and culture results). Copy of the chest radiograph report will be included in the CRF.
3. Vital signs (heart rate, respiratory rate, blood pressure, temperature) will be recorded in study documents at least once each day for each study subject.
 - a. For patients who have multiple measures of vital signs on a single day, both the high and low results for the day will be recorded, along with dates and times of measurements.
 - b. If vital signs for a study subject are not otherwise documented in the medical chart, then these will be measured by study personnel.
4. Pulse oximetry measurement of oxygen saturation will be performed and recorded (along with FiO₂) at least once each day on all study patients. For patients who have multiple measures of oxygen saturation on a single day, both the high and low results for the day will be recorded, along with dates and times of measurements.
5. If new signs or symptoms of treatment-emergent pulmonary adverse events are noted during the daily evaluation conducted by study personnel, then a CXR will be performed, if:
 - a. Respiratory rate is increased to greater than 24 per minute and sustained for more than 60 minutes.

Exceptions: CXR will not be required if oxygen saturation on room air is > 92% and the increased respiratory rate is documented as probably/definitely due to other, non-pulmonary cause (e.g., metabolic acidosis, severe pain, anxiety, etc.).

- b. Chest pain, dyspnea, bronchospasm, wheezing, and/or persistent cough are increased from the time of previous study assessment.
- c. Oxygen saturation is $\leq 92\%$ on room air.
- d. Patient uses supplemental oxygen.
- e. Patient requires mechanical assisted ventilation (e.g., positive-pressure ventilation (PPV) with intubation, helmet, or tight-fitting facemask).
 - i. A CXR will be recorded from the medical record if obtained on first day of mechanical ventilation and for any day on which P/F ratio is ≤ 300 .

- ii. If ALI criteria have been met previously during the study period or pulmonary status is unchanged since last CXR, then an additional CXR is not required but may be performed as determined by patient's physician.

B3 When a patient enrolled in the clinical trial requires mechanical assisted ventilation (positive pressure ventilation with intubation or tight-fitting face mask or helmet) during the study period, then:

1. Information regarding changes in pulmonary status will be collected and entered into study CRF once every 3 days for the duration of this study period.
2. Oxygen saturation (and FiO₂) will be measured and recorded at least once every 12 hours during the period of mechanical assisted ventilation
3. Arterial blood gas (ABG) will be recorded at least once each day for the first 3 days of the period of mechanical assisted ventilation
4. Fluid intake and output will be measured and recorded each study day during which mechanical assisted ventilation support is required.
5. An echocardiogram will be performed on any day on which P/F ratio is ≤ 300
 - a. If a study patient has previously met requirements for ALI or if pulmonary status is unchanged since previous echocardiogram, then another echocardiogram is not required for study purposes on days when P/F ratio is ≤ 300
 - b. If pulmonary artery wedge pressure is available, then echocardiogram not required.

B4 If a patient has P/F of ≤ 300 during the study period, then:

1. A chest radiograph will be performed. However, if ALI criteria have been met previously or if the pulmonary status is unchanged since last CXR, then an additional CXR is not required but may be performed as determined by patient's physician.
2. Fluid intake and output will be measured and recorded each day.
3. An echocardiogram will be performed. However, if pulmonary artery wedge pressure is available or if ALI criteria have been met previously during study period, then an additional echocardiogram will not be required but may be performed as determined by patient's physician.

B5 Additional Information Recorded in the CRF

Blood products administered, such as fresh frozen plasma, red blood cells

Any information regarding positive blood cultures, positive bronchoalveolar lavage cultures or positive stains for pneumocystis, positive tracheal aspirate cultures

Ventilator-free days, if ventilator support is discontinued during study period

Use of total body irradiation

Exposure to pharmacologic agents associated with pulmonary toxicity

