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FDA Review Team
 For P010003
 CryoLife BioGlue®

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The memos that follow are from each of the reviewers on the review team.

Team Leader Memo

Device Description

The device is a two-part glue of purified bovine serum albumin (45% w/v or 8 ml/10 ml) and glutaraldehyde (10% w/v or 2 ml/10 ml) in a dispensing system that keeps the two parts separated until ready to use, at which time they pass through a tortuous path applicator tip (available in standard and extended lengths of 10, 20, and 27 cm) where they are mixed in the appropriate proportions and dispensed onto the tissue needing repair/bonding. The bonding is via a localized chemical reaction resulting in covalent bonding (conjugated Schiff's bases or amino bonds of the N-terminal amino acids and α -amino groups of lysine) of tissue surface proteins to the protein in the glue. The chemical reaction begins immediately after the two compounds are mixed, setting up in 20-30 seconds, and is completely reacted within 2 minutes. The first 5 layers of the tissue are involved in the bond. It will bond the tissue (closing the false lumen), reinforce tissue for facilitating suturing of a graft, and seal the anastomoses. The compounds are in separate plastic chambers in a dual-chamber cartridge, capped, double pouched (foil pouch inside). A separate double pouch will contain the twist ring tool (for tightening the tip) and a delivery device (glue gun). All components are gamma irradiated (25 kGy dose with ^{60}Co), with the exception of the applicator tips with extended lengths, which are sterilized by 100% ETO. Also available will be replacement tips and refill cartridges. The glue has a 36-month shelf life date.

History

This product has a regulatory history with the Agency, and has also undergone some modifications in formulation, which are important to understand in the review of the memos that follow. The sponsor first submitted an Investigational Device Exemption (IDE) for a clinical study for use of BioGlue® as an adjunct in repair of Type A (ascending) aortic dissections. Once FDA approved the clinical study for that cohort, the sponsor submitted a Humanitarian Device Exemption (HDE) for use of the BioGlue® as an adjunct in repair of both Type A and Type B (descending) aortic dissections. An HDE is a mechanism of granting limited marketing approval for a restricted indication for treating a disease or patient population that has an incidence rate of 4000 or less in the U.S. per year. In granting such approvals, the Agency considers only the safety and

probable benefit of the device, but not the effectiveness. These approvals are granted for devices when there are no alternatives, or the alternatives are inadequate to treat this limited population. Since the existing sealants and glues available (fibrin and thrombin based) are slow-acting, FDA granted the approval in this case. However, should a competitor gain marketing approval via the Premarket Approval (PMA) process, in which both safety and effectiveness are established, then all existing products cleared via the HDE process become null and void, since an alternative will then be available.

Once the HDE approval was granted for essentially the same patient population as was being assessed in the IDE study, the sponsor had difficulty enrolling patients into the IDE study, maintaining randomization, and obtaining patient consent, and had protocol violations with off-label use. FDA advised the sponsor that if they were to seek a broader market for the BioGlue® that they would have to ensure compliance with the protocol and regulations, or propose an alternative study for the desired label. They then proposed the cardiac and vascular study, for which the PMA is being considered today.

The formulation of the BioGlue® has undergone some modifications, which should also be understood for your review. The sponsor began with the BG1000 outside of the U.S. This was essentially the same as the BG2000, but the user had to fill the syringes prior to using. The BG2000 was supplied aseptically filled into the syringes for immediate use. The BG3000 is gamma irradiated to achieve sterility, and also available for immediate use. Both the BG2000 and the BG3000 are supplied in cartridges that are loaded into a delivery system that has long, tortuous applicators to pre-mix the two solutions in the correct ratios. The BG2000 formulation gave toxic results in many of the biocompatibility tests, but FDA decided to allow its use in the aortic dissection indication, since the probable benefit of having a sealant that cures immediately was believed to outweigh the risk of toxicity to this patient population. However, when the sponsor decided to expand the indication to sealing anastomoses, FDA's concern about the potential toxicity was heightened. The gamma irradiated product was re-assessed for the acute toxicity tests, and passed them all. We remain concerned about the potential for an immune response, however.

Report of Prior Investigations

Prior investigations of this material included an animal study, biocompatibility testing, and bench studies on biodegradation, residual glutaraldehyde, and visual, analytical, and functional testing, described below.

Biocompatibility Testing

The sponsor claims that ISO 10993-1/FDA Blue Book memo #G95-1 was followed for this testing, but some of the applicable tests were not performed, and no

rationale was given for omission of the studies (specifically, no carcinogenicity or chronic toxicity testing was done, nor was biodegradation). Testing was done on the glue, and on the delivery system. The glue was allowed to cure prior to testing, which is probably not the preferred approach. FDA is developing a guidance for testing of cured products such as the BioGlue®, but it is not completed. The results of testing of the BG2000 formulation, the BG3000 formulation, and the delivery system components are tabulated on the next several pages.

The BG2000 implantable glue gave a toxic response in many of the tests, specifically, in the cytotoxicity, genotoxicity, sub-chronic, hemolysis, and thrombogenicity tests. The components in the delivery system were not toxic, with the exception of the mixing chamber in the tip for cytotoxicity (this component was not assessed for 5 day implant). The sponsor does not provide a rationale to explain why these toxic results are acceptable, nor is there any rationale provided for not conducting the carcinogenicity, chronic, and biodegradation tests. Despite these omissions and the toxic results, as noted above, FDA granted permission to begin the aortic dissection study (1) because there is a history of use of similar products for the same indication in Europe, and (2) the formulations used in Europe contain similar amounts/doses of glutaraldehyde to that proposed in the BioGlue, and (3) the probable benefits (based on the European use and the animal study below) outweigh the disadvantages. We did not request further toxicological testing for this device for the dissection indication.

The BG3000, assessed only for acute toxicity, passed all tests.

To justify omitting implant and chronic studies of the modified BG3000 formulation (gamma sterilized), testing was done in-house to compare the extractables in the polymerized BG2000 series to the BG3000 series. Testing involved pH, GC assay, UV-VIS scans, and HPLC analyses. The firm concludes that there were no significant differences between the two formulations. I referred this attachment to FDA chemists to verify that the correct tests methods and conclusions were made. Dr. Chen indicated that chemically, the BG2000 and BG3000 were not similar, but it was agreed that since the physical and toxicological properties were not altered, that we would allow this justification, and no further testing was sought, with the exception of testing to assess the immunogenic potential.

Table 1
Results of Toxicity Testing of the Implantable **BG2000** Glue Material

Test Performed/Lab Report # (literature citation, standard reference*)	Extract(s) if used (polar, non-polar) or animal model	Extract conditions (time, temperature, area or mass to volume ratio compared to in-use conditions)	Test and Control(s) used	Results/Comments (units when appropriate)
Cytotoxicity (Chrysalis)	MEM	6 cm ² /ml fresh cured at 37 °C for 24 hours	neat and 1:4 dilution of test and blank, CdCl ₂ 1000 µg/ml (+) vehicle blank (-)	very cytotoxic reaction (Grade 4) after 48 hours with L-929 cells
Cytotoxicity (Toxicon)	MEM	2.0 g air dried for 24 hours after curing in 10 ml at 37 °C for 24 hours	neat test extract natural rubber (+) silicone (-)	moderate reaction (Grade 3) after 48 hours with L-929 cells
Sensitization (Chrysalis Buehler method*)	saline	fresh cured 3 cm ² /ml ratio at 50°C for 72 hours	DNCB (+) saline blank (-)	0.3 ml dermal doses, no sensitization observed
Irritation/Intracutaneous Toxicity (Chrysalis)	saline, 1:20 EtOH/saline, PEG, and sesame oil	ratio of 3 cm ² /ml at 50 ° for 72 hours	vehicle blanks (-)	No difference in the control and test extracts were observed at 24, 48, or 72 hours for any vehicle.
Systemic toxicity	saline, 1:20 EtOH/saline, PEG, and sesame oil	ratio of 3 cm ² /ml at 50 ° for 72 hours	vehicle blanks (-)	No difference in the control and test extracts were observed at 24, 48, or 72 hours for any vehicle.
Sub-chronic toxicity	90 day subcutaneous implant in rat model (n=20 each test and control)	~1.8 ml	Saline	Implant area remained raised and firm for most animals; some had erythema and abrasions at the site. Changes were noted in the RBC and WBC values. No effect on body weight or weight gain, hematology, coagulation, clinical chemistry other than noted. No gross pathology or organ weight differences. The implant was encapsulated with some mineralization and evidence of a foreign body reaction.
Genotoxicity				
gene mutation Ames	saline	4 g/20 ml ratio at 37 °C for 24 hours	saline (-) 2-aminoanthracene (+) Sodium Azide (+) 2-Nitrofluorene (+) 9-Aminoacridine (+)	no genotoxicity in TA 98, TA100, TA1535, and TA1537 strains of <i>S. typhimurium</i> with and without metabolic activation

Test Performed/Lab Report # (literature citation, standard reference*)	Extract(s) if used (polar, non-polar) or animal model	Extract conditions (time, temperature, area or mass to volume ratio compared to in-use conditions)	Test and Control(s) used	Results/Comments (units when appropriate)
gene mutation Ames	saline and DMSO	6 cm ² /ml ratio at 50 °C for 72 hours in doses of 1.67, 5.00, 16.7, 50.0, 100, and 200 µl/plate	saline (-) 2-anthramine (+) Sodium Azide (+) 2-Nitrofluorene (+) 9-Aminoacridine (+) Mitomycin C (+) ENNG (+) 2-aminofluorene (+)	TA1535, TA1537, TA98, TA100 and TA102 strains of <i>S. typhimurium</i> and WP2 <i>uvrA</i> strain of <i>E. coli</i> with and without metabolic activation were assessed. Possible genotoxic result was found in strain TA1535 with activation in saline, and for TA1535, TA 1537 and WP2 <i>uvrA</i> in DMSO. Retesting gave normal results except for TA1537 with S9, and thus considered to be marginally positive for strains TA1535 and TA1537.
AS52 Chinese Hamster Ovary Cell/XPRT Mammalian Cell Forward Mutation Assay	Ham's F12 treatment medium	120 cm ² /40 ml (3 cm ² /ml) ratio for 24 hours at 37 ° C Ten final concentrations ranging from 0.00333 to neat for toxicity screen, then between 0.0000500 and neat with S9 and 0.500 to 66.7 with S9 for the initial assay, then 0.500-83.3% with and without S9	sham F12 vehicle (-) ethyl methanesulfonate (+) dimethylnitrosamine (+)	Genotoxic results (dose-dependent increase in mutants) observed
Chromosome aberration in Human Lymphocytes	RPMI 1640	6 cm ² /ml at 37 ° C for 24 hours Tox pre-screen in ten concentrations with and without S9 Initial assay using 5.00-75% concentration. Confirmatory assay involved concentrations of 5.00-50%	cyclophosphamide (+) mitomycin C	Tox pre-screen revealed toxic effects, but not due to pH or osmolality. Genotoxic effect (dose dependent increase in porportion of aberrant metaphase and aberrations/cell) were noted in initial assay. Results of confirmatory assay same as initial
DNA damage <i>in vivo</i> assay				
Implantation	N/A	N/A	USP polyethylene (-)	At 120 hours (5 days) the BioGlue gave results similar to the negative controls (macroscopic assessment only)
Hemocompatibility				
hemolysis	human and monkey blood	1 cm x 1 mm pieces in 5 ml	USP polyethylene (-)	No hemolysis of human blood, but monkey blood inconclusive, since negative control caused hemolysis.
Complement activation	rat serum samples from subchronic study animals below	subcutaneous implant of ~1.8 ml	saline injection	5 µl samples were analyzed for hemolytic complement with the EZ Complement CH50 assay kit on day 30 and day 90 after implant. No difference was observed between test and control animals on day 30 or day 90

Test Performed/Lab Report # (literature citation, standard reference*)	Extract(s) if used (polar, non-polar) or animal model	Extract conditions (time, temperature, area or mass to volume ratio compared to in-use conditions)	Test and Control(s) used	Results/Comments (units when appropriate)
thrombogenicity	rabbit carotid artery (n=5)	1.0-1.8 ml injected into carotid artery	N/A	aPTT, platelets, HCT, ACT, and bleeding time were measured before and after injection (at 15, 60 and 120 minutes), as were systemic arterial pressure, heart rate, and lead II ECG (after 30 minutes of stabilization, for 10 minutes). No effect on any parameter except for blood flow, which was 85% reduced in 2, and temporarily increased in 2. Microscopically the endothelial cells were affected (cells lost with thrombi formed on the wall, i.e., this material caused thrombus formation)
Pyrogenicity				
Chronic toxicity				
Carcinogenicity				
Biodegradation				

Table 2
Results of Toxicity Testing of the Delivery System

Test Performed/Lab Report # (literature citation, standard reference*)	Extract(s) if used (polar, non-polar) or animal model	Extract conditions (time, temperature, area or mass to volume ratio compared to in-use conditions)	Test and Control(s) used	Results/Comments (units when appropriate)
Cytotoxicity	MEM	6 cm ² /ml ratio at 37 ° C for 24 hours	vehicle (-) 2.00 µl/ml CdCl ₂ (+)	eight components tested separately. All components gave a mild or less reaction (non-toxic) after 48 hours of exposure, except for the mixing tip, which gave a moderate score of 3.0 (toxic).
Sensitization (Buehler method*)	saline	"appropriate surface-to-volume ratios" at 50 ° C for 72 hours	saline (-) DNCB in EtOH (induction) or acetone (challenge)	None of the test or negative control animals had a sensitization reaction; most of the positive control animals had a reaction.

Test Performed/Lab Report # (literature citation, standard reference*)	Extract(s) if used (polar, non-polar) or animal model	Extract conditions (time, temperature, area or mass to volume ratio compared to in-use conditions)	Test and Control(s) used	Results/Comments (units when appropriate)
Irritation/Intracutaneous Toxicity	saline, 1:20 EtOH:saline, PEG 400, sesame oil	all components tested separately at 50 ° C for 72 hours extraction using the following ratios: 1 cartridge/41.06 ml 10 pieces (5 each piston A and B)/23.5 ml 40 pieces/20 ml O-ring (EPDM and ± lubricant) 5 pieces/23.6 ml mixing tip housing 24 pieces/20.2 ml mixing tip mixing chamber 1 piece/36.65 ml plunger	vehicle controls (-); PEG blank and test diluted 7:4	No difference between results in the test and control animals at 24, 48 or 72 for any component, except for the O-ring in sesame oil (0.3 control vs. 1.3 test, repeat 0.5 control, 1.7 test)
Systemic toxicity	saline, 1:20 saline:EtOH, PEG, sesame oil	same as intracutaneous test above	same as above	no difference between control and test animals for any component
Sub-chronic toxicity	N/A	1 x 10 mm strips of the cartridge, pistons A and B, O-ring (EPDM and ± lubricant) mixing tip housing, , and plunger	USP polyethylene high density plastic	After 120 hours (5 days) implant, sites were examined macroscopically only for capsule, hemorrhage, infection, and necrosis. There was no encapsulation of any component, and healing was similar to the negative control in all cases. No results were provided for the mixing tip mixing chamber

Table 3
Results of Toxicity Testing of the **BG3000** BioGlue

Test Performed/Lab Report # (literature citation, standard reference*)	Extract(s) if used (polar, non-polar)	Extract conditions (time, temperature, area or mass to volume ratio compared to in-use conditions)	Test and Control(s) used	Results/Comments (units when appropriate)
Cytotoxicity (ISO)	MEM	9.6g in 48.0 ml at 37 °C for 24 hours	Natural rubber (+) Silicone tubing (-)	L-929 cells gave a grade 2 (mild) reactivity score with test extract at 48 hours

Test Performed/Lab Report # (literature citation, standard reference*)	Extract(s) if used (polar, non-polar)	Extract conditions (time, temperature, area or mass to volume ratio compared to in-use conditions)	Test and Control(s) used	Results/Comments (units when appropriate)
Sensitization (Maximization)	Saline and CSO	4g/20ml 37 °C 72 hours	Saline and CSO (-) DNCB (+) with and without activation	No sensitization was observed
Intracutaneous Toxicity (ISO/USP)	Saline and CSO	4g/20ml at 37 °C for 72 hours	Saline and CSO	No toxicity observed in either extract
Systemic Toxicity (ISO/USP)	Saline (IV) and CSO (IP)	4g/20ml at 37 °C for 72 hours	Saline and CSO	No signs of toxicity
Hemolysis (DHEW)	Saline	4g/20ml at 37 °C for 72 hours	Saline (-) Water (+)	4.45% hemolysis, considered to be non-hemolytic
Rabbit pyrogen (ISO)	saline	4g/20ml at 37 °C for 72 hours	Saline (-)	<0.5 °C rise in all rabbits-non pyrogenic

Animal Study

A previously reported sheep model for acute descending aortic dissection was used for this study, involving 30 sheep (30 enrolled to guarantee a minimum of 12 animals in both the test and control groups). Dissections were created and verified to meet criteria (false lumen volume <50% of total aortic volume, length of <7.1 cm, and width of <2.6 cm). Animals were then randomized in a blinded fashion into either the surgical repair of the proximal flap alone, or repair by gluing the layers of the dissection together and surgical repair of the proximal flap. A total of 13 animals were randomized into each group (4 dissections did not meet criteria). A 40% mortality rate was anticipated either due to failure of the model to perform or failure of the treatment. Failure of the model to perform was defined as progression of the dissection past 7 cm, 50% of total aortic volume, spontaneous rupture, or other reason for instability of the dissection. At explant, the aortas were excised and gross and microscopic histopathology assessments made. Dissections varied from 5.0 to 7.0 cm long (mean 5.2 cm), 1.2-2.5 cm wide (mean 1.4 cm), and 20-50% (mean 35%) of the aortic volume (statistically similar among the two groups).

In the test group, 5 died (3 pneumonia/weather stress, 1 tension pneumothorax, and 1 chronic aneurysm rupture at 51 days), all with healed or obliterated dissections except for the animal with the rupture (due to mycotic super-infection of a technical glue failure [this event deemed related]) In the 8 animals surviving to the 90 day sacrifice period, the dissection was completely healed without signs of progression.

In the control/surgical group, 1 animal died of pneumonia/weather stress, and 4 of proximal or distal aneurysm progression and rupture [all deemed related]. Eight animals survived to 90 days. Of these, 2 dissections healed after 3-3.5 cm of progression; 2 had a chronic dissection formed at the distal suture line; in 4 a chronic dissection formed at re-entry points unrelated to the surgical repair site. The false lumen made up 25-75% of the total aortic lumen in these chronic cases, and the aortic wall was thinning and the diameter expanding.

Histologically, various parameters were given a numeric score for comparison purposes (intimal hyperplasia, fibrin deposition, thrombus, acute inflammation, fibrosis, neovascularization, granulomatous inflammation, giant cells, lymphoid nodules, tissue necrosis, hemorrhage, and mineralization). Other parameters which were scored for statistical analysis included cross-clamp duration, intra-operative blood loss, complications, mortality, treatment failure, animal gender, age, weight, and hematocrit, dissection length, width, area. The early related mortality rate was significantly different using 1 sided Fisher's Exact test p values, but not for 2 sided (sample size too small), suggesting a trend toward lower

mortality with BioGlue than surgical repair. All mortality combined was not statistically different between groups. Late death was not significantly different (related or overall). When treatment related mortality and morbidity were considered together, the difference between the groups was significant, with 12/13 animals in the control group experiencing either a related death or morbidity, compared to only one related death in the test group, whether or not animals not completing the study were considered in the analysis. Histological analysis revealed differences in the severity and frequency of lymphoid nodule formation; there was an inflammatory reaction associated with the BioGlue; mineralization and tissue necrosis was more frequent and severe in the BioGlue samples. In areas where the BioGlue was applied, there was fibrosis in the media, and thinner walls were observed. The conclusion by the pathologist was that these observations were consistent with a foreign body reaction, and that the wall thickness in the BioGlue group is consistent with the variation in the surgical procedure for creation of the dissection. Intimal hyperplasia was markedly reduced in the lesions treated with BioGlue.

Additional animal studies were done to assess sealing at the anastomosis area (coagulopathic sheep model and sutureless porcine CABG model) in support of modifying the protocol for the cardiac and vascular study.

In vitro Testing of the BioGlue

Tests were done on the components (albumin and glutaraldehyde), on the adhesive, and on the packaging, described below. Sample sizes were mostly chosen using the MIL Standard 105E.

1. Tests on albumin component

- physical inspection—is done throughout the manufacturing process. The entire lot is inspected after the BSA is filled into the vial for damage and volume, and portions of the lot are checked for integrity of the crimp and seal.
- UV-Vis spectrophotometric profile—Each batch is checked in the range of 200-700 nm for its spectrophotometric profile at 10 mg/ml dilution. In order to be acceptable, peaks only at 212 (peptide bonds) and 280 (aromatic amino acid side chains) are acceptable. Additional peaks would mean impure albumin.
- PH [information redacted]. The pH is monitored throughout the process (raw material, filtered bulk and heat treated vial).
- Purity (SDS-PAGE—sodium dodecyl sulfate-polyacrylamide gel electrophoresis)—this method is used to determine the amount of polymerization/non-monomer form of the albumin after filtration and heat treatment [information redacted]. Bands forming in locations

other than 66,000 kDa are indicative of impurities or polymers. [information redacted].

- Protein concentration (Colorimetric)—this method verifies the concentration of the protein to ensure adequate mixing and dispensing (too high a concentration will result in too viscous a mixture which will not flow through the mixing tip; too low a concentration results in poor bonding strength). This method was done throughout the process at dilutions of 0.45 mg/ml. The specification is $45 \pm 3\%$ protein.
- Bioburden—this is assessed by filtration of the 45% bulk material. [Information redacted] aerobic, anaerobic, mold and yeast. There is no mention of fungi.
- Sterility of filled vials—the USP sampling procedure (10% of the lot, maximum 20) is used to verify sterility of the filled vials, by either filtration or direct inoculation.

2. Tests on Glutaraldehyde component:

- physical inspection—inspection similar to the albumin component is done throughout the process, both on the 50% and the 10% solutions in the filled vials.
- glutaraldehyde concentration (hydroxylamine assay)—the adhesive properties needed are dependent on the appropriate amount of glutaraldehyde, [information redacted] so the specification chosen was 10%. This titration assay is used to verify the concentration of the raw stock solution [information redacted] and that in the vials and diluted stock solution [information redacted].
- extent of autopolymerization (absorbance ratio)—The monomer has an absorbance peak at 280 nm, whereas polymerized material peaks at 235 nm, so the ratio of the peak at 280 to 235 nm is measured to give an indication of the percentage of polymerization. [Information redacted].
- Sterility of filled vials—This is similar to the testing for the albumin component; no aerobic or anaerobic growth after 14 days

3. Functional testing of Adhesive

- Shear strength—initial strength tests were done during muscle samples, but these samples were variable in their stretch, fiber orientation, and fat content, which led to variable results. A method using porcine dermal tissue was validated to give more reliable results, [information redacted]. Samples are tested for shear strength after applying glue to a 1 cm^2 [information redacted].

4. Packaging

- Inspection (subassemblies and final)—packages are inspected for damage, component verification and seal closure. Sample size varies

with lot. Also checked is the labeling, radiation dose (delivery system).

- Applicator tip pouch sub-assembly—as above
- BioGlue delivery tray package and label—as above
- delivery tray bagged for irradiation—as above
- inspection of irradiated tray—the count is verified to be the same as was sent out for sterilization
- Adhesive package and label—as above, shelf life date also checked.
- Seal Peel test—both the pouch and the tray are peel tested to verify that a minimum of 1.1 PSI is achieved (n=13/lot).

Clinical Review

The sponsor has reported the results of a prospective, multicentered, randomized, blinded clinical trial of BioGlue Surgical Adhesive in patients undergoing cardiac or vascular surgical procedures. The purpose of the study was to collect data to support the safety and effectiveness of BioGlue when used as an anastomotic sealant to provide hemostasis. A total of 151 patients who were to undergo a cardiac or vascular surgical procedure were randomized in a 1:1 ratio to surgical treatment with or without BioGlue. The patients were blinded as to which treatment they had received until after the study was complete. Follow-up was at discharge from the hospital, 1 month and 3 months. Enrollment of patients began April 25, 2000 and was completed on September 15, 2000. A total of 6 investigational sites participated.

The primary response variable was the proportion of vascular anastomoses achieving hemostasis without the need for additional measures to control bleeding. This was assessed just before skin closure after blood pressure had been restored to the vessel. The study hypothesis was that the proportion of anastomoses achieving hemostasis would be higher in the patients treated with BioGlue. The required sample size was estimated based on the assumption that the rate of continued bleeding at the anastomotic site would be 5% in the patients treated with BioGlue and 15% in the patients treated without BioGlue. Using a two-tailed test and an alpha of 0.05, a sample size of 141 anastomoses per treatment group was required to provide a statistical power of 80%. Assuming that there would be 2 anastomoses per patient, this required 71 patients in each treatment group. This number was increased by 10% to account for patients who crossed over from one group to the other because of uncontrolled bleeding. The number was increased by an additional 10% to allow for patients lost to follow-up. The resulting estimated sample size was 86 patients per treatment group.

The number of patients who crossed over was less than expected. Only one patient, who had been randomized to treatment without BioGlue, had uncontrolled bleeding. That patient was crossed over to the BioGlue treatment arm. Enrollment in the study was stopped when 151 patients had been treated and evaluated for the primary response variable. At that time, 76 patients had been randomized to the test group treated with BioGlue and 75 patients had been randomized to surgery without BioGlue. These numbers exceeded the estimated required sample size of 71 patients.

The randomization of patients was controlled so that each clinical center enrolled approximately the same number of patients into each treatment group. The number of patients enrolled per clinical center differed considerably, with one center enrolling 32% of the patients and one center enrolling only 2% of the patients. Three of the six clinical centers enrolled more than the expected average of 17% of the total; therefore, the multicentered nature of the study was fairly well maintained.

Patient Population

Baseline demographic and clinical data are presented, allowing a comparison of the treatment groups. The following table summarizes these data. CAD, COPD, CRF, and CHF mean coronary artery disease, chronic obstructive pulmonary disease, chronic renal failure, and congestive heart failure. The numbers in parentheses are the percent of the total number of patients in the treatment group.

Comparison of Treatment Groups

Patient Parameter	BioGlue Group (n=76)	Control Group (n=74)	Crossover (n=1)
Mean Age (years)	63.4	66.3	73
Men	49 (64%)	48 (65%)	0
Women	27 (36%)	26 (35%)	1
Caucasian	67 (88%)	66 (89%)	1
Smoker	60 (79%)	57 (77%)	1
Diabetic	12 (16%)	11 (15%)	0
Hypertensive	55 (73%)	57 (77%)	1
Aortic aneurysm	56 (74%)	49 (66%)	1
CAD	34 (45%)	30 (41%)	1
Patient Parameter	BioGlue Group (n=76)	Control Group (n=74)	Crossover (n=1)
Visceral Occlusive Disease	4 (5%)	3 (4%)	0
CHF	16 (21%)	11 (15%)	0
CRF	6 (8%)	7 (9%)	0
Cerebrovascular Disease	7 (9%)	12 (16%)	0

Peripheral Vascular Disease	15 (20%)	19 (26%)	0
Stroke	8 (11%)	12 (16%)	0

The patients in the treatment groups appear fairly well matched in terms of these factors.

The preoperative indications for which the patients underwent surgery are summarized in the following table. The numbers in parentheses are the percent of the total number of patients in the treatment group.

Indications for Surgery

Parameter	BioGlue® Group (n=76)	Control Group (n=74)	Crossover (n=1)
Thoracoabdominal aneurism	16 (21%)	19 (26%)	0
Ascending aortic aneurism	23 (30%)	19 (26%)	1
Other aortic aneurism	16 (21%)	13 (18%)	0
Peripheral occlusive disease	4 (5%)	7 (9%)	0
Carotid occlusive disease	10 (13%)	9 (12%)	0
Aortic root surgery	4 (5%)	3 (4%)	0
Aortic valve disease	7 (9%)	14 (19%)	0
Other surgery	7 (9%)	7 (9%)	0
Total	87	91	1

Since the total number of indications for surgery is greater than the number of patients, some of the patients had more than one indication for their surgery. Surgery for aortic aneurisms predominated in this series of patients. The indications for surgery were fairly well balanced between the treatment groups.

The preoperative laboratory values for the patients who underwent surgery are summarized in the following table.

Preoperative Laboratory Values

Parameter	BioGlue® Group (n=76)	Control Group (n=74)	Crossover (n=1)
Mean Hemoglobin (g/dl)	13.2	13.2	14.4
Mean hematocrit (%)	39.7	39.6	42.0
Mean WBC (10 ³ /ul)	7.8	7.4	6.9
Mean platelet count (10 ³ /ul)	238.1	227.5	212.0
Mean PT (sec)	11.9	11.9	12.7

Mean PTT (sec)	29.0	28.9	23.2
Mean fibrinogen (mg/dl)	384.2	383.6	320.0

The preoperative laboratory values appear fairly well balanced between the treatment groups.

At least one laboratory value was abnormal in 28% of the patients treated with BioGlue and 17% of the patients treated without BioGlue. Any abnormal laboratory value was commented on as to its clinical significance and whether it contributed to an adverse event. There were only three instances of laboratory values contributing to an adverse event; all of these were in the group treated without BioGlue. In one patient, a preoperative hemoglobin of 9.1 g/dl and hematocrit of 27% was thought to have contributed to an adverse event. Two patients had low postoperative platelet counts which were thought to have contributed to adverse events.

Treatment Parameters

The surgical procedures were characterized in terms of the type of procedure, the amount of BioGlue used, and the types of materials bonded. The following table summarizes the types of surgical procedures. The numbers in parentheses are the percent of the total number of procedures in the treatment group.

Types of Surgery

Parameter	BioGlue® Group (n=76)	Control Group (n=74)	Crossover (n=1)
Cardiac Procedures	24 (23%)	25 (26%)	0
Aortic Procedures	57 (54%)	47 (49%)	1
Peripheral Vascular Procedures	25 (24%)	23 (24%)	0
Total	106	95	1

The proportion of patients having each of the three general types of procedures seems to be similar in the two treatment groups. The cardiac procedures included 4 aortic root replacements, 1 aortoplasty, 5 aortic valve annuloplasties, 1 aortic valve resuspension, 23 aortic valve replacements, 2 Bentall procedures, 8 composite valve conduit procedures, 2 mitral valve replacements, 2 Ross procedures, and 1 coronary artery bypass grafting procedure. The aortic procedures included 21 abdominal aortic aneurism repairs, 21 ascending aortic aneurism repairs, 9 repairs of aneurisms of the ascending and aortic arch, 12 repairs of aneurisms of the transverse aortic arch, 1 repair of an aneurism of the aortic arch and descending aorta, 8 repairs of aneurisms of

the descending aorta, 32 thoracoabdominal aneurism repairs and 1 repair of a type B aortic dissection. The peripheral vascular procedures included 5 aorto-femoral bypasses, 2 aorto-iliac bypasses, 1 aorto-innominate artery bypass, 1 carotid bypass, 19 carotid endarterectomies, 3 femoral-distal bypasses, 2 femero-femoral bypasses, 5 femero-popliteal bypasses, 1 hepato-renal bypass, 1 popliteal-dorsalis pedis bypass, 1 profunda femoris endarterectomy, 6 renal artery bypasses, and 1 renal artery endarterectomy.

The sponsor reports that the average number of cartridges of BioGlue used per surgical repair was 2.6, with a range of from 1 to 4 cartridges. The types of material connected in the anastomoses are summarized in the following table. The numbers in parentheses are the percent of the total number of anastomoses in the treatment group. PTFE means polytetrafluoroethylene.

Type of Material in Anastomoses

Parameter	BioGlue® Group (n=76)	Control Group (n=74)	Crossover (n=1)
Tissue-to-polyester	147 (73%)	127 (69%)	3
Tissue-to-tissue	19 (9%)	28 (15%)	0
Tissue-to-PTFE	14 (7%)	15 (8%)	0
Other	17 (8%)	13 (7%)	0
Data Missing	5 (2%)	1 (1%)	0
Total	202	184	3

The proportion of the various types of material connected in the anastomoses seems to be similar in the two treatment groups. In most anastomoses, the materials being connected were tissue-to-polyester, with some anastomoses involving tissue-to-tissue and tissue-to-PTFE. The other materials connected included 21 anastomoses of polyester to polyester, 3 tissue to porcine tissue, 2 cryopreserved homograft to tissue, 2 prosthetic valve to tissue, 1 polyester to PTFE, and 1 polyester to bovine pericardium.

Safety Results

The safety data consisted of the number of patient deaths and the other adverse events. There were 5 patient deaths in each of the two treatment groups. There was one crossover patient who was originally randomized to be treated without BioGlue. Because of uncontrolled bleeding, the patient was crossed over and successfully treated with BioGlue. This patient is considered to be part of the BioGlue treatment group for safety purposes. She is one of the patients who died during the study.

A brief case history is presented for each of the ten patients who died during the study. The following table summarized the patient deaths in this study. TAA, AAA, and TAAA mean thoracic aortic aneurism, abdominal aortic aneurism, and thoracoabdominal aortic aneurism. CHF is congestive heart failure.

Patient Deaths

AAAT1 treatment1	Age	Sex	Lesion	Days	Cause of Death	Procedure Related
BioGlue®	76	M	TAA	1	CHF, unable to wean	Yes
BioGlue®	42	F	TAA	1	CHF, cardiac arrest	Yes
BioGlue®	79	M	AAA	1	Bradycardia, asystole	Yes
BioGlue®	78	F	TAAA	42	Stroke	No
Crossover	74	F	TAA	43	Sepsis	Yes
Control	70	F	TAAA	15	Multiorgan failure	Yes
Control	72	F	AVR	109	Cardiac arrest	No
Control	79	M	TAA	85	Respiratory failure	Yes
Control	70	F	AAA	109	Cardiac arrest	No
Control	75	M	TAA	17	Spinal infarction	Yes

Nine of the ten patients who died had aortic aneurisms, seven of which involved the thoracic aorta. There were a variety of causes of death, mostly related to the patient's comorbid condition exacerbated by the surgery. None of the patients died of complications of surgical bleeding.

The following table summarizes the other adverse events. The numbers of patients having the adverse events are shown in order of their frequency of occurrence in the total of the two treatment groups. The crossover patient is included in the BioGlue treatment group. The numbers in parentheses are the percent of the total number of patients in the treatment group.

Other Adverse Events

Parameter	BioGlue® Group (n=77)	Control Groups (n=74)
Pleural effusion	20 (26%)	21 (28%)
Cardiac arrhythmia	17 (22%)	14 (19%)
Respiratory failure	13 (17%)	12 (16%)
Infection	13 (17%)	10 (14%)
Renal failure	13 (17%)	9 (12%)
Neurological deficits	5 (6%)	16 (22%)
Atelectasis	6 (8%)	3 (4%)
Hemorrhage	3 (4%)	3 (4%)
Incisional pain	2 (3%)	4 (5%)
Anemia	4 (5%)	2 (3%)
Ileus	3 (4%)	2 (3%)
Organ system failure	3 (4%)	2 (3%)
Myocardial ischemia	3 (4%)	2 (3%)
Myocardial infarction	3 (4%)	1 (1%)
Dysphagia	4 (5%)	1 (1%)
Pneumothorax	2 (3%)	2 (3%)
Altered mental status	2 (3%)	2 (3%)
Stroke	1 (1%)	3 (4%)
Paraplegia	1 (1%)	2 (3%)
Depression	3 (4%)	0

Only the rate of occurrence of neurological deficits appears to be significantly different in the two treatment groups. The reason for this is not clear, but is probably not related to the use of the BioGlue. The safety data do not raise any significant concerns about the safety of the use of BioGlue within the three month follow-up period.

Effectiveness Results

The primary response variable was the proportion of vascular anastomoses achieving hemostasis without the need for additional measures to control bleeding. The following table summarizes the hemostasis data on an intent-to-treat basis.

Anastomoses Achieving Hemostasis

Parameter	BioGlue® Group	Control Group	p-value
Proportion of Anastomoses	164/202 (81.2%)	105/184 (57.1%)	<0.001
Proportion of Patients	46/76 (60.5%)	29/74 (39.2%)	0.014

The sponsor has calculated the P-value based on Fisher's Exact Test. The proportion of patients and the proportion of anastomoses achieving hemostasis are both statistically significantly greater in the group of patients treated with BioGlue.

Secondary effectiveness endpoints including the intra-operative use of blood products, additional hemostatic measures, and re-operation because of bleeding at the anastomotic site. The following table summarizes the results of the secondary effectiveness endpoints. CPB means cardiopulmonary bypass. The procedure times are measured in minutes; the blood products are measured in units.

Secondary Effectiveness Endpoints

Parameter	BioGlue® Group	Control Group	p-value
Blood products, mean value			N.S.
Red blood cells	2.3	1.85	N.S.
Platelets	5.13	5.19	N.S.
Fresh frozen plasma	3.79	3.29	N.S.
Cryoprecipitate	4.32	2.03	N.S.
Donor exposures	20.6	14.2	N.S.
Pledgets used initially	53/202 (26%)	66/184 (36%)	0.047
Mean CPB Time	168.1	144.2	N.S.
Mean cross-clamp time	74.0	69.1	N.S.
Mean total operative time	237.7	228.7	N.S.
Mean days in ICU	3.9	4.8	N.S.
Mean days in hospital	9.5	10.9	N.S.
Reoperation for bleeding	0	1	N.S.

Only the proportion of anastomoses in which pledgets were initially used to reinforce the anastomoses was statistically significantly different between the two treatment groups.

Limitations of the Study

A limitation of this clinical study was the choice of a surrogate primary effectiveness endpoint. This endpoint was the proportion of surgical anastomoses which achieved hemostasis without additional measures to control bleeding. Although the study showed a statistically significant improvement in the primary effectiveness endpoint, there was no apparent improvement in most of the clinically significant secondary effectiveness endpoints, particularly the use of blood products. This may have occurred because the amount of blood saved by the improved sealing of the surgical anastomoses was small compared to the variability of blood loss between the different

surgical procedures. This would have made it difficult to demonstrate a reduction in the use of blood products without a much larger sample size.

Another limitation of the study was the relatively small number of anastomoses joining tissue-to-PTFE. The sponsor has submitted additional clinical data from the commercial use of BioGlue in the international market. This included 35 cases of the anastomosis of tissue-to-PTFE grafts, 17 cases of tissue-to-tissue anastomoses, and 3 cases which involved a combination of tissue-to tissue and tissue-to-polyester anastomoses. When the users were asked whether BioGlue was effective, the response was affirmative in all cases where a response was recorded. This included 31 of the 35 cases of tissue-to-PTFE anastomoses, 13 of the 17 cases of tissue-to-tissue anastomoses, and 2 of the 3 combined anastomoses.

Another limitation of the study was that the follow-up period of three months did not allow assessment of long term safety data.

Conclusion

The sponsor has developed a surgical glue intended to be used as an adjunct to standard methods of cardiac and vascular repair such as sutures or staples by sealing and reinforcing anastomoses. They conducted a multicenter, randomized, blinded clinical trial of the surgical glue in 151 patients undergoing cardiac or vascular surgical procedures. The data show that using the surgical glue caused an improvement in the proportion of surgical anastomoses which achieved hemostasis without additional measures to control bleeding; however, the study did not demonstrate significant clinical benefits such as reduced blood use or reduced operative time. No significant safety concerns were evident during the 3 months of follow-up.

Immunology Review

The studies submitted were appropriate and well done. However I disagree with the interpretation of the results. The company does not consider the inflammatory responses and the immune response significant. I disagree with this. The studies indicate that Bioglue is a potent immunogen. It has the potential to sensitize patients to bovine products. This may result in hypersensitivity responses and immune complex disease occurring a year or more after implantation. It may also result in making the patient allergic or sensitive to other bovine products. This Bioglue should be used with extreme care and the patient adequately informed of the risk of allergy to bovine products, including milk. The most likely consequences will be anaphylactic shock,

immune complex disease with liver, lung, and joints most commonly affected. There also may be disturbances in the lymphoid tissue of the gastrointestinal tract with various clinical signs and symptoms. These may occur long after the initial surgery and should not be ignored or forgotten.

Review of the document.
Attachment 4

The standard tests for delayed hypersensitivity reactions using the Kligman maximization test in the guinea pig and the Buehler test in the guinea pig showed no evidence of delayed contact hypersensitivity. A study was then undertaken in Japan to assess humoral responses in the guinea pig.

Animals were divided into 3 groups, one receiving extracts of Bioglue, one receiving BSA, and one receiving saline all with Freund's complete antigen. The humoral immunity was assessed by challenge for acute anaphylactic shock. They also drew blood just prior to the shock challenge. The results of the test showed that the negative control of saline produced no signs of anaphylactic shock and no antibody titers. The animals immunized with BSA had high titers of antibody and severe anaphylactic shock. The animals receiving Bioglue did not have antibody titers as high, nor anaphylactic responses as severe as those receiving BSA, but nevertheless, they were significantly above the negative control.

The testing lab commented that the Bioglue seemed to sensitize but not induce the anaphylactic shock, but expressed concern that it could sensitize to other bovine products.

The company indicates that this test indicates the product is not immunogenic.

I have great concerns about this product and agree with the testing house. Patients receiving Bioglue could have hypersensitivity responses to other bovine products, including milk, sometime later after receiving the Bioglue.

Attachment 5 is a peer review pathology study focusing on the skin sites and the kidney. It was very important that they did the detailed review of the kidney sections. One of the concerns in the biological response to immunogenic materials is the formation of immune complex disease. The kidneys are a prime target for the effects of this. The detailed study showed no evidence of immune complex disease. The detailed analysis of the skin sites showed some inflammatory cells.

Attachment 3.

Describes the Buehler test and results which showed no contact sensitivity, the total complement assay which showed no differences, and then the 90 day subcutaneous

study in the rat for implantation toxicity. The gross findings indicate that Bioglue is an irritant. The hematologic and clinical chemistry vary some between the groups but is not consistent. The pathology summary indicates that there is a lot of inflammation at the site of the Bioglue. But they dismiss it as not significant. The tabulated animal data shows a lot of sites where no data was entered or the tissues were unavailable for evaluation.

The pathology summary is suspect since so many data points are missing. However, it is evident that a chronic inflammatory response is mounted against the Bioglue.

The study in the sheep is a little confusing to sort out. It would appear that the inflammatory response to the Bioglue occurs late, about the time that resorption is occurring. This is expected. However, since the resorption occurs so late, the most severe immunologic potential may not appear until a year or more after implantation.

Statistics Review

Question 9

The logistic regression model for binary data was used to compare two treatment groups while adjusting for study site and treatment by site interactions:

$$\text{Log} \left\{ \frac{p}{(1-p)} \right\} = \text{intercept} + \text{trtgrp} + \text{site} + \text{trtgrp} * \text{site}$$

The hypothesis testing was clearly addressed.

This is no treatment by site interaction ($p=0.6804$). Therefore, the data can be pooled across sites. This reviewer is satisfied with the response.

Question 10

A logistic regression analysis was performed to determine if any preoperative factors, had an influence on the primary outcome (anastomotic hemostasis). Prognostic factors are listed on page 30. The sponsor said that site 03 was not in the model is because site 03 did not routinely collect fibrinogen values.

This clinical decision of fibrinogen values should be decided by a physician.

Question 11

A Table... on page 31 depicts the observed adverse events. There were no statistically significant differences between the BioGlue group and the Conventional group except the Neurological depicts ($p=0.0091$). The ratio is 6.5% (BioGlue group) vs. 21.6% (control groups). This reviewer is satisfied with the response.

Question 12

For the purpose of documenting complication related to the surgical procedure, the sponsor combined the data collected at hospital discharge, at 30 days postoperatively, and 3 months postoperatively. This reviewer is satisfied with the response.

Chemistry Reviews

Review of comparative chemistry for BG2000 and BG3000

1. This file has a technical defect to convince the claims made by the CryoLife. The evidences that the company provided have revealed the deficiency. The claimed equivalency of the new product of BG 3000 to the marked product BG 2000 is very questionable. The evidences are:
 - The pH test results (page 5.1 Table 1) [Information redacted]. The ΔpH is the pH change after 72 hr extraction compared with 24 hr extractions. The pH is a sensitive issue in the protein chemistry area, especially for the value of pI (isoelectric point). As known the pI value for serum albumin is 4.9¹ and 5.89 is for tryptophan. N-acetyl-L-tryptophan is a stabilizer added to the BSA solution. The BSA reacts with glutaraldehyde (GLU) to form biopolymers. As extraction time increase from 24 hr to 72 hrs, the pH of the extraction solution from the BioGlue implant adhesive is expected to change, toward more basic. The polymerized product consists of Schiff base. The pH of the extract should be more basic than BSA. In any case, the pH is expected to change, either due to the leaching of the monomer, or the stabilizer. As documented in Table 1, [information redacted]. The unusual phenomena may explain the potential damage of the BioGlue through γ -irradiation. The denaturation of BSA and reduction of the activity of the GLU by light irradiation has been cited in the textbooks. The GLU consists of two aldehyde groups that are very easily oxidized by either oxidants or light irradiation, to become a carboxylic acid. Therefore, this pH experiment revealed the defect for equivalency claim between BG2000 and BG 3000 with γ -irradiation.

- The GC Analysis:
The GC results from Table 2 Page 6 and the GC chromatogram has provided strong evidence that the BG3000 is NOT equivalent to BG2000. The peak intensity difference between BG2000 and BG3000 (without γ -irradiation) is [information redacted] for with γ -irradiation at 72 hr extraction. The explanation of the difference can not based on the assumption it is that due to age difference, without data being provided. As a matter of fact, the three GC chromatograms are not equivalent based on the number of peaks and the peak intensity. When GLU been deactivated by the γ -irradiation, and forms acid, the boiling point will be increased, therefore, less volatile, the peak intensity of the GLU will be 10 to 20 times reduced (Attachment 1). There is no consistency to confirm the conclusion that BG3000 is equivalent to BG2000 from the GC data.
- UV-VIS Analysis:
The UV spectrum (attachment II) has shown the typical tryptophan at 280 nm, which is the N-acetyl-L-tryptophan from both products. That only confirmed the stabilizer was added to both BG2000 and 3000. [Information redacted]. This information revealed that the BG2000 has more conjugated system with GLU and Lysine unit (BSA contains 59 units) than BG3000. The peak intensity of BG2000 is 2 times of the BG3000. If the company added same amount of the stabilizer into BG2000 and 3000, then the huge difference of the peak intensity and the shift of the λ_{\max} can only confirm that BG 3000 is not equivalent to BG2000.
- The HPLC Analysis:
The HPLC analysis data was confirmed again that the BG3000 is different from BG2000. They are not even close to an equivalency claim. I do not think that the HPLC is a good means for this claim. One reason is the complexity of the peaks that have not been able to identify exclusively. Second reason is the retention time that was changing.

2. My Suggestions:

- NMR analysis. The GLU has typical peaks at δ 9-10 if without light irradiation, otherwise the peak would have a very different δ if the GLU has been oxidized.
- IR analysis. The normal GLU has IR peak at 2720 and 1725 cm^{-1} , if after γ -irradiation, then the peak positions are changed drastically.
- CE (Capillary Electrophoresis) analysis. This is a typical tool for analysis of proteins. The BSA forms Schiff base with GLU would have different R_f value for the one with aldehyde group and the one only has carboxyl groups if an oxidation occur during the irradiation. If self polymerized of GLU, then the R_f value should be totally different from either of other peaks. This method is very sensitive and economical.

¹ Donald Voet and Judith G. Voet **Biochemistry**, second ed. John Wiley & Sons, Inc, page 77, 1994.

Review for the change in shelf life due to the change in polymer to monomer absorbance ratio.

The chemical constituents of BioGlue[®] Surgical Adhesive are purified bovine serum albumin (BSA), a protein and glutaraldehyde (GA), a cross-linking agent. When activated by mixing and dispensed to target tissue sites, GA covalently cross-links the surface-proteins of the patient's tissue to the proteins of BSA. Thus, BSA acts as a protein matrix for the cross-linking agent, GA.

In order to thoroughly review the Shelf Life of the device, parts of Analytical and Functional testing have been evaluated.

Conclusions are:

1. Adhesive cure rate (or set time approximately 2 minutes) and adhesive shear strength [information redacted] meets the acceptance criteria.
2. The extent of autopolymerization (the final polymer to monomer ratio normalized against the initial polymer to monomer ratio) of glutaraldehyde also meets the acceptance criteria.
3. In response to question # 3 of the initial scientific review of PMA application # P010003, the company submitted an amendment # 2, a detailed (step-by-step) mathematical approach to show how they obtained the Arrhenius shift factor [information redacted] for autopolymerization of glutaraldehyde at 37 C relative to 25 C, the reference temperature. Hence, a 36 month shelf-life under the specific storage condition (25 C) is justifiable.

**COMMENTS OF DR. HOMBERGER, A PANEL MEMBER FROM ANOTHER PANEL,
REGARDING IMMUNOLOGY:**

The following comments are in response to your request to review information submitted to FDA relating to toxicological testing of CryoLife BioGlue Surgical Adhesive. In particular, you have directed me to review studies performed by the proponent to ascertain the potential for “immunological reactions” that may attend the use of BioGlue in broader clinical applications including cardiac and vascular repair; and you have requested that I address 3 questions related to the information presented in the PMA. Regarding the likelihood that a clinical immunologic response may occur following the use of BioGlue, I believe the data presented are not sufficient to reach a firm conclusion. The data do indicate that the immune systems of test animals respond to BioGlue with formation of specific antibodies which react with glutaraldehyde-polymerized BSA (BioGlue) and BSA (cf. Study #I-00-082 Part II). Since these antibodies are of the IgG isotype, I believe it is justifiable to conclude that there is recruitment of antigen-specific T lymphocytes some of which are likely to persist for extended periods of time as “memory cells” capable of responding to subsequent or persistent exposures to BioGlue, BSA and structurally-related antigens. This finding does not necessarily indicate an increased risk of a clinically significant immunologic reaction. The presence of immunologic “memory” is a prerequisite for having an ongoing or subsequent immunologically-mediated reaction, but further exposure to the eliciting antigen is usually required for such a reaction to occur in vivo. The distinction here is subtle but important. A transitory immune response to BioGlue is not likely to be clinically significant except as it may prime the immune system to respond in a more vigorous way to subsequent antigen exposure. In addition, if there is sufficient antigen available at the surgical site to continuously stimulate an already responsive immune system, there is at least a theoretical risk of developing a clinically significant immune reaction locally or a systemic reaction such as immune complex mediated disease. I do not believe the data presented are sufficient to determine the risk of such an event occurring clinically. In view of the foregoing, it is tempting to advise that further animal studies be performed. I hesitate to make such a recommendation because I believe it would be very difficult to design a study that would yield data relevant to the issue of defining the magnitude of risk in human subjects. Nevertheless, it is prudent and advisable to provide extensive product labeling with specific references to the risks of using this material in patients with known sensitivity to bovine antigens, prior histories of immune-mediated diseases including diseases characterized by vascular inflammation, or generalized atopy. It is also prudent to caution against the repeated use of this material in the same individual since animal studies show that BioGlue does elicit an immune response in animal experiments. Regarding the advisability of performing post-market studies, I would recommend that human patients treated with BioGlue be tested at appropriate times following surgical use of the material for the presence of specific antibodies to BioGlue and BSA, and that in vitro studies of delayed hypersensitivity to BioGlue also be performed. The latter can be performed by measuring lymphocyte blastogenesis to several concentrations of BioGlue in serum-free medium with sensitivity defined by calculating a stimulation index. Please contact me if you have questions about my comments or wish to discuss the recommendations for product labeling or post-market studies.