

## Infusion of P-Capt prion-filtered red blood cell products demonstrate acceptable in vivo viability and no evidence of neoantigen formation

Jose A. Cancelas, Neeta Rugg, P. Gayle Pratt, D. Nicole Worsham, Joan C. Pehta, Kate Banks, Robertson D. Davenport, and W. John Judd

**BACKGROUND:** Transmission of variant Creutzfeldt-Jacob disease (vCJD) is a major concern in blood transfusion. The P-Capt filter has been shown to remove around 4 log ID<sub>50</sub> prion infectivity from prion-spiked human red blood cells (RBCs).

**STUDY DESIGN AND METHODS:** Two independent, single-center, randomized, open-label studies were designed to analyze the safety of P-Capt-filtered RBCs. RBCs prepared from leukoreduced whole blood from 43 eligible subjects were randomly assigned to P-Capt filtration and/or storage in plasma or SAGM and stored for 28 or 42 days. Stored RBCs were analyzed for in vivo 24-hour recovery, hemolysis, metabolic variables, blood group antigen expression, neoantigen formation, and safety after autologous infusion.

**RESULTS:** Mean P-Capt filtration times for leukoreduced RBCs were 41 (SAGM) to 51 (plasma) minutes. Thirteen of 14 subjects receiving P-Capt-filtered RBCs had 24-hour RBC recoveries of 75% or more after 42-day storage, with a mean hemolysis of less than 0.6%. No loss of RBC antigen expression or formation of neoantigens was observed. In both studies, RBCs had white blood cell counts of less than  $1 \times 10^6$ /unit after leukofiltration. P-Capt prion filtration provided an additional greater than 0.8 log leukoreduction. No serious or unexpected adverse events were observed after infusion of P-Capt-filtered full-volume RBC units.

**CONCLUSIONS:** P-Capt-filtered, stored RBCs demonstrated acceptable viability and no detectable neoantigen expression, immunogenic responses, or safety issues after infusion of a complete unit. The additional filtration time and modest reduction in RBC content are within acceptable levels for implementation in countries with transfusion transmission of vCJD.

**H**uman transmissible spongiform encephalopathies (TSEs) are a group of related diseases that include kuru and sporadic and variant Creutzfeldt-Jacob Disease (vCJD).<sup>1</sup> vCJD, a disease with a clinical course and neurologic profile distinct from sporadic CJD, is a zoonosis derived from bovine spongiform encephalopathy (“mad cow disease”).<sup>2,3</sup> Although the incidence of vCJD has waned in the past few years, the number of individuals infected with vCJD in the United Kingdom was estimated by survey of surgically removed tissues to be around 100 per million in the population of age-related high risk,<sup>4,5</sup> far greater than the incidence observed, and forewarns that blood donations from asymptomatic individuals may present a serious threat of human-to-human transmission.<sup>6</sup>

Sixty-six recipients of blood donated by persons who later died of vCJD have been identified. Of the 19 recipients who lived long enough to present evidence of vCJD, four cases of transfusion-transmitted vCJD have been diagnosed either at autopsy or clinically.<sup>7,8</sup> This is consistent with a transmission rate of at least 20%.<sup>9</sup> In nonhuman primates, transmissibility of TSE by transfusion

**ABBREVIATIONS:** TSE(s) = transmissible spongiform encephalopathy(-ies); vCJD = variant Creutzfeldt-Jacob disease; WB = whole blood.

From the Hoxworth Blood Center, University of Cincinnati, Cincinnati, Ohio; The Alpha Bio Group, New Canaan, Connecticut; and the Department of Pathology, University of Michigan, Ann Arbor, Michigan.

*Address reprint requests to:* Jose A. Cancelas, MD, PhD, Hoxworth Blood Center, Research Division, University of Cincinnati, 3130 Highland Avenue, Cincinnati, OH 45267-0055; e-mail: jose.cancelas@uc.edu.

Received for publication October 26, 2010; revision received February 10, 2011, and accepted February 16, 2011.  
doi: 10.1111/j.1537-2995.2011.03133.x

**TRANSFUSION** 2011;51:2228-2236.

appears to be as high as the oral route.<sup>10</sup> Non-white blood cell (WBC)-bound prion protein infectivity is not removed by leukofiltration, and therefore leukoreduction alone may have little impact on transmission risk.<sup>11,12</sup>

Although testing of individual donations is desirable, the technical challenges in developing a sensitive test to identify prion protein remain formidable,<sup>13</sup> and although a new assay for CJD detection in cerebrospinal fluid of patients has been recently reported,<sup>14</sup> at this point in time, there is no credible assay for vCJD detection in blood donors. The lack of success with leukoreduction, the inherent difficulties in developing chemical-based pathogen inactivation protocols for red blood cell (RBC) containing products that would be effective on prion infectivity, and the inability thus far to develop a method to detect prion infectivity in blood means prion removal is the only practical solution to further reduce the risk of transfusion transmission. An appropriately designed and validated prion filtration strategy might also provide greater protection from these low-titer, long-incubating agents than is theoretically possible by diagnostic strategies.

A prion-specific affinity resin developed by Pathogen Removal and Diagnostic Technologies, Inc. for the selective adsorption of prions was incorporated into a filter device format, P-Capt, by Macopharma (Tourcoing, France), which was approved for use in the European Union (CE marked) in 2006. The resin, integrated into the prion filter, has been shown to remove approximately 4 log ID<sub>50</sub> of prion infectivity from human blood spiked with a hamster brain homogenate from symptomatic hamsters previously infected with 263K scrapie, a commonly used model to test prion infectivity.<sup>15</sup> The resin also demonstrated the ability to remove endogenous infectivity from hamster leukoreduced RBCs to below the limit of detection of the bioassay used to determine the infectivity titer.<sup>11</sup> RBC storage variables after leukoreduced RBCs were filtered with the P-Capt prion filter and then stored with either plasma-CPD or SAGM to outdate have been evaluated.<sup>16-18</sup> All of the final variables (hemoglobin [Hb] g/unit, hematocrit [Hct], residual WBCs, total volume) as well as end of shelf life product variables (hemolysis, adenosine 5'-triphosphate [ATP], 2,3-diphosphoglycerate acid [2,3-DPG], potassium, and pH) were within the UK and Council of Europe Guidelines.<sup>19,20</sup> No effects on complement or coagulation factor activation were observed, and no platelet (PLT) activation was identified.<sup>21</sup> These results indicate that RBC stability is not adversely affected by exposure to the P-Capt prion filter. More recently, the use of the P-Capt prion filter has been shown to have no detrimental effect on the *in vitro* variables of RBC quality, nor did its use appear to induce any relevant immunohematologic changes, although it did result in a loss of Hb. Despite this loss, the units remained within UK specifications throughout storage.<sup>18</sup>

Here, we report two studies designed to assess the effects of prion filtration on RBCs stored in nonadditive or additive solution by analysis of *in vivo* recovery, neoantigenicity, *in vitro* biochemical, and hematologic stability after 28 days in CPD-plasma or 42 days in SAGM solution and *in vivo* safety and neoantigenicity after infusion.

## MATERIALS AND METHODS

Two independent, single-center, randomized, open-label studies were performed under the authorization of the institutional review board at the University of Cincinnati. All subjects gave written informed consent and were eligible to donate according to the criteria of the Food and Drug Administration and the AABB (with the exception of travel-related deferrals). After screening, all subjects had 1 unit of whole blood (WB; 450 mL ± 10%) drawn using a CPD-SAGM collection set (DQE 6282LB, Macopharma) with an integral WB WBC removal filter (LST2-WB).

The primary endpoint of the chromium-51 (<sup>51</sup>Cr)-labeled RBC 24-hour recovery study (Study 1) was to examine the *in vivo* 24-hour recovery after infusion of autologous RBCs that had been filtered using the P-Capt prion filter and document that there were no more than 20% of the components with an *in vivo* 24-hour recovery of less than 75%.

In Study 1, after WBC filtration, subjects were randomized into one of four cohorts:

- Cohort 1: P-capt prion-filtered blood; 28-day storage in plasma (P-Capt-plasma)
- Cohort 2: non-P-capt prion-filtered blood; 28-day storage in plasma (control-plasma)
- Cohort 3: P-capt prion-filtered blood; 42-day storage in SAGM (P-Capt-SAGM)
- Cohort 4: non-P-capt prion-filtered blood; 42-day storage in SAGM (control-SAGM)

A second filtration step using the P-Capt prion filter (PSE 3080XB, Macopharma) was performed immediately thereafter in Cohorts 1 and 3. An aliquot from the prepared RBC units was taken on Days 0, 14, and 27 and tested for biochemical and hematologic variables. Results of prion-filtered units were compared to control (non-prion-filtered). All the processes were performed within 8 hours of blood collection. Sterility of the stored RBC units was tested 5 to 10 days before reinfusion of an aliquot of <sup>51</sup>Cr-radiolabeled RBCs. The blood was stored under normal blood bank conditions (1-6°C).

In the immunohematologic study (Study 2), after WBC filtration, blood units were stratified based on ABO blood group and randomly assigned to one of two cohorts:

- Cohort 1: P-Capt prion-filtered blood; 28-day storage of RBCs in plasma
- Cohort 2: P-Capt prion-filtered blood; 42-day storage of RBCs in SAGM

This process was designed so that for each cohort, there would be a minimum of two subjects with blood group A, one with blood group B, and two with blood group O. Half of the subjects had SAGM added to their RBCs and the other half had plasma added to their RBCs followed by another filtration step using the P-Capt prion filter (PSE 3080XB, Macopharma). Samples were drawn for testing before leukofiltration (baseline), before prion filtration, at the start of storage, and after storage. Blood was stored for either 28 days (plasma) or 42 days (SAGM) under normal blood bank conditions (1-6°C). Sterility of the stored RBC units was tested 5 to 10 days before reinfusion. At the end of storage and before reinfusion, each unit was inspected for any signs of unusual hemolysis or discoloration, and sample was drawn to evaluate changes from the baseline sample. The entire autologous RBC unit was then reinfused into the subjects. Follow-up consisted of the evaluation of safety events and laboratory assays (including evidence of neoantigenicity) for 6 weeks after infusion.

For both Study 1 and Study 2, analysis of hematology, spun Hct, postfiltration WBC count, plasma Hb, sodium, potassium, glucose, lactate, pH, 2,3-DPG, and ATP were performed according to standard procedures and/or manufacturer's instructions. For both studies, bacterial cultures were performed on all units during the last week of storage.

### WBC content

Remaining WBC content was determined by high sensitivity fluorescence-activated cell sorting analysis of propidium iodide-positive events using calibrated beads (Becton Dickinson, San Jose, CA).<sup>22</sup>

### 24-hour recovery of <sup>51</sup>Cr-labeled RBCs (Study 1)

On Day 28 or 42, each unit was inspected for any signs of unusual hemolysis or discoloration indicative of bacterial growth. Each unit was well mixed by hand (1 min), and approximately 15 mL of the RBCs was labeled with approximately 15 (10-20)  $\mu$ Ci <sup>51</sup>Cr using standard techniques.<sup>23,24</sup> The labeling agent, sodium chromate <sup>51</sup>Cr, was mixed aseptically with the RBCs at room temperature for 30 minutes and washed once with a double volume of saline. An aliquot of the final volume was reserved as a standard, and the remaining labeled cells (approx. 10 mL) were injected into a free-flowing peripheral vein. Samples (5 mL each) were taken from a contralateral vein at 5, 7.5, 10, 12.5, 15, 20, and 60 minutes, as well as at 24 hours. The samples were counted in a gamma counter to determine <sup>51</sup>Cr activity. The activity of the samples from the first 15 minutes was back-extrapolated to determine T<sub>0</sub>. A total of 27 subjects were eligible and evaluated. One recovery, from a subject belonging to the nonfiltered 42-day SAGM

group (Study 1, Cohort 4), could not be evaluated due to difficulties with venous access.

### Immunohematologic studies (Study 2)

The expression of C, e, and k antigens was determined in agglutination tests with monoclonal reagents (Hemo Bioscience, Durham, NC). The expression of D, E c, K, Fya, Fyb, S, and s antigens was determined by gel technique using polyclonal reagents (Hemo Bioscience) prediluted in AB serum to a titer of 4 to 8 versus single-dose RBCs. Reactions were graded and scored using the method of Marsh and Jenkins.<sup>25</sup> Expression of Band 3 and CD47 antigens was determined by flow cytometry using fluorescein isothiocyanate (FITC)-conjugated antibody to Band 3 (BRIC-6, International Blood Group Reference Laboratory, Bristol, UK) and CD47 (MIMA-49, New York Blood Center, New York, NY) with FITC-conjugated goat anti-mouse immunoglobulin (IgG) (Abcam, Cambridge, MA). Direct antiglobulin tests (DAT) were performed using tube technique with polyclonal reagent (anti-IgG plus C3, Ortho Clinical Diagnostics, Raritan, NJ). Gel tests for unexpected antibodies and DAT were performed on pretransfusion and on Day 42 posttransfusion samples with Group O R1R1, R2R2, and rr reagent RBCs. In addition, these samples were tested by gel technique against P-Capt prion filtered RBCs. Tests between the prion filtered RBCs and 12 randomly selected AB plasma units were also performed using the gel technique.

### Statistical analysis

Fisher's exact test or paired t test (SAS Version 8.20 or higher, SAS Institute, Inc., Cary, NC) were used for statistical analyses. Unless otherwise specified, all statistical tests were conducted using a two-sided alternative hypothesis and had a significance level of at least 0.05.

## RESULTS

### Demographics and baseline characteristics

In Study 1, 33 subjects were screened and 29 were enrolled. One subject withdrew from the study due to an inability to return within the time frame required for reinfusion, this subject was replaced. The mean age of all subjects was  $39.2 \pm 13.4$  years. There were no significant differences in demographic variables between the cohorts in baseline vital signs, screening Hct, or laboratory variables. In Study 2, 16 subjects were screened, 14 were enrolled, and seven were assigned to each treatment group. The mean age of all subjects was  $44.1 \pm 12.3$  years. Distribution of donors according to their ABO group was as follows: five blood group A (two SAGM and three

plasma), two blood group B (one each SAGM and plasma), and seven blood group O (four SAGM and three plasma). For both studies, all inclusion-exclusion criteria were met for each subject.

**WBC reduction and RBC yield**

For both studies, all units had WBC counts of less than  $5 \times 10^6$ /unit after leukofiltration. The mean prion filtration time was  $41.8 \pm 6.2$  and  $42.6 \pm 16.2$  minutes for the SAGM groups on Studies 1 and 2, respectively, and  $41.4 \pm 16.2$  and  $51.4 \pm 16.4$  minutes for the plasma groups in Studies 1 and 2. For Study 1, the mean WBC count after leukofiltration was  $0.11 \pm 0.09$  cells/ $\mu$ L in the P-Capt 28-day storage group and  $0.16 \pm 0.19$  cells/ $\mu$ L in the P-Capt 42-day storage group. After prion filtration the WBC counts were  $0.04 \pm 0.04$  and  $0.01 \pm 0.02$  cells/ $\mu$ L, respectively. This resulted in an 86.7% reduction ( $p = 0.0004$ ) in the P-Capt 28-day and a 94.7% reduction ( $p = 0.0001$ ) in the P-Capt 42-day group. For Study 2, the WBCs remaining after prion filtration were  $0.04 \pm 0.04$  cells/ $\mu$ L (83.9%,  $p = 0.001$ ) in the SAGM group and  $0.18$  cells/ $\mu$ L (93.1%,  $p < 0.001$ ) in the plasma group.

We next analyzed the volume of RBCs after filtration. It should be noted that 40 mL of the SAGM solution in the prion filter remains in the final product. For both studies, the mean starting volume was similar across the four cohorts, as were the mean volumes of the units before prion filtration, if applicable (Table 1). The mean volume of RBCs recovered after prion filtration, for plasma- and SAGM-containing products, after correction for the additional fluid in the prion filter was 86.2%, and 87.0%, of the recoveries of the corresponding non-prion-filtered, WBC-filtered RBC products, respectively. Despite this loss of Hb (Table 1), all units had 40 g Hb/unit or more at the time of storage, which is consistent with the UK specifications for leukoreduced units.

**Poststorage biochemical variables**

There were no significant changes in mean RBC content, Hb level, PLT numbers, and spun Hct over time in the prion-filtered units stored in either SAGM or plasma (data not shown). The results of testing for Hct, pH, and ATP showed significant decreases from the time of initial storage to immediately before reinfusion for both SAGM- and plasma-stored samples (Table 2). In all groups, the level of 2,3-DPG decreased after storage, as expected, to less than 1  $\mu$ mol/g Hb (data not shown). Mean glucose consumption ranged from 35% to 69%, mean lactate concentration increased from 6.1 to 15.8-fold, and mean potassium increased from 12.8- to 29.9-fold (data not shown) without any significant difference between P-Capt-filtered and control units and with values within the expected values for the length of storage and storage

**TABLE 1. Processing of donated blood by treatment group and Hb content of units after filtration(s)**

Variable	Statistic	Study 1				Study 2			
		P-Capt plasma 28 days (n = 7)	Control plasma 28 days (n = 7)	P-Capt SAGM 42 days (n = 8)	Control SAGM 42 days (n = 6)	P-Capt plasma 28 days (n = 7)	P-Capt SAGM 42 days (n = 7)	P-Capt plasma 42 days (n = 7)	P-Capt SAGM 42 days (n = 7)
WB volume (mL) before leukoreduction	Mean (SD) Range	516.2 (7.5) 504.8-527.5	519.1 (4.4) 514.9-528.4	522.8 (6.6) 510.9-531.9	522.8 (6.2) 513.6-528.6	522.0 (4.8) 514.3-529.4	522.8 (6.6) 513.6-528.6	522.0 (4.8) 514.3-529.4	522.4 (3.4) 518.0-528.3
RBC unit volume (mL) before leukoreduction	Mean (SD) Range	292.0 (17.6) 271.5-316.5	N/A N/A	286.4 (20.5) 265.8-331.5	NA NA	300.7 (18.5) 269-319.6	NA NA	300.7 (18.5) 269-319.6	290.4 (8.3) 279.7-303.2
RBC unit volume (mL) before storage after filtration(s) (after prion filtration if applicable)*	Mean (SD) Range	291.6 (16.1) 268.5-315.9	301.8 (17.5) 285.6-330.6	289.1 (20.4) 267.2-332.4	309.0 (15.6) 286.7-330.4	299.6 (14.3) 274.6-316.8	309.0 (15.6) 286.7-330.4	299.6 (14.3) 274.6-316.8	293.2 (7.7) 284.4-304.9
Hb (g/dL) after filtration(s) before storage	Mean (SD) Range	16.9 (0.9) 16.0-18.3	18.9 (0.8) 18.1-20.2	16.2 (0.9) 15.1-18.2	19.8 (1.0) 18.5-21.3	17.5 (0.9) 16.2-18.8	19.8 (1.0) 18.5-21.3	17.5 (0.9) 16.2-18.8	17.1 (1.0) 14.9-17.9
Hb (g) per unit after filtration(s) before storage (after prion filtration if applicable)*	Mean (SD) Range	49.3 (5.0) 43.0-55.5	57.1 (5.2) 52.2-66.8	47.3 (5.9) 41.7-60.5	61.2 (5.8) 54.2-70.4	52.4 (5.1) 44.5-58.6	61.2 (5.8) 54.2-70.4	52.4 (5.1) 44.5-58.6	50.2 (3.5) 43.2-53.7

\* Determinations were performed immediately before storage of RBC units. Although it appears that there was minimal loss of volume through the filters, it is noted that 40 mL SAGM is contained within the prion filter. The net minimal loss of volume is the result of the subtraction of added SAGM volume minus RBC volume loss. NA = not analyzed.

**TABLE 2. Laboratory tests on processed and/or stored unit at start and finish by treatment group: variables with significant changes over storage time**

Variable	Time point	Statistic	Study 1				Study 2			
			P-Capt plasma		Control plasma		P-Capt plasma		Control plasma	
			28 days (n = 7)	42 days (n = 7)	28 days (n = 7)	42 days (n = 7)	28 days (n = 7)	42 days (n = 7)	28 days (n = 7)	42 days (n = 7)
Hct (%)	Baseline	Mean (SD)	55.6 (1.9)	53.4 (2.3)	64.1 (1.2)	61.4 (2.9)	55.9 (5.6)	55.9 (3.7)	55.9 (3.7)	
	Day 27 or 41 of Storage	Mean (SD) p value*	54.6 (1.7) 0.02	53.2 (2.1) 0.57	62.2 (1.6) 0.002	62.7 (3.0) 0.09	54.8 (3.2) 0.46	56.4 (2.4) 0.61	56.4 (2.4) 0.61	
pH	Baseline	Mean (SD)	6.9 (0.02)	6.9 (0.03)	6.9 (0.02)	7.0 (0.04)	6.98 (0.03)	6.97 (0.04)	6.97 (0.04)	
	Day 27 or 41 of Storage	Mean (SD) p value*	6.5 (0.02) <0.01	6.3 (0.03) <0.01	6.5 (0.02) <0.01	6.4 (0.03) <0.01	6.93 (0.025) <0.01	6.52 (0.04) <0.01	6.52 (0.04) <0.01	
ATP (μmol/gHb)	Baseline	Mean (SD)	3.1 (0.5)	3.7 (0.9)	3.0 (1.0)	3.1 (0.6)	3.6 (0.5)	3.3 (0.8)	3.3 (0.8)	
	Day 27 or 41 of Storage	Mean (SD) p value*	1.8 (0.5) 0.0001	2.0 (0.2) 0.007	1.4 (0.3) 0.004	1.7 (0.4) <0.01	2.1 (0.2) <0.01	1.9 (0.5) <0.01	1.9 (0.5) <0.01	
Hemolysis (%)	Baseline	Mean (SD)	0.034 (0.019)	0.029 (0.003)	0.032 (0.018)	0.020 (0.003)	0.036 (0.020)	0.025 (0.003)	0.025 (0.003)	
	Day 27 or 41 of Storage	Mean (SD) p value*	0.400 (0.314) 0.02	0.409 (1.125) 0.0001	0.327 (0.182) 0.004	0.370 (0.191) 0.003	0.559 (0.326) 0.005	0.271 (0.196) 0.02	0.271 (0.196) 0.02	

\* p value based on paired t test.

solution used. The percentage of hemolysis showed a significant increase during storage that was within reported ranges for 28- and 42-day storage in CPD plasma and SAGM, respectively (Table 2). All units had 0.8% or less hemolysis at baseline. On the day before the end of storage 4 of 15 units in the P-Capt SAGM cohorts had a value of more than 0.8% and 2 of 15 units had a value higher than 1%.

**In vivo 24-hour <sup>51</sup>Cr-labeled RBC recovery: Study 1**

In Study 1, one subject in Cohort 4 (SAGM control arm) was unable to complete the RBC recovery procedure due to difficulties with venous access. The percentage of subjects with an RBC recovery of 75% or more at 24 hours was 87.5% in both the prion-filtered and non-prion-filtered 28-day plasma storage groups, and 100% in the prion-filtered SAGM and 80% in the control SAGM arm (Table 3). There were no significant differences in the number of subjects with an RBC recovery of 75% or more at 24 hours among the four cohorts (Table 3).

**Immunohematologic studies: Study 2**

In the neoantigenicity analysis performed in Study 2, no major differences were seen between pre- and post-prion filtration RBC antigen scores (Figs. 1 and 2) or in Band 3 and CD47 percentage of positive cells or mean fluorescent intensity (Table 4). All pre- and 6-week posttransfusion DATs and antibody screens were negative. No reactivity was seen with AB plasma and prion-filtered (and non-prion-filtered) control cells. Finally, tests to detect antibody formation to prion-filtered RBCs, performed between control prion-filtered RBCs from random group-compatible donors and postinfusion subject plasma, were nonreactive.

**Infusion tolerability and adverse events**

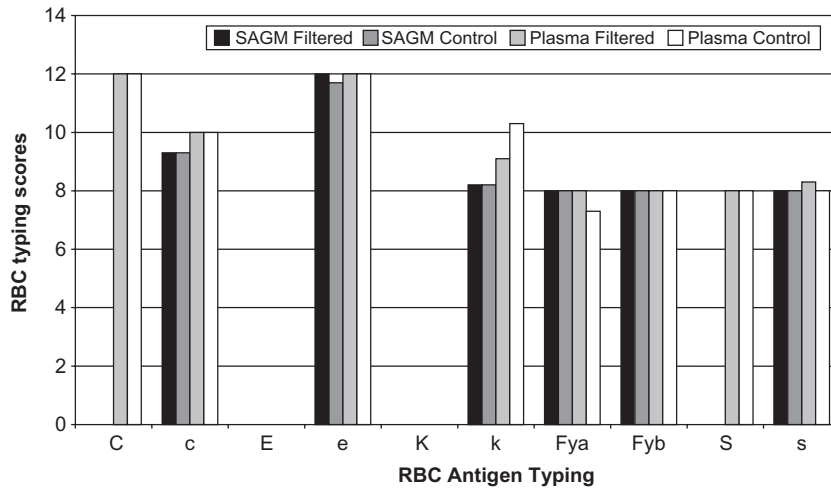
For Study 1, there were no serious adverse events reported. There were 14 adverse events reported in eight subjects (11 events in the P-Capt arms and three in the control arms). One of the 14 total adverse events was considered as “unlikely related” to study treatment (sinusitis), the remaining 13 events were considered to be “unrelated” to the study treatment. The most frequently reported adverse events were headache (x6), sinusitis (x2), dyspepsia (x2), and dysmenorrhea (x2). There was one report each of rhinitis and pain. There were five events classified as Grade 2 (dysmenorrhea [x2], headache [x2], and myalgia), all of which occurred in two subjects randomly assigned to Cohort 3 (P-Capt filter-SAGM), and none were thought to be related to study treatment. The remaining nine events were classified as Grade 1. There were no apparent relationships between the adverse events

**TABLE 3. Study 1: 24-hour recovery of <sup>51</sup>Cr-radiolabeled RBC infused at the end of storage periods**

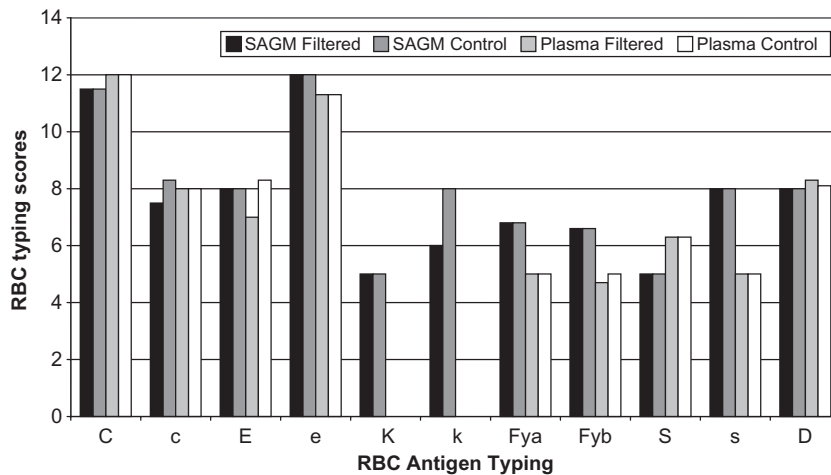
	P-Capt plasma (n = 7)	Control plasma (n = 7)	p value*	P-Capt SAGM (n = 8)	Control SAGM (n = 5†)	p value*
Mean ± SD	85.1 ± 6.1	83.0 ± 9.3	0.62	84.0 ± 6.3	78.6 ± 2.9	0.10
Range	74.6-93.8†	65.9-93.2†		78.7-97.4	73.6-81.1†	
Percentage with recovery ≥ 75%						
No	1 (14.3%)	1 (14.3%)	1.0	0	1 (20%)	0.38
Yes	6 (85.7%)	6 (85.7%)		8 (100%)	4 (80%)	

\* p value based on Fisher's exact test.

† RBC recovery for one subject could not be completed due to an inability to withdraw blood samples due to difficulties with venous access.



**Fig. 1. RBC antigen typing scores: stored prion-filtered and non-prion-filtered (controls) from apparent homozygous subjects.**



**Fig. 2. RBC antigen typing scores: stored prion-filtered and non-prion-filtered (controls) from heterozygous subjects.**

reported and the donation of the WB unit or the reinfusion of the stored, radiolabeled RBC. The time lapse between the withdrawal of the WB unit and the onset of the adverse event ranged from 2 to 43 days, and the time between the RBC reinfusion and the onset of the adverse event ranged from 1 to 40 days. All vital signs remained stable during

infusion. There were no serious adverse events reported and all bacterial cultures were sterile after 5 days of incubation. There were no significant changes before or after infusion in mean systolic or diastolic blood pressure, pulse, or temperature. None of the values for these variables were outside of the normal range after reinfusion.

For Study 2, there were five Grade 1 (mild and generally transient deviation from typical or normal clinical course) adverse events (three in the SAGM group and two in the plasma group) reported in four subjects, headache (×2), sore throat (pharyngitis), knee pain (arthralgia), and myalgia. All of the events occurred either 20 or more days before infusion (arthralgia and myalgia) or 30 or more days after infusion. All vital signs remained stable during infusion. There were no serious adverse events reported and all bacterial cultures were sterile after 5 days of incubation. There were no significant changes before or after infusion in mean systolic or diastolic blood pressure, pulse, or temperature. None of the values for these variables were outside of the normal range after reinfusion.

## DISCUSSION

The P-Capt prion filter is a sterile dockable filter for the removal of prions from leukoreduced RBCs. The P-Capt filter was designed to help further reduce the risk of transmission of vCJD through transfusion of RBC units by the selective adsorption of prions. In the filter, ligands specific for prion proteins are covalently attached to a resin. The resin is impregnated between nonwoven media, which is then integrated and placed in an outer polyvinylchloride housing to form the P-Capt prion filter.

**TABLE 4. Flow cytometry results with anti-IAP (CD47) and Band 3: stored prion-filtered and non-prion-filtered (control) RBCs**

Antigen	Variable	Statistic	P-Capt SAGM (n = 7)	Control SAGM (n = 7)	SAGM p value*	P-Capt plasma (n = 7)	Control plasma (n = 7)	Plasma p value*
CD47	Percentage positive	Mean ± SD Range	99.14 ± 2.20 94.16-100.00	99.41 ± 1.25 96.58-99.98	0.49	94.97 ± 2.30 91.32-97.32	96.58 ± 2.52 92.62-99.35	0.11
	Mean fluorescent intensity	Mean ± SD Range	78.42 ± 22.83 31.61-106.65	76.58 ± 24.42 29.74-100.26	0.72	27.51 ± 2.57 24.21-31.30	29.98 ± 4.87 25.52-38.17	0.26
Band 3	Percentage positive	Mean ± SD Range	100 ± 0.0 99.99-100	99.99 ± 0.01 99.96-100	0.06	99.99 ± 0.02 99.96-100	100 ± 0.01 99.97-100	0.86
	Mean fluorescent intensity	Mean ± SD Range	865 ± 195 569-1037	927 ± 248 685-1355	0.37	1198 ± 361 854-1917	1348 ± 405 855-1995	0.51

\* p value based on paired t test.

Although standard leukoreduction has been demonstrated to remove WBC-associated TSE infectivity in WB collected and pooled from scrapie-infected hamsters, only 42% to 72% of the total blood-borne infectivity is removed.<sup>14,18</sup> Studies evaluating the effectiveness of the P-Capt prion filter have demonstrated a greater than 99.9% (>3 log) reduction of infectivity in human RBCs spiked with a hamster-derived scrapie infected homogenate.<sup>15</sup> In another study, none of 100 hamsters inoculated with P-Capt prion-filtered leukoreduced WB from TSE-infected animals became infected, while 15 of 99 hamsters inoculated with leukoreduced blood from TSE-infected animals that had not been treated with the P-Capt prion filter contracted TSE infections from the contaminated blood.<sup>11</sup> Additionally, the removal of endogenous blood-borne infectivity, a more representative test of performance, was demonstrated to remove more than 1.2 log of infectivity, which is below the detection limit of the most sensitive bioassay systems available for the measurement of prion infectivity.<sup>15</sup>

Overall, in this study, prion-filtered units had an approximately 15% lower content of RBCs than non-prion-filtered units, while maintaining Hb at or above 40 g/unit. Hemolysis was less than 0.8% for all units stored in plasma for 28 days, but was higher than 0.8% in 4 of 15 units and more than 1% in 2 of 15 units stored in SAGM. The increased hemolysis of these 42-day-stored units may be a result of the additional manipulation during storage where sampling for blood cultures was required before in vivo administration. A recent study on 272 P-Capt-filtered units that had been stored unmanipulated for up to 42 days in SAGM has shown that this method may meet the UK specifications.<sup>18</sup>

The primary endpoint of Study 1 was to examine the 24-hour recovery after infusion of radiolabeled autologous RBCs after filtration with the P-Capt prion filter and to document that there were no more than 20% components with a recovery of less than 75%. Across all subjects, the mean RBC recovery was 83%, 84.5% in all subjects in the P-Capt groups and 81.2% in all subjects in the non-P-Capt groups. There were no significant differences in RBC recovery between the P-Capt 28-day (85.1%) and the non-P-Capt 28-day groups (83.0%) or between the P-Capt 42-day (84.0%) and non-P-Capt 42-day (78.6%) groups.

The percentage of subjects with RBC recovery of 75% or more at 24 hours was 87.5% in both 28-day treatment groups, 100% in the P-Capt 42-day treatment group, and 80% in the non-P-Capt 42-day group. There were no significant differences in the number of subjects with RBC recovery of 75% or more at 24 hours between the P-Capt 28-day group (85.7%; 95% confidence interval [CI], 59.8 to [-100]) and the non-P-Capt 28-day group (85.7%; 95% CI, 59.8 to [-100]; p = 1.00), nor between the P-Capt 42-day (100%; 95% CI, 100 to [-100]) and the non-P-Capt 42-day

groups (80%; 95% CI, 44.9 to [-100];  $p = 0.38$ ). Therefore, the primary endpoint was met.

In Study 2, a full unit of prion-filtered RBCs was well tolerated after autologous infusion without any relevant adverse events. Analysis showed an absence of changes in RBC antigens after storage, and DAT and antibody screening for RBC antigens or neoantigens 6 weeks after infusion of autologous prion-filtered RBCs were negative. These results are in line with the data from Cahill and colleagues<sup>16</sup> who found no significant adverse events in a group of 20 patients transfused with 1 unit, including six who received transfusion of a second prion-filtered unit. Additional studies are required to rule out the development of antibody formation after repeated infusions and analysis of their impact on the RBC recovery in vivo.

In conclusion, the use of the P-Capt prion filter resulted in an adequate safety profile with acceptable storage variables assessed by in vivo methods and no indication of the development of neoantigenicity after P-capt filtration and reinfusion of 1 complete unit of autologous RBCs in normal volunteers.

#### ACKNOWLEDGMENTS

The authors thank Michael Mathews, BSc, MSc, for his assistance in providing study management, regulatory advice, and logistics during the conduct of this clinical trial and Margaret O'Leary for editorial assistance.

#### CONFLICT OF INTEREST

JAC, NR, PGP, and DNW have no relevant conflicts of interest to declare. WJJ is Consultant of Alpha Bio Group, LLC. JCP and KB are consultants of ProMetic Life Sciences, Inc. This study was fully conducted at Hoxworth Blood Center, University of Cincinnati, while immunohematologic analyses were performed at the Department of Pathology, University of Michigan. The study was supported with funding from Pathogen Removal and Diagnostic Technologies, Inc./ProMetic Life Sciences, Inc./MacoPharma.

#### REFERENCES

1. Prusiner SB. Prions. *Proc Natl Acad Sci U S A* 1998;95:13363-83.
2. Will RG, Ironside JW, Hornlimann B, Zeidler M. Creutzfeldt-Jakob disease. *Lancet* 1996;347:65-6.
3. Gibbs CJ Jr, Gajdusek DC, Asher DM, Alpers MP, Beck E, Daniel PM, Matthews WB. Creutzfeldt-Jakob disease (spongiform encephalopathy): transmission to the chimpanzee. *Science* 1968;161:388-9.
4. de Marco MF, Linehan J, Gill ON, Clewley JP, Brandner S. Large-scale immunohistochemical examination for lymphoreticular prion protein in tonsil specimens collected in Britain. *J Pathol* 2010;222:380-7.
5. Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, Ritchie D, Penney M, Hegazy D, Ironside JW. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol* 2004;203:733-9.
6. Llewelyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, Mackenzie J, Will RG. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 2004;363:417-21.
7. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG. Creutzfeldt-Jakob disease and blood transfusion: results of the UK transfusion medicine epidemiological review study. *Vox Sang* 2006;91:221-30.
8. Peden A, McCardle L, Head MW, Love S, Ward HJ, Cousens SN, Keeling DM, Millar CM, Hill FG, Ironside JW. Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. *Haemophilia* 2010;16:296-304.
9. Wilson K, Ricketts MN. Transfusion transmission of vCJD: a crisis avoided? *Lancet* 2004;364:477-9.
10. Herzog C, Sales N, Etchegaray N, Charbonnier A, Freire S, Dormont D, Deslys JP, Lasmézas CI. Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. *Lancet* 2004;363:422-8.
11. Gregori L, Gurgel PV, Lathrop JT, Edwardson P, Lambert BC, Carbonell RG, Burton SJ, Hammond DJ, Rohwer RG. Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins. *Lancet* 2006;368:2226-30.
12. Gregori L, McCombie N, Palmer D, Birch P, Sowemimo-Coker SO, Giulivi A, Rohwer RG. Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood. *Lancet* 2004;364:529-31.
13. Gregori L, Gray BN, Rose E, Spinner DS, Kascsak RJ, Rohwer RG. A sensitive and quantitative assay for normal PrP in plasma. *J Virol Methods* 2008;149:251-9.
14. Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, Matsubara T, Nakagaki T, Yamanaka H, Shirabe S, Yamada M, Mizusawa H, Kitamoto T, Klug G, McGlade A, Collins SJ, Nishida N. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med* 2011;17:175-8.
15. Gregori L, Lambert BC, Gurgel PV, Gheorghiu L, Edwardson P, Lathrop JT, Macauley C, Carbonell RG, Burton SJ, Hammond D, Rohwer RG. Reduction of transmissible spongiform encephalopathy infectivity from human red blood cells with prion protein affinity ligands. *Transfusion* 2006;46:1152-61.
16. Cahill MR, Murphy T, Khan M, Fagan J, Murphy WG. Phase I/II safety study of transfusion of prion-filtered red cell concentrates in transfusion-dependent patients. *Vox Sang* 2010;99:174-6.



17. Murphy CV, Eakins E, Fagan J, Croxon H, Murphy WG. In vitro assessment of red-cell concentrates in SAG-M filtered through the MacoPharma P-CAPT prion-reduction filter. *Transfus Med* 2009;19:109-16.
18. Wiltshire M, Thomas S, Scott J, Hicks V, Haines M, Cookson P, Garwood M, Cardigan R. Prion reduction of red blood cells: impact on component quality. *Transfusion* 2010;50:970-9.
19. UK-Blood-Services. Evaluation of new red cell components for transfusion. Guidelines for the Blood Transfusion Services in the UK TSO (The Stationery Office). 7th ed. London: The Stationery Office; 2005. p. 119-21.
20. Council of Europe. Red cells, in additive solution. Guide to the preparation, use and quality assurance of blood components. 2007;Chapter 12-14:113-28.
21. Lathrop M, Poncheri M, Gheorghiu L, Hammond D. Storage study of red blood cells treated with the MacoPharma P-Capt prion removal filter. *Transfus Med* 2006;16:36.
22. Barclay GR, Walker B, Gibson J, McColl K, Turner ML. Quality assurance by a commercial flow cytometry method of leucodepletion of whole blood donations: initial application of universal testing and proposals for a batch-release sampling plan. *Transfus Med* 2000;10:37-48.
23. International Committee for Standardization in Haematology. Recommended method for radioisotope red-cell survival studies. *Br J Haematol* 1980;45:659-66.
24. Moroff GS, Sohmer PR, Button LN. Proposed standardization of methods for determining the 24-hour survival of stored red cells. *Transfusion* 1984;24:109-14.
25. Marsh WL, Jenkins WJ. Anti-i: a new cold antibody. *Nature* 1960;188:753. 