■ CLINICAL INVESTIGATION

Use of Perflubron Emulsion to Decrease Allogeneic Blood Transfusion in High-blood-loss Non-Cardiac Surgery

Results of a European Phase 3 Study

Donat R. Spahn, M.D.,* Klaus F. Waschke, M.D.,† Thomas Standl, M.D.,‡ Johann Motsch, M.D.,§
Léone Van Huynegem, M.D.,‖ Martin Welte, M.D.,# Hans Gombotz, M.D.,** Pierre Coriat, M.D.,††
Lev Verkh, Ph.D.,‡‡ Simon Faithfull, M.D., Ph.D.,§§ Peter Keipert, Ph.D.,‖‖‖
the European Perflubron Emulsion in Non-Cardiac Surgery Study Group##

Background: This single-blind randomized study in general surgery evaluated the efficacy of perflubron emulsion (PFC) as an artificial oxygen carrier being used to augment preoperative acute normovolemic hemodilution to reduce and avoid transfusion of both allogeneic erythrocytes and erythrocytes from preoperative autologous donation compared with standard of care.

Methods: Subjects (N = 492) with hemoglobin concentrations of 12–15 g/dl undergoing noncardiac surgical procedures with 20 ml/kg or greater expected blood loss were randomized into two groups. Control patients were transfused intraoperatively at a hemoglobin concentration less than 8.0 ± 0.5 g/dl or at protocol-defined, physiologic triggers. PFC-treated patients first underwent acute normovolemic hemodilution to hemoglobin of 8.0 ± 0.5 g/dl, followed by dosing with perflubron emulsion (1.8 g/kg). When hemoglobin reached less than 6.5 ± 0.5 g/dl, an additional 0.9-g/kg dose was given. PFC patients were transfused at hemoglobin less than 5.5 ± 0.5 g/dl or at predefined physiologic triggers. After surgery, hemoglobin was maintained at 8.5 ± 0.5 g/dl or greater in all patients until discharge. Efficacy endpoints included the number of allogeneic and preoperative autologous donation units transfused and the percentage of subjects avoiding transfusion.

Results: Both groups had similar hemoglobin concentrations at screening (13.5 ± 1.0 g/dl) and at discharge: 10.8 ± 1.2 g/dl (PFC) and 11.1 ± 1.3 g/dl (control). At 24 h, more patients in the PFC group avoided allogeneic and preoperative autologous donation erythrocyte transfusions (53% *vs.* 43%, $P < 0.05$), and fewer erythrocytes were transfused (1.5 ± 4.8 *vs.* 2.1 ± 3.9 units; median, 0 *vs.* 1 unit; $P = 0.013$). By day of discharge, these differences were not significant in the intent-to-treat population, but overall there were less allogeneic and preoperative autologous donation erythrocyte transfusions in the PFC group (696 *vs.* 846 units). In the protocol-defined target population (n = 330 subjects with blood loss $\geq$ 20 ml/kg), significantly greater avoidance of any erythrocyte transfusion was maintained through day of hospital discharge (26% *vs.* 16% in the PFC and control groups, respectively; $P < 0.05$), and there was also a significant reduction in the number of erythrocyte units transfused (3.4 ± 2.9 *vs.* 4.9 ± 2.4 units; median 2 *vs.* 4 units; $P < 0.001$). Adverse events rates were similar in the PFC (86%) and control (81%) groups; however, more serious adverse events were reported in the PFC group (32%) than in controls (21%; $P < 0.05$). Overall mortality was 3%, and the difference between groups (PFC, 4% *vs.* controls, 2%) was not statistically significant.

Conclusions: Augmented acute normovolemic hemodilution with PFC reduces transfusion needs in patients undergoing noncardiac surgical procedures with blood loss 20 ml/kg or greater.

This article is accompanied by an Editorial View. Please see:
Tremper KK: Perfluorochemical "red blood cell substitutes": The continued search for an indication. ANESTHESIOLOGY 2002; 97:1333–4

* Former: Professor, Institute of Anesthesiology, UniversitätsSpital Zürich, Zürich, Switzerland, Current: Professor and Chairman, Department of Anesthesiology, University Hospital Lausanne (CHUV), CH-1011 Lausanne, Switzerland. † Former: Associate Professor, Department of Anesthesiology, Ruprecht-Karls-Universität Heidelberg, Mannheim, Germany, Current: Professor, Department of Anesthesiology, Universitatsklinikum Mannheim, Mannheim, Germany. ‡ Professor, Department of Anesthesiology, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany. § Former: Professor, Department of Anesthesiology, Ruprecht-Karls-Universität Heidelberg, Heidelberg, Germany, Current: Professor of Anesthesiology, Vice Chairman, Department of Anesthesiology, University Hospital Heidelberg, Heidelberg Germany. ‖ Professor, Department of Anesthesiology, Hôpital de Tivoli, La Louvière, Belgium. # Former: Professor, Department of Anesthesiology & Operative Intensive Care Medicine, Universitätsklinikum Benjamin Franklin, Berlin, Germany, Current: Director, Institut für Anaesthesiologie und operative Intensivmedizin, Klinikum Darmstadt, Darmstadt, Germany. ** Former: Professor, Department of Anesthesiology, Landeskrankenhaus Graz, Graz, Austria, Current: Professor and Chairman, Department of Anesthesiology, Allgemeines öffentliches Krankenhaus der Stadt Linz, Linz, Austria. †† Professor and Chairman, Department of Anesthesiology, Hôpital Pitié-Salpêtrière, Paris, France. ‡‡ Former: Director, Clinical Research, Oxygen Carriers Development, Alliance Pharmaceutical Corp., Current: Verkh's Consulting Services, Clinical Trials and Project Management, Escondido, California. §§ Vice President, Medical Affairs, ‖‖‖ Program Director, Oxygen Carriers Development, Alliance Pharmaceutical Corp., San Diego, California. ## Members of the European Perflubron Emulsion in Non-Cardiac Surgery Study Group are listed in Appendix 1.

Address reprint requests to Dr. Spahn: Service d' Anesthésiologie, Centre Hospitalier Universitaire Vaudois CHUV, CH-1011 Lausanne, Switzerland. Address electronic mail to: donat.spahn@chuv.hospvd.ch. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

ALLOGENEIC erythrocyte transfusions represent a limited resource[1,2] and are associated with adverse events[3,4] such as acute transfusion reactions,[5] transmission of infectious diseases,[3] immunosuppression,[6,7] and postoperative infections.[8] They are also associated with significant cost[9] and may have limited or delayed oxygen transport efficacy because of the storage lesion effect.[10] Alternatives to erythrocyte transfusion are therefore desirable,[1] driven in part by increasing public concern[11] about safety and availability of donor erythrocytes. Although "artificial blood" is not yet a clinical reality, several temporary "artificial oxygen carriers" are in late-stage clinical development.[1,2]

Artificial oxygen carriers may be grouped into two categories: hemoglobin-based solutions and perfluoro-

1338

chemical-based emulsions.[1] This study focuses on the efficacy and safety of perflubron emulsion (PFC) administered following preoperative acute normovolemic hemodilution (ANH). PFC has previously been shown to improve tissue oxygenation in canine models[12,13] and to reverse physiologic transfusion triggers in surgical patients.[14] Administering an artificial oxygen carrier following ANH is expected to augment oxygen delivery and thereby safely allow lower intraoperative hematocrit concentrations during the period of major blood loss. As a result, autologous blood harvested during ANH may be retransfused toward the end of the surgical procedure, when surgical control of bleeding has been achieved. The endpoint for this phase 3 study was to demonstrate that PFC administered following ANH can decrease allogeneic erythrocyte transfusions in major noncardiac surgical procedures associated with significant blood loss.

Materials and Methods

Study Population

The study was performed at 34 centers in 8 European countries from November 1998, to June 2000. All local ethics committees approved the protocol. After we obtained written informed consent, 492 patients undergoing major noncardiac surgical procedures with expected blood loss between 20 and 70 ml/kg were enrolled. Additional inclusion criteria included preoperative hemoglobin concentration between 12 and 15 g/dl, age between 18 and 80 yr, weight between 50 and 125 kg, American Society of Anesthesiologists physical status I–III, and estimated blood volume sufficient to allow removal of at least 2 units of autologous blood during preoperative ANH.

Exclusion criteria were pregnancy or lactation; refusal of allogeneic erythrocyte transfusion; history of myocardial infarction within 6 months; unstable angina or coronary artery disease, placing the subject at risk of myocardial ischemia at the hemoglobin concentration defined by the protocol (8.0 $\pm$ 0.5 g/dl); severe chronic obstructive pulmonary disease or other pulmonary condition placing the subject at risk due to low hemoglobin concentration and inability to substantially increase their arterial oxygen partial pressure with a fraction of inspired oxygen (F_{IO_2}) of 1.0; carotid artery disease or history of transient ischemic attacks or amaurosis fugax; systemic infection, clinical signs of sepsis, leukocytosis, or fever greater than 38.5°C; trauma within 72 h of surgery; history of bleeding disorder; preoperative platelet count less than 150,000/μl; significant hepatic disease, defined as aspartate aminotranferase or alanine aminotranferase greater than twice the upper limit of normal; significant renal disease, defined as a creatinine concentration greater than 180 μM/l; use of cell salvage; pharmacologically induced hypotension; participation in other studies involving an investigational drug or device

within 30 days or within 12 months when an artificial oxygen carrier was involved; and history of hypersensitivity to egg yolk or any constituent of PFC.

Study Protocol

This was a prospective, multicenter, single-blind, randomized, controlled, parallel-group, phase 3 study. PFC (Oxygent™; Alliance Pharmaceutical Corp., San Diego, CA) is a 60% wt/vol perfluorochemical emulsion based on perflubron emulsified with 3.6% wt/vol egg lecithin in phosphate-buffered saline, and was provided by the manufacturer, who also sponsored the study. After providing written informed consent, each patient was randomized within 48 h before surgery to either standard of care (control) or PFC treatment in conjunction with ANH. All patients had radial artery and central venous catheters in addition to standard anesthesia monitoring. Neither anesthetic drugs nor infusions (crystalloids and colloids) were specified by protocol. Hemoglobin concentration (HemoCue; AB Leo Diagnostics, Helsinborg, Sweden) was measured after each unit of blood removed during ANH (PFC group) and intraoperatively at least every 30 min.

Control Group (Standard of Care)

Patients were maintained at an F_{IO_2} of 0.4 and transfused with erythrocytes from preoperative autologous donation (PAD), if available, or allogeneic erythrocytes for each intraoperative transfusion trigger, i.e., either a hemoglobin concentration less than 8.0 $\pm$ 0.5 g/dl or any one of the protocol-defined intraoperative physiologic triggers not easily reversed by fluid adminiatration or adjustment of anesthesia. These included tachycardia (heart rate $\geq$ 100 beats/min or $\geq$ 135% of postanesthesia induction value), hypotension (mean arterial pressure $\leq$ 60 mmHg or $\leq$ 65% of postinduction value), mixed venous oxygen partial pressure 38 mmHg or less (if a pulmonary artery catheter was used), or ST-segment depression ($>$ 0.1 mV) or elevation ($>$ 0.2 mV).

Perflubron Emulsion Treatment Group

Prior to surgical incision, patients underwent ANH to a hemoglobin concentration of 8.0 $\pm$ 0.5 g/dl at an F_{IO_2} of 1.0. Investigators were free in their choice of standard crystalloids and colloids to replace the blood withdrawn during ANH according to recommended guidelines for volume replacement. Clinical signs of normovolemia (stable heart rate and blood pressure) further guided amounts of replacement fluids administered during ANH; central venous pressure was not monitored systematically during ANH but was available later to help assess volume status. At skin incision, a 1.8-g/kg dose (3 ml/kg) of PFC was given. This represents approximately 0.94 ml/kg of the PFC active ingredient, i.e., nonemulsified perfluorochemical. When hemoglobin reached 6.5 $\pm$ 0.5 g/dl during surgery, a second 0.9-g/kg

(1.5 ml/kg) dose of PFC was administered (~0.47 ml/kg of neat perfluorochemical). When hemoglobin concentration was less than 5.5 ± 0.5 g/dl or when the physiologic triggers (described in the previous paragraph for the control group) were met, patients were transfused with ANH or PAD units, if available, before receiving allogeneic erythrocytes. All units of autologous blood collected during ANH were retransfused after surgery.

In both groups, a hemoglobin concentration of 8.5 ± 0.5 g/dl or greater was targeted at the end of surgery, maintained through postoperative day 3, and was required by protocol as the minimum concentration at hospital discharge. The ranges in hemoglobin transfusion triggers (± 0.5 g/dl) were not applicable for individual patients but were provided to allow centers to adjust transfusion decisions (equally in both groups) according to local standards. Investigators were required to measure hemoglobin concentrations postoperatively to ensure that protocol-mandated transfusion requirements were followed, but this person was not blinded to the group assignment of the patient. No specific criteria for the perioperative administration of fresh frozen plasma, cryoprecipitate, or platelet transfusions were defined in the protocol.

An intraoperative hemoglobin trigger difference of 2.5 g/dl between control and PFC-treated groups was based on pharmacodynamic and pharmacokinetic data from previous phase 2 studies. These data included physiologic efficacy of perflubron emulsion in orthopedic and urologic patients receiving a 1.8-g/kg dose of PFC (details provided in Appendix 2). Physiologic modeling of a 2.7-g/kg dose predicted that a hemoglobin equivalency of 2.5 g/dl or greater after blood loss would decrease hemoglobin to 5.5 g/dl. As a result, PFC-treated patients with a hemoglobin concentration of 5.5 g/dl have similar oxygen dynamics as control patients with a hemoglobin concentration of 8.0 g/dl.

Blood samples were collected in both groups for laboratory analyses at several time points: after anesthesia induction but prior to ANH, at arrival in the recovery room or intensive care unit, and on postoperative day (POD) 1, 2, 3, 7, and 21 or day of discharge (DD), whichever came first. Patients were also contacted 3 months after surgery to obtain additional follow-up information regarding postoperative adverse events (AEs). AEs and serious AEs were reported according to standard regulatory guidelines for current Good Clinical Practice. No predefined definitions of clinically relevant AEs were provided to investigators. An independent data safety monitoring board was used to periodically review laboratory and safety data during the conduct of this study.

Statistical Analysis

The sample size for this study was based on the primary efficacy endpoint, with a type 1 error (α) = 0.049 (adjusted from 0.05 due to the interim analysis), and

power (β) = 90%. The minimum clinically meaningful difference in mean reduction in the number of erythrocyte units transfused is $\delta = 1.0$. The estimate of the pooled SD for a comparison of the mean difference in the number of erythrocyte units transfused is $S_p = 3.0$; this estimate was obtained from phase 2 clinical studies. A two-sample t test for sample size estimation yielded n $= 2 (Z_{\alpha/2} + Z\beta)^2 S_p^2/\delta^2 = 2 (1.97 + 1.28)^2 \cdot 9/1.0$, about 190 per group. Assuming a 5% dropout rate increased this to 200 per group, and allowing for 20% of subjects to have a blood loss less than 20 ml/kg increased the required sample size to about 240 per group.

The primary efficacy endpoint for this protocol was the number of allogeneic or PAD units transfused during the acute study period (24 h following skin incision). Secondary endpoints included the percentage of subjects avoiding allogeneic erythrocyte transfusions during the acute study period, the percentage of subjects avoiding allogeneic erythrocyte and PAD unit transfusions during the acute study period, and the elapsed time from immediate arrival in the recovery room to DD or POD 21. In addition, transfusion outcome (number of allogeneic or PAD units transfused and percentage of patients avoiding any allogeneic or PAD transfusion) on PODs 1, 3, and 7 and on DD (or POD 21, whichever came first) was prospectively recorded and analyzed. The primary efficacy population was defined as the intent-to-treat population, which included all randomized subjects. The secondary efficacy population prospectively defined in the protocol was all randomized subjects with estimated blood loss of 20 ml/kg or greater. The safety population included all treated subjects who underwent surgery and, if randomized to receive PFC, who also received at least the first dose of PFC.

Treatment groups were compared for the number of erythrocyte or PAD units transfused using an analysis of covariance, with treatment group, site, and screening hemoglobin in the model. A rank transformation of the number of units transfused was used to be able to account for outliers. To estimate the mean and mean difference effects, a log transformation was used. A logistic regression was used to compare treatment groups for the number of subjects avoiding erythrocyte and PAD transfusions. Treatment group, site, and type of surgery were in the model. For demographic and safety data, treatment groups were compared using the Fisher exact test for categoric data and t tests for continuous data. All data are presented as means $\pm$ SD unless otherwise indicated.

Results

Patient demographics were similar in both groups at screening and baseline (table 1), as were types of surgeries (table 2). A total of 24 patients (14 PFC and 10

Table 1. Patient Characteristics at Baseline for all Randomized (Intent-to-treat) Patients

Patient Characteristics	PFC Group (N = 241)	Controls (N = 251)	P Value
Age (yrs)	59 ± 11	59 ± 13	0.461
> 65-yr-old (%)	38.6	39.0	0.918
Female (%)	34	29	0.279
ASA Class 1/2/3 (%)*	15/72/13	22/63/15	0.063
PAD (% of subjects)†	3.3	6.0	0.163
Height (cm)	171 ± 9	171 ± 9	0.434
Weight (kg)	76 ± 13	75 ± 12	0.464
BSA (m²)‡	1.9 ± 0.2	1.9 ± 0.2	0.787
Estimated blood volume (l)	5.1 ± 0.9	5.3 ± 1.0	0.014
Screening Hb (g/dl)	13.5 ± 1.0	13.5 ± 0.9	0.689
Platelets (10^9/l)	258 ± 77	266 ± 80	0.224
PT (%)§	98 ± 12	99 ± 12	0.550
APTT (s)‖	30 ± 6	31 ± 5	0.192
Fibrinogen (g/l)	3.6 ± 1.3	3.7 ± 1.6	0.348

Data are mean ± SD or percentages.

*American Society of Anesthesiologists; †Preoperative autologous (blood) donation; ‡Body surface area; §Prothrombin time; ‖Activated partial thromboplastin time

controls) were withdrawn before treatment or surgery, either at the subject's request or because clinical condition did not warrant surgery at the time (these subjects are all included in the efficacy analysis for the intent-to-treat group but are excluded from the safety analysis). During preoperative ANH, 1,618 ± 558 ml (range, 450–3,374 ml) of blood was withdrawn and replaced by 1,312 ± 680 ml (range, 100–3,500 ml) colloid and 2,418 ± 1627 ml (range, 100–10,000 ml) crystalloid infusions. The mean post-ANH hemoglobin concentration achieved was 8.1 ± 0.5 g/dl. All 241 PFC-treated patients received the 1.8-g/kg dose of PFC, but only 177 subjects lost enough blood to require the 0.9-g/kg dose. Hemoglobin concentrations, per protocol, were lower intraoperatively in the PFC group ($P < 0.001$) but returned to similar values postoperatively (fig. 1).

Efficacy: Transfusion Requirements

In the intent-to-treat population (n = 492), the PFC group (n = 241) required fewer transfusions than con-

Table 2. Surgery Information

	PFC Group (N = 227)*	Controls (N = 241)*	P Value
Operations	—	—	—
Malignancies (%)	67	69	0.660
Abdominal (%)	82	81	0.690
Orthopedic (%)	18	20	0.690
Infection potential	62	62	0.900
Duration of surgery (min)	243 ± 119	235 ± 119	0.456
Hospitalization	—	—	—
Duration (days)	14.7 ± 5.3	14.4 ± 5.5	0.538

Data are mean ± SD or percentages.

*Treated patients (randomized patients who underwent surgery and if randomized to the perflubron group also received at least the first dose of perflubron).
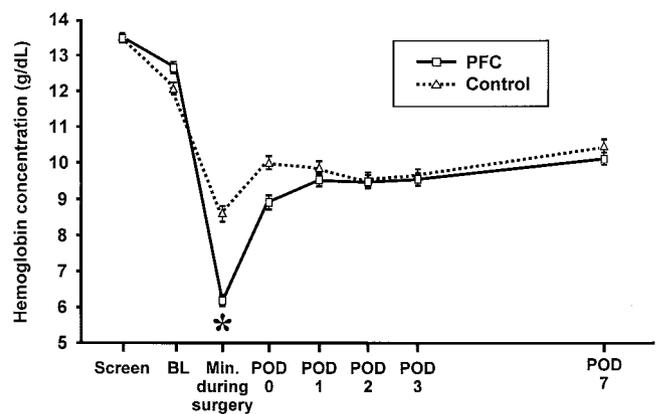
Fig. 1. Perioperative hemoglobin concentrations in the control group (open triangle) and in PFC-treated group (open square) at preoperative screening (Screen), at baseline just before anesthesia induction (BL), at intraoperative nadir (Min. during surgery), after the operation (POD 0), and on postoperative days (POD) 1, 2, 3, and 7. Data are mean ± SD. *$P < 0.01$ between groups.

trols (n = 251) despite a higher estimated intraoperative blood loss (2.7 ± 2.7 vs. 2.3 ± 2.0 l, respectively; $P < 0.05$). The primary endpoint (reduction in number of allogeneic–PAD units transfused at 24 h) was achieved: the PFC group received 1.5 ± 4.8 versus 2.1 ± 3.9 units (median 0 vs. 1 unit) in controls ($P = 0.013$), representing a reduction of 26% (table 3). After POD 3, although differences were still present, they were no longer statistically significant. However, in the protocol-defined target population (blood loss ≥ 20 ml/kg; n = 330, or 67% of all randomized subjects), the PFC group had a larger reduction in allogeneic and PAD units transfused (mean, 2.0 ± 4.0 vs. 3.3 ± 3.0; median, 1 vs. 3 units; $P < 0.001$) on POD 1 (table 3), and this difference remained significantly different from controls through discharge (mean, 3.4 ± 2.9 vs. 4.9 ± 2.4; median, 2 vs. 4 units at POD 21 or DD; $P < 0.001$). In total, for the intent-to-treat population through POD 21 or DD, PFC-treated subjects required 696 units versus 846 units in the control group.

Regarding complete avoidance in the intent-to-treat population, approximately 21% more patients in the PFC group avoided allogeneic and PAD transfusions compared with controls ($P < 0.05$) during the acute study period (24 h). Later, the difference was not significant any more (fig. 2). However, in the protocol-defined target population, a significantly ($P < 0.05$) greater percentage (almost twice as many) of patients avoided transfusion at all time points from POD 1 through POD 21 or DD (fig. 2). A post hoc analysis to identify patients who benefited from PFC treatment indicated that when estimated surgical blood loss was 10 ml/kg or greater (n = 424; 86% of all randomized subjects), transfusion was significantly reduced in PFC-treated patients versus controls at all time points through POD 21 or DD (fig. 2).

Table 3. Number of Allogeneic Erthrocytes and/or PAD Units Transfused

Study Day	PFC Group Mean ± SD*	Median	Controls Mean ± SD*	Median	Mean % Reduction	P Value
Intent-to-treat (N = 492)					—	—
1	1.5 ± 4.8	0	2.1 ± 3.9	1	26.1%	0.013
3	2.1 ± 3.8	1	2.6 ± 3.4	2	19.5%	0.052
7	2.5 ± 3.4	1	3.0 ± 3.1	2	15.9%	0.128
21 or DD†	2.7 ± 3.3	1	3.2 ± 3.0	2	15.5%	0.162
Target population (N = 330)	—	—	—	—	—	—
1	2.0 ± 4.0	1	3.3 ± 3.0	3	40.8%	<0.001
3	2.7 ± 3.3	2	4.1 ± 2.7	3	33.2%	<0.001
7	3.2 ± 3.0	2	4.6 ± 2.5	4	30.3%	<0.001
21 or DD†	3.4 ± 2.9	2	4.9 ± 2.4	4	30.3%	<0.001

Information on the percentage of avoidance is shown in fig. 2.

*Mean adjusted for covariates (analysis of covariance) using a natural log transformation; †Whichever occurred sooner; DD is day of discharge.

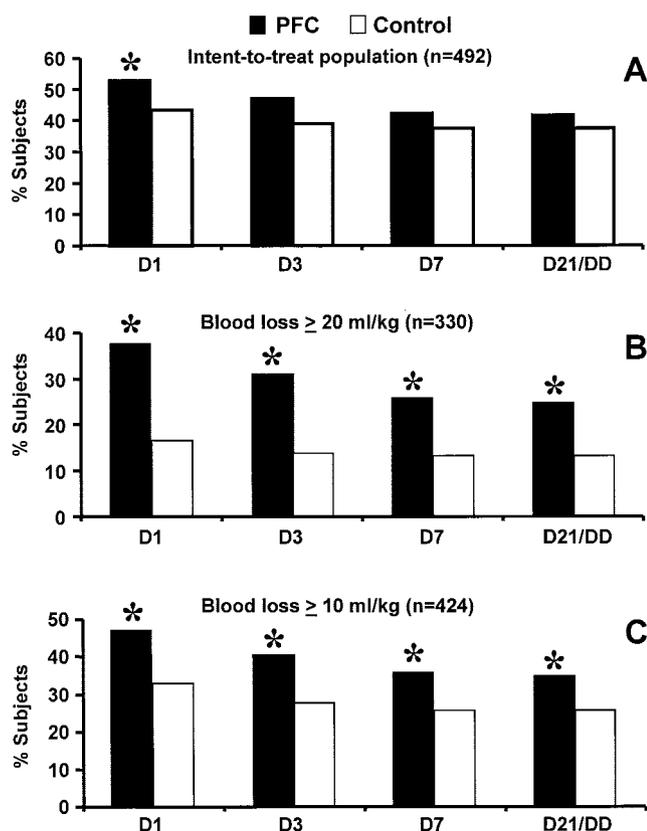PAD = preoperative autologous donation.

In addition to 11 subjects (3 in the PFC group, 8 controls) who received PAD units, some patients in each group received other blood components intraoperatively: fresh frozen plasma (12.8% in the PFC group and 12.5% in the control group), cryoprecipitate (1 patient in the PFC group), and platelets (7 in PFC group, 7 in the control group; 300 ml for each subject in each of the groups). The number of patients avoiding all types of transfusions of blood and blood components was significantly higher in the PFC group at 24 h, but the difference was not significant at DD (table 4). In contrast, in the protocol-defined target population (blood loss ≥ 20 ml/kg), the percentage of patients avoiding transfusion of any blood and blood components remained significantly greater until DD (table 4).

Platelet counts were significantly lower, by approximately 15–25% on PODs 1–3, in the PFC *versus* control groups ($P < 0.01$; fig. 3). By POD 7, platelet counts were still lower in the PFC group, but both groups had recovered to normal screening levels.

Safety: Adverse Events

As expected, a high number of AEs were reported in this study in patients undergoing major surgery with substantial (≥ 20 ml/kg) blood loss. The incidence of AEs (table 5) was similar in the PFC group (86%) compared with controls (81%). More serious AEs were reported in the PFC group than in controls (32% *vs.* 21%; $P < 0.05$; table 5). However, only the category "Digestive System" was significantly different from controls, mostly because of a higher reported occurrence of postoperative ileus. Four serious AE cases of ileus were

Fig. 2. Percent of subjects avoiding any allogeneic and preoperative autologous donation transfusions in the (*A*) intent-to-treat population (n = 492), (*B*) protocol-defined target population with blood loss 20 ml/kg or greater (n = 330), and (*C*) clinical benefit group (*post hoc* analysis), *i.e.*, patients with surgical blood loss 10 ml/kg or greater (n = 424). *$P < 0.05$ between groups.

Table 4. Number of Patients Avoiding Transfusion of all Types of Blood and Blood Components

Study Day	PFC Group	Controls	P Value
Intent-to-treat (N = 492)	—	—	—
1	126/241 (52%)	109/251 (43%)	0.048
3	112/241 (47%)	98/251 (39%)	0.087
7	101/241 (42%)	94/251 (38%)	0.288
21 or DD*	99/241 (41%)	94/251 (38%)	0.382
Target population (N = 330)	—	—	—
1	66/178 (37%)	25/152 (16%)	<0.001
3	54/178 (30%)	21/152 (14%)	<0.001
7	45/178 (25%)	20/152 (13%)	<0.001
21 or DD*	43/178 (24%)	20/152 (13%)	0.011

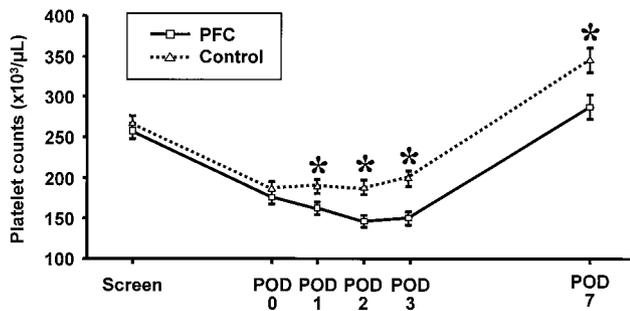*Whichever occurred sooner; DD is day of discharge.

Fig. 3. Perioperative platelet count in the control group (open triangle) and in PFC-treated patients (open square) at preoperative screening (Screen), after the operation (POD 0), and on postoperative days (POD) 1, 2, 3, and 7. Data are means ± SD. *P < 0.01 between groups.

reported in the PFC group (*vs.* none in controls), one after a rectum amputation, two after major gynecologic tumor excision, and one after radical cystectomy.

The independent data safety monitoring board noted some group imbalances in certain AEs but concluded that there was no clinically consistent pattern or significance. They also concluded that since the investigators were not blinded to treatment allocation, there was a possibility that the Hawthorne effect[15,16] may have influenced reporting. Overall mortality was 3% (PFC, 4% *vs.* controls, 2%); the difference between groups was not

Table 5. Adverse and Serious Adverse Events (Listed by COSTART Body System)

	PFC (N = 227)*	Control (N = 241)*	P Value§
Any adverse event†, N, (%)	195 (86%)	195 (81%)	0.172
Body as a whole	114 (50%)	110 (46%)	0.355
Cardiovascular system	90 (40%)	73 (30%)	0.041
Digestive system	101 (45%)	84 (35%)	0.038
Hemic and lymphatic system	72 (32%)	68 (28%)	0.421
Metabolic and nutritional disorders	101 (45%)	91 (38%)	0.159
Nervous system	51 (23%)	37 (15%)	0.058
Respiratory system	37 (16%)	39 (16%)	1.000
Skin and appendages	19 (8%)	15 (6%)	0.380
Urogenital system	51 (23%)	62 (26%)	0.450
Any serious adverse event‡, N, (%)	72 (32%)	51 (21%)	0.012
Body as a whole	32 (14%)	25 (10%)	0.258
Cardiovascular system	16 (7%)	12 (5%)	0.436
Digestive system	16 (7%)	5 (2%)	0.013
Hemic and lymphatic system	3 (1.3%)	3 (1.2%)	1.000
Metabolic and nutritional disorders	7 (3%)	5 (2%)	0.567
Musculoskeletal system	4 (2%)	3 (1%)	0.717
Nervous system	4 (2%)	2 (1%)	0.438
Respiratory system	9 (4%)	7 (3%)	0.163
Urogenital system	9 (4%)	5 (2%)	0.283
Mortality, N, (%)	10 (4%)	5 (2%)	0.192

*Treated patients (randomized patients who underwent surgery and if randomized to the perflubron group also received at least the first dose of perflubron); †Adverse events listed if present in >5% of patients in either the PFC or the control group (statistical analysis was based on all reported treatment-emergent adverse events); ‡Serious adverse events listed if present in >1% of patients in either the PFC or the control group (statistical analysis was based on all reported treatment-emergent adverse events); §P value calculated from Fisher exact test.

COSTART = Coding symbols for a thesaurus of adverse reaction terms.

statistically significant. Tumor progression, sepsis, multiorgan failure, and cardiopulmonary complications were responsible for the deaths, and all were considered by the investigators to be a result of underlying disease or condition and to be unrelated to the study drug.

Discussion

This pivotal phase 3 study has demonstrated that transfusion requirements during high-blood-loss noncardiac surgery can be decreased by using PFC as an intravenous oxygen carrier to enable more extensive preoperative harvesting of autologous blood during hemodilution.

In the intent-to-treat population, PFC-treated patients required fewer erythrocyte units compared with controls in the initial 24 h, but erythrocyte transfusion rates were similar at hospital discharge (table 3). In contrast, overall reduction and avoidance of erythrocyte transfusions were significantly greater in PFC-treated patients in the protocol-defined subgroup of patients experiencing blood loss of 20 ml/kg or greater, and this benefit persisted through hospital discharge. This outcome agrees with mathematical modeling predictions that blood-sparing efficacy of (augmented) ANH can only be achieved when there is high surgical blood loss.[17–19] The efficacy of ANH augmented by PFC is derived from the hemodilution itself, based on the fact that more dilute blood is being lost during surgical blood loss, which minimizes loss of erythrocytes. Concomitant intraoperative treatment with PFC and high F_{IO_2} provides improved oxygenation, which in turn permits lower hemoglobin concentrations to be safely tolerated.

Since no significant difference in erythrocyte transfusions was noted after POD 1 in the intent-to-treat population (which included 162 subjects who had < 20 ml/kg blood loss), additional analyses were performed in a *post hoc* analysis to identify the blood loss level where using augmented ANH with PFC resulted in a significant reduction in transfusion. These analyses demonstated that significant savings were still observed at an estimated blood loss of 10 ml/kg or greater (fig. 2C), which represents 86% (n = 424) of all subjects in the study. For a large number of major noncardiac operations, ANH augmented by using PFC as a temporary intravascular oxygen carrier may therefore represent a new autologous method to decrease allogeneic erythrocyte transfusions.

Regarding safety, a high incidence of AEs was observed in this study (table 5), as expected during major noncardiac surgery,[20–22] since two thirds of the patients were undergoing extensive surgical procedures for malignancies (table 2). The higher rate of serious AEs reported in the category "Digestive System" was mostly due to postoperative ileus. A 2% incidence of ileus is not unusual for these operations, but, surprisingly, no ileus was reported in the control group. Reports of sepsis and infection are

also of interest, since emulsion particles are initially cleared from the circulation by the phagocytic cells (macrophages in the spleen and Kupffer cells in the liver) of the reticuloendothelial system,[23] which could theoretically increase susceptibility to postoperative infections. However, aggregates of all infectious complications were similar in both groups (32%), supporting earlier clinical findings that immune function is not compromised by PFC.[24] Overall mortality at 3 months was 3%, which is consistent with a recent report in a similar surgical population.[20] Tumor progression, sepsis, multiorgan failure, and cardiopulmonary complications were responsible for these deaths, and no deaths were considered to be related to drug treatment.

Overall, the incidence of AEs was equal in both groups (table 5), although more were reported in the PFC group for the categories "Cardiovascular System" (hypertension) and "Digestive System" (ileus). Hypertension occurred mainly postoperatively and was likely related to mandatory retransfusion of all remaining ANH blood. The clinical consequences in the ileus cases were minor, and no general pattern or pathophysiological mechanisms were found in *post hoc* analyses that could link these events to treatment.

The transient decrease in platelet counts due to splenic sequestration and clearance occurring a few days after surgery was expected based on previous phase 2 clinical data.[14] Since the number of platelet transfusions and the incidence of bleeding events were similar in both groups, it would appear that this moderate decrease in postoperative platelet count is of little clinical relevance. In addition, the difference in the number of erythrocyte units transfused between the groups persisted (table 3), and the hemoglobin was similar throughout the postoperative period (fig. 1). This indicates that the lower platelet count in the PFC group was not associated with any increased transfusion requirements. This is consistent with earlier studies in human volunteers demonstrating that PFC does not compromise platelet function or increase bleeding time.[25]

The question remains why augmented ANH with PFC successfully reduced erythrocyte transfusions but was associated with a somewhat higher incidence of AEs. It is possible that several centers did not have sufficient previous experience with the degree of ANH and the lower intraoperative hemoglobin concentrations mandated by the protocol. A potential reporting bias (since the study was not double-blinded) to ascribe untoward postoperative events to a new procedure (augmented ANH) using an experimental drug (PFC), as well as a lack of experience with either, may explain some of the increased AE reporting in the PFC-treated group. While the principles of ANH appear simple, there are fluid management and blood volume issues that deserve special attention: hypovolemia must be prevented during blood harvesting and throughout the operation, and hy-

pervolemia must be avoided during retransfusion of ANH units. Performing ANH involves considerable fluid and electrolyte shifts that have to be controlled, and this is where previous experience is helpful to ensure euvolemia. Centers without previous experience may have therefore performed suboptimal ANH and had more problems maintaining euvolumia throughout the procedure, and thus may have encountered more AEs. The data safety monitoring board noted a trend (statistically nonsignificant) for the PFC group to receive smaller volumes of both colloids and crystalloids, although one might have expected the PFC group to receive more fluids intraoperatively because of the 500-ml greater estimated blood loss. This may be an important contributor to some of the AEs reported, because hypovolemia is known to be associated with adverse postoperative outcomes from major surgery.[26] There was also a trend ($P = 0.063$) for the PFC group to have less patients with American Society of Anesthesiologists physical status I and more with status II compared with the control group (table 1), which could potentially be associated with greater postoperative morbidity.

For the conduct of this study, the preoperative ANH procedure delayed surgery by approximately 45–60 min, since it was mandated by protocol to complete the ANH prior to skin incision. In future clinical practice, however, it would only be important to perform ANH prior to major blood loss; thus, it could easily be completed during the first hour of major tumor operations, since the major bleeding phase in most of these operations is not immediately after incision. The future clinical use of artificial oxygen carriers following ANH is therefore not necessarily associated with extra time requirements and would not have to delay the start of surgery.

Postoperative hemoglobin values were slightly higher (~9.5 g/dl) than the protocol specified hemoglobin-based transfusion trigger (8.5 ± 0.5 g/dl; fig. 1). At each time point only a certain percentage of patients had a hemoglobin concentration less than 8.5 ± 0.5 g/dl and thus would be transfused, while the other patients had hemoglobin values greater than 8.5 ± 0.5 g/dl. Since hemoglobin was measured frequently in the early postoperative period, transfused patients were likely to have a hemoglobin value greater than or close to 8.5 ± 0.5 g/dl in subsequent measurements. Group mean hemoglobin values would therefore be expected to be higher than the protocol-specified postoperative hemoglobin transfusion trigger. More importantly, the postoperative hemoglobin concentrations were similar in both groups. Hence, the different transfusion needs between groups is not related to different transfusion strategies postoperatively, but rather to the lesser intraoperative transfusion needs in the PFC group.

The single-blinded nature of this study deserves comment. Intraoperative blinding was essentially impossible, given the fact that the patients had to be treated differ-

ently in the PFC and control groups (preoperative ANH, different intraoperative hemoglobin transfusion triggers). It is therefore difficult to determine whether the estimation of blood loss was inaccurate or potentially biased, or whether blood loss was actually somewhat greater in the PFC group undergoing preoperative ANH, which is associated with vasodilation, decreased blood viscosity, and eventually a reduced margination of platelets at low hematocrit.[27,28] Also, AE reporting might have been biased or influenced by the Hawthorne effect.[15,16] Finally, the investigators responsible for monitoring postoperative hemoglobin concentrations, to ensure that protocol-mandated transfusion requirements were strictly followed, were not blinded. This might introduce bias as well. However, this study used prospectively defined postoperative hemoglobin transfusion triggers, and the protocol was generally well followed, which resulted in nearly identical postoperative hemoglobin concentrations in both groups (fig. 1). Hence, the potential for bias to influence the efficacy (*i.e.*, erythrocyte transfusion) outcome of the study appears to be minor.

In the current study, efficacy was greatest when estimated surgical blood loss was 20 ml/kg or greater, but significant transfusion avoidance was also achieved in a broader subset of patients undergoing procedures with a blood loss of at least 10 ml/kg. If blood loss is lower than that, there is no real need to transfuse erythrocytes, and, therefore, there is no chance to improve on the transfusion outcome with any intervention. For this reason, the intent-to-treat analysis was compromised in its ability to demonstrate significant avoidance through discharge, since 33% of the patients bled less than 20 ml/kg and 14% lost less than 10 ml/kg. The efficacy findings in this phase 3 study suggest that the use of PFC as an intravenous oxygen therapeutic to augment autologous blood harvesting may represent a new alternative for the growing number of patients seeking to avoid or minimize the risks of allogeneic erythrocyte transfusions in high-blood-loss elective surgery.

References

1. Klein HG: The prospects for red-cell substitutes. N Engl J Med 2000; 342:1666–8

2. Winslow RM: Blood substitutes. Adv Drug Deliv Rev 2000; 40:131–42

3. Goodnough LT, Brecher ME, Kanter MH, AuBuchon JP: Transfusion medicine: First of two parts—Blood transfusion. N Engl J Med 1999; 340:438–47

4. Spahn DR, Casutt M: Eliminating blood transfusions: New aspects and perspectives. Anesthesiology 2000; 93:242–55

5. Williamson LM, Lowe S, Love EM, Cohen H, Soldan K, McClelland DB, Skacel P, Barbara JA: Serious hazards of transfusion (SHOT) initiative: Analysis of the first two annual reports. BMJ 1999; 319:16–9

6. Klein HG: Immunomodulatory aspects of transfusion. Anesthesiology 1999; 91:861–5

7. Blumberg N, Heal JM: Blood transfusion immunomodulation: The silent epidemic. Arch Pathol Lab Med 1998; 122:117–9

8. Innerhofer P, Walleczek C, Luz G, Hobisch Hagen P, Benzer A, Stockl B, Hessenberger G, Nussbaumer W, Schobersberger W: Transfusion of buffy coat-depleted blood components and risk of postoperative infection in orthopedic patients. Transfusion 1999; 39:625–32

9. Vamvakas EC, Carven JH: Allogeneic blood transfusion, hospital charges, and length of hospitalization: A study of 487 consecutive patients undergoing colorectal cancer resection. Arch Pathol Lab Med 1998; 122:145–51

10. Hebert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, Tweeddale M, Schweitzer I, Yetisir E, the Transfusion Requirements in Critical Care Investigators for the Canadian Critical Care Trials Group: A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. N Engl J Med 1999; 340:409–17

11. AuBuchon JP: Public health, public trust, and public decision making: Making hepatitis C virus lookback work. Transfusion 1999; 39:123–7

12. Habler OP, Kleen MS, Hutter JW, Podtschaske AH, Tiede M, Kemming GI, Welte MV, Corso CO, Batra S, Keipert PE, Faithfull NS, Messmer KF: Hemodilution and intravenous perflubron emulsion as an alternative to blood transfusion: Effects on tissue oxygenation during profound hemodilution in anesthetized dogs. Transfusion 1998; 38:145–55

13. Holman WL, Spruell RD, Ferguson ER, Clymer JJ, Vicente WV, Murrah CP, Pacifico AD: Tissue oxygenation with graded dissolved oxygen delivery during cardiopulmonary bypass. J Thorac Cardiovasc Surg 1995; 110:774–85

14. Spahn DR, van Bremt R, Theilmeier G, Reibold JP, Welte M, Heinzerling H, Birck K, Keipert PE, Messmer K, Perflubron Emulsion Study Group: Perflubron emulsion delays blood transfusion in orthopedic surgery. Anesthesiology 1999; 91:1195–208

15. De Amici D, Klersy C, Ramajoli F, Brustia L: The awareness of being observed changes the patient's psychological well-bing in anesthesia. Anesth Analg 2000; 90:739–40

16. Parsons HM: What happened at Hawthorne? Science 1974; 183:922–32

17. Weiskopf RB: Efficacy of acute normovolemic hemodilution assessed as a function of fraction of blood volume lost. Anesthesiology 2001; 94:439–46

18. Brecher ME, Goodnough LT, Monk T: The value of oxygen-carrying solutions in the operative setting, as determined by mathematical modeling. Transfusion 1999; 39:396–402

19. Spahn DR, Willimann PF, Faithfull NS: Wirksamkeit der Augmentierten Akuten Normovolämen Hämodilution (A-ANH). Anaesthesist 2001; 50(suppl 1): S49–54

20. Bennett-Guerrero E, Welsby I, Dunn TJ, Young LR, Wahl TA, Diers TL, Phillips-Bute BG, Newman MF, Mythen MG: The use of a postoperative morbidity survey to evaluate patients with prolonged hospitalization after routine, moderate-risk, elective surgery. Anesth Analg 1999; 89:514–9

21. Bennett Guerrero E, Panah MH, Barclay R, Bodian CA, Winfree WJ, Andres LA, Reich DL, Mythen MG: Decreased endotoxin immunity is associated with greater mortality and/or prolonged hospitalization after surgery. Anesthesiology 2001; 94:992–8

22. Takala J, Meier-Hellmann A, Eddlestone J, Hulstaert P, Sramek V, for the European Multicenter Study Group on Dopexamine in Major Abdominal Surgery: Effect of dopexamine on outcome after major abdominal surgery: A prospective, randomized controlled multicenter study. Crit Care Med 2000; 28:3417–23

23. Keipert PE: Perfluorochemical emulsions: Future alternatives to transfusion. Blood Subst Princ Meth Prod Clin Trials 1998; 2:127–56

24. Noveck RJ, Shannon EJ, Leese PT, Shorr JS, Flaim KE, Keipert PE, Woods CM: Randomized safety studies of intravenous perflubron emulsion. II. Effects on immune function in healthy volunteers. Anesth Analg 2000; 91:812–22

25. Leese PT, Noveck RJ, Shorr JS, Woods CM, Flaim KE, Keipert PE: Randomized safety studies of intravenous perflubron emulsion. I. Effects on coagulation function in healthy volunteers. Anesth Analg 2000; 91:804–11

26. Venn R, Steele A, Richardson P, Poloniecki J, Grounds M, Newman P: Randomized controlled trial to investigate influence of the fluid challenge on duration of hospital stay and perioperative morbidity in patients with hip fractures. Br J Anaesth 2002; 88:65–71

27. Valeri CR, Crowley JP, Loscalzo J: The red cell transfusion trigger: Has a sin of commission now become a sin of omission? Transfusion 1998; 38:602–10

28. Eberst ME: Hemostasis on renal disease: Pathophysiology and management. Am J Med 1994; 95:168–79

Appendix 1

European Perflubron Emulsion in Non–Cardiac Surgery Study Group

Principal Investigator	Co-PI	Site
Alan Aitkenhead, M.D., B.Sc., M.B. Ch.B., F.R.C.A. Professor of Anesthesia	Victoria L. Webster, M.B.B.S., F.R.C.A. Specialist Registrar in Anesthesia	Queen's Medical Centre University Department of Anaesthesia Nottingham, NG7 2UH United Kingdom
Philippe Baele, M.D., Professor of Anesthesia	Luc Van Obbergh, M.D., Ph.D. Professor of Anesthesia	Cliniques Universitaires St. Luc Service d' Anésthesiologie 1200 Bruxelles Belgium
Björn Biber, M.D., Ph.D. Professor of Anesthesiology and Intensive Care	Roman A´Roch, M.D. Anesthesiologist	Umeå University Hospital Department of Anaesthesiology and Intensive Care 901 85 Umeå Sweden
Joachim Boldt, M.D., Ph.D. Professor of Anesthesiology and Intensive Care Medicine	Stefan Suttner, M.D. Anesthesiologist	Klinik für Anästhesiologie u. Operative Intensivmedizin Klinikum der Stadt Ludwigshafen a. Rh. GGmbH 67063 Ludwigshafen Germany
Bernard Bourréli, M.D. Anesthesiologist	None	CHRU - Hotel Dieu Dep. d'Anaesthésie - Réanimations 44035 Nantes Cedex 01 France
Xavier Capdevila, M.D., Ph.D. Professor of Anesthesia and Intensive Care	Nathalie Bernard, M.D., Ms.C. Assistant Professor of Anesthesia	Département d'Anesthésie-Réanimation A Hôpital Lapeyronie CHU Montpellier 34295 Montpellier France
Pierre Coriat, M.D. Professor of Anesthesiology	Philippe LeCosquer, M.D. Anesthesiologist	Groupe Hospitalier Salpetrière Dépt. Anesthésie & Réanimation 75651 Paris cedex 13 France
Phillipe Duvaldestin, M.D. Professor of Anesthesia	Motamed, Cyrus, M.D. Anesthesiologist	Hôpital Henri Mondor Service d'Anesthésie et Réanimation 94010 Creteil Cedex France
Magnus Garrioch, M.B. Ch.B., F.R.C.A., F.R.C.P.(Glas) Consultant of Anaesthesia and Intensive Care	None	Southern General Hospital NHS Trust and University of Glasgow Dept. of Anaesthesia and Intensive Care Glasgow, G51 4TF United Kingdom
Hans Gombotz, M.D. Professor of Anesthesiology	Michael Zink, M.D. Anesthesiologist	Department of Anesthesiology and Intensive Care University of Graz, Austria 8036 Graz Austria
Gunter Hempelmann, M.D., Dr. h.c. Professor of Anesthesia	Antje Reske, M.D. Anesthesiologist	Universitätsklinikum Anästhesiologie und Operative Intensivmedizin 35385 Giessen Germany
Petra Innerhofer, M.D. Anesthesiologist	Markus Mitteermayer, M.D. Anesthesiologist	Universitätsklinik für Anästhesie und Allgemeine Intensivmedizin 6020 Innsbruck Austria
Benoît Lambrigts, M.D. Anesthesiologist	Hugues Versailles, M.D. Anesthesiologist	Centre Hospitalier de Jolimont Dépt d'Anesthésie 7100 Haine St Paul Belgium
Maurice Lamy, MD Professor of Anesthesiology and Intensive Care Medicine	Robert Larbuisson, MD Professor of Anesthesiology and Intensive Care Medicine	CHU de Liège Service d'Anesthésie-Réanimation 4000 Liège Belgium

(continues)

(continued)

Principal Investigator	Co-PI	Site
Reinhard Larsen, M.D. Professor of Anesthesiology	Markus Paxian, M.D. Anesthesiologist	Klinik fur Anasthesiologie und Intensivmedizin Universitatskliniken Des Saarlandes D66421 Homburg/Saar Germany
André Lienhart, M.D. Professor of Anesthesiology	Marc Beaussier, M.D. Anesthesiologist	Hôpital St. Antoine Département Anesthésie et Réanimation 75571 Paris cedex 12 France
Paul Lieverse, M.D. Anesthesiologist	None	Daniel Den Hoed Ziekenhuis Groene Hilledijk 301 3075 EA Rotterdam The Netherlands
Claude Martin, M.D. Professor of Anesthesiology	Serge de Moro, M.D. Anesthesiologist	Hôpital Nord Service de Réanimation Polyvalente 13915 Marseille cedex 20 France
Charles McCollum, M.D. Professor of Vascular Surgery	Nabil El-Makatti, M.D. Consultant Anaesthetist	South Manchester University Hospitals – Withington Hospital Department of Surgery West Didsbury Manchester, M20 2LR United Kingdom
Claude Meistelman, M.D. Professor of Anesthesiology	Roland Kipper, M.D. Anesthesiologist	Dépt. d'Anesthésie et de Réanimation Hopital Brabois 54511 Vandoeuvre France
Jörg Meyer, M.D. Professor of Anesthesiology	Hugo Van Aken, M.D., F.R.C.A., F.A.N.Z.A. Professor of Anesthesiology	Westfälische Wilhelms-Universität, Klinik und Poliklink für Anästhesiologie und Operative Intensivmedizin 48149 Münster Germany
Johann Motsch, M.D., Ph.D. Professor of Anesthesiology	Isabella Jung, M.D. Anesthesiologist	Ruprecht-Karls-Universität Heidelberg Klinik für Anästhesiologie 69120 Heidelberg Germany
Douglas Newton, M.B.B.S., F.R.C.A. Anesthesiologist	David Vaughan, M.B.B.S., F.R.C.A. Anesthesiologist	Northwick Park & St. Marks NHS Trust Anaesthetic Research Department Harrow, HAI 3UJ United Kingdom
Konrad Reinhart, M.D. Professor of Anesthesiology	Waheedullah Karzai, M.D. Anesthesiologist	Direktor, Klinik für Anästhesiologie und Intensivtherapie Universitätsklinikum Jena 07740 Jena Germany
Pierre Schoeffler, M.D. Professor of Anesthesiology	Hélène Beaujard, M.D. Anesthesiologist	Département d'Anesthésie et de Réanimation CHU de Clermont-Ferrand 63003 Clermont-Ferrand Cedex France
Jochen Schulte am Esch, M.D. Professor of Anesthesiology	Thomas Standl, M.D. Professor of Anesthesiology	Universitätsklinikum Hamburg-Eppendorf Klinik für Anästhesiologie Martinistrasse 52 20246 Hamburg Germany
Donat R. Spahn, M.D. Professor of Anesthesiology	Patrick Willimann, M.D. Anesthesiologist Mattias Casutt, M.D. Anesthesiologist	Institut für Anästhesiologie UniversitätSpital Zürich CH-8091 Zürich Switzerland
Klaus Unertl, M.D. Professor of Anesthesiology and Transfusion Medicine	Markus Müller, M.D. Anesthesiologist	Klinik für Anästhesiologie und Transfusionsmedizin Universitäts-Klinikum Tübingen 72076 Tübingen Germany
Eugene Vandermeersch, M.D., Ph.D. Anesthesiologist	Esther Geerts, M.D. Anesthesiologist	U.Z. Gasthuisberg (K.U. Leuven) 3000 Leuven Belgium

(continues)

(continued)

Principal Investigator	Co-PI	Site
Léone Van Huynegem, M.D., Ph.D. Anesthesiologist	Isabelle Rausin, M.D. Anesthesiologist	Hôpital de Tivoli Dépt d'Anesthésie 7100 La Louvière Belgium
Maarten van Wijk, M.D. Anesthesiologist	Franklin van Dorsten, M.D. Anesthesiologist	Bosch Medicentrum Location GZG Afd. Anesthesiologie 5200 ME's Hertogenbosch The Netherlands
Klaus Waschke, M.D. Professor of Anesthesiology	Thomas Frietsch, M.D. Anesthesiologist	Institut für Anästhesiologie und Operative Invensivmedizin Universitätsklinikum Mannheim Ruprecht-Karls-Universität Heidelberg 68135 Mannheim Germany
Martin Welte, M.D. Professor of Anesthesiology	Tatajana Luck, M.D. Anesthesiologist	Klinik fuer Anaesthesiologie und operative Intensivmedizin Klinikum Benjamin Franklin Freie Universitaet Berlin Hindenburgdamm 30 12200 Berlin Germany
Andreas Zollinger, M.D. Associate Professor of Anesthesiology	Christoph Hofer, M.D. Anesthesiologist	Stadtspital Triemli Institut für Anästhesiologie 8063 Zurich Switzerland

Appendix 2

Oxygen Off-loading Capacity of Perflubron Emulsion: Hemoglobin Equivalency

In large phase 2 studies in hemodiluted surgical patients[14], it was demonstrated that, after administration of PFC, patients with low hemoglobin concentrations were improved physiologically (based on mean arterial pressure, heart rate, cardiac output, electrocardiogram changes, and mixed venous oxygen tension). In these studies, subjects underwent preoperative ANH to a hemoglobin concentration of 9.0 g/dl. When the first physiologic transfusion trigger (protocol-defined changes in mean arterial pressure, heart rate, cardiac output, mixed venous oxygen tension, electrocardiogram) occurred during surgery, subjects were randomized to one of four groups: PFC at one of two doses, 1.8 or 0.9 g PFC/kg with F_{IO_2} of 1.0, colloid plus F_{IO_2} of 1.0, or autologous blood plus F_{IO_2} of 0.4. The primary endpoints were an assessment of the efficacy in reversing the physiologic triggers and the duration of the reversal (*i.e.*, the period of time before a second physiologic trigger occurred, at which time all subjects received autologous blood). These studies thus provide a means of assessing the activity and efficacy of PFC by measuring its ability to reverse and maintain the reversal of the physiologic transfusion triggers during a measured decline in hemoglobin.

Computer Model to Assess Hemoglobin Equivalency

A computer model was developed to provide a means of quantitating the added oxygen off-loading capacity provided by the PFC as a "hemoglobin equivalent" value. The percent contribution of the PFC-carried oxygen to the total body oxygen consumption ($\dot{V}_{O_2}$) is expressed in terms of the percent contribution of 1 g/dl erythrocyte hemoglobin to the total $\dot{V}_{O_2}$. For instance, if a 1-g/kg dose of PFC provides 10% of $\dot{V}_{O_2}$ and each 1-g/dl of erythrocyte hemoglobin provides 5% of the $\dot{V}_{O_2}$, then the hemoglobin equivalent is 10/5 = 2 g/dl. The "effective hemoglobin" can then be considered the sum of the erythrocyte hemoglobin and the hemoglobin equivalent. For example, if the hemoglobin equivalent of a dose of PFC is 2 g/dl and the

erythrocyte hemoglobin is 8 g/dl, the effective hemoglobin would be 8 g/dl plus 2 g/dl = 10 g/dl.

Analysis of the data from the two phase 2 clinical studies with PFC indicated that the 1.8- and 0.9-g/kg doses of PFC yielded hemoglobin equivalence values of approximately 2.7 g/dl and 1.4 g/dl, respectively, at the time the dose was administered. These data support the conclusion that the intended clinical dose of PFC, 2.7 g/kg, can provide an initial mean hemoglobin equivalent on the order of 4 g/dl at the time of dosing. With simultaneous surgical bleeding and clearance of PFC from the blood by the RES, the analysis indicates that sufficient PFC will remain to provide a hemoglobin equivalent of at least 2.5 g/dl at the time the circulating erythrocyte hemoglobin decreases to 5.5 g/dl. In combination with the available erythrocyte hemoglobin, the remaining effective hemoglobin will be at least 8.0 g/dl (which matched the intraoperative target hemoglobin concentration for transfusing blood in the control group).

Calculation of Hemoglobin Equivalents

In the phase 2 studies, PFC was administered when any one of several predefined physiologic "transfusion triggers" was reached during surgery. After the dose had been administered (during ongoing surgical blood loss), a set of hemodynamic and oxygenation measurements was taken to assess trigger reversal. By knowing the cardiac output, arterial and mixed venous blood gases, body temperature, hemoglobin, and the PFC concentration in the blood at any given time point, it was possible to calculate the contribution of both hemoglobin and PFC to total body $\dot{V}_{O_2}$. Using data for the 73 subjects for whom full data sets were available, the projected mean hemoglobin equivalent of a 2.7-g/kg dose of PFC was determined to be 2.4 ± 1.7 g/dl, by extrapolation from hemoglobin equivalent values calculated for the patients receiving 1.8- and 0.9-g/kg PFC doses.

This prediction of hemoglobin equivalence actually underestimates the potential hemoglobin equivalence because, at the time the blood samples for PFC concentration were obtained, circulating perflubron concentrations had already been reduced from the immediate postdosing levels due to ongoing blood loss and clearance by the RES. Also, the

time elapsed before the postdosing sample was collected varied from patient to patient. Hence, a more relevant estimate of the total initial hemoglobin equivalence can be obtained by calculating the PFC concentration based on each subject's estimated blood volume and the known total dose of PFC administered. Using this approach, the mean predicted hemoglobin equivalence for a total 2.7-g/kg dose of PFC was found to be 4.0 ± 2.6 g/dl.

Losses of Perfluorochemical

Removal of PFC from the circulation by the RES exhibits dose-dependent pharmacokinetics, and phase 1 studies with PFC demonstrated that the half-life of PFC in the blood is related to the absolute dose (*i.e.*, total grams of PFC) administered.[25] Its clearance from the circulation can therefore be predicted, as can its removal from the circulation as surgical bleeding occurs. It is therefore possible to estimate the hemoglobin equivalent adjusted for RES clearance and surgical blood loss at any point during the surgical procedure.

Extensive calculations of hemoglobin equivalence were performed for the proposed dosing regimen of administering a 1.8-g/kg dose of PFC at a erythrocyte hemoglobin concentration of 8.0 g/dl, with a second dose of 0.9 g/kg PFC given at an erythrocyte hemoglobin concentration of 6.5 g/dl. These calculations were performed at differing rates of blood loss for patients with different blood volumes and indicated that it was safe under all circumstances to allow the PFC-treated patient's erythrocyte hemoglobin to decrease to the point at which erythrocyte transfusion was mandated in the protocol, *i.e.*, 5.5 g/dl, as the effective hemoglobin remained greater than 8.0 g/dl.

Model Verification

Verification of the accuracy of the model for calculation of the hemoglobin equivalence has been accomplished by comparison to experimental clinical data from the phase 2 studies. In the 48 subjects for whom data were available, the hemoglobin equivalence at the second postdosing transfusion trigger, calculated from the clinical data, was then compared with the hemoglobin equivalence calculated by the model. There were no significant differences in hemoglobin equivalence (model *vs.* clinical); the mean difference was -0.2 ± 0.7 g/dl, and there was a highly significant correlation between the two variables ($R^2 = 0.90$). Hence, the model appears to incorporate all of the critical parameters necessary to produce acceptable predictions of hemoglobin equivalency. This model was therefore used to design the current study and to ensure that the PFC-treated patients could safely be taken to the low hemoglobin concentrations that were targeted in this study.