REVIEW OF STUDY D92-048

Title of the Study: "a Multi-center Randomized Double-blind, Placebo-controlled Group Comparison Study to Investigate the Effect of Aprotinin on Graft Patency in Patients Undergoing Primary Coronary Bypass Surgery (CABG) for Myocardial Revascularization".

Background Information: Numerous clinical trials have demonstrated that aprotinin effectively reduces the postoperative bleeding and the transfusion requirements associated with coronary artery bypass surgery. At same time, a trend toward increased risk of graft closure and MI for the groups treated with aprotinin has been observed in most studies. However, none of these studies was sufficiently powered to detect a statistically significant difference between Trasylol and placebo.

Because of variability among investigators of definition of MI, a blinded prospective analysis of MI based on ECG, SGOT, LDH and CK-MB values was performed in two US studies (D92-008 and D92-016). The incidence rates of MI for the pooled data from the two studies are summarized in the following table:

	Treatment			
	Aprotinin High Dose	Aprotinin Low Dose	Aprotinin Pump Prime	Placebo
% Definite, Probable or Possible MI	41/236(17%)	45/241(19%)	43/237(18%)	38/240(16%)

p-value: High Dose Aprotinin vs Placebo=0.526

On 1-10-1995, the sponsor submitted the results of the Canadian study No.TRA-B05 (Bayer AG Study No. Bay a 0128/0434) which had assessed the efficacy and safety of aprotinin in 122 patients undergoing repeat valve replacement or repeat CABG surgery. The study showed a significantly higher incidence of death, MI, and saphenous vein graft (SVG) closure in the aprotinin-treated group compared to placebo. Six aprotinin-treated patients experienced graft thrombosis versus none in the placebo group and six patients developed MI compared to 3 in the placebo group. Four of the six MI in the aprotinin group occurred with graft thrombosis. Nine deaths occurred in the aprotinin-treated group

compared to two in the placebo group (p-value=0.023 by chi-square test, 0.024 by M-H test, and 0.028 by Fisher's Exact test).

Following the report of study TRA-B05, on March 1, 1995, the sponsor was requested by the Agency to perform a combined safety analysis of death, MI, and graft closure comparing all aprotinin-treated patients, and each aprotinin dose regimen, to placebo-treated patients from all available US and non-US studies. The overall data pool derived from 6 US and 22 non-US studies included a total of 2204 aprotinin-treated patients and 1267 placebo-treated patients.

The analyses of the data from the six US studies showed that in the high-dose aprotinin group, the incidence rate of MI was significantly higher in the aprotinin-treated group compared to placebo (55/519 (11%) versus 34/515 (7%); p-value= 0.022). For the low-dose and for the pump prime only aprotinin groups, the incidence rates of death, MI, and graft closure were not different from placebo. There were no statistically significant differences in the rates of death, MI, and occluded grafts when the combined study population treated with all regimens of aprotinin was compared to the placebo group.

In the non-US Studies, the incidence rates of death or MI for all patients valid for safety analysis randomized to aprotinin (n=830) or to placebo (n=792) were not significantly different from those of the placebo group. A significantly higher rate of graft closure was found in the aprotinin than in the placebo group: 8/830 or 1%, versus 1/792 or 0%, respectively; p-value= 0.023). Six of the eight patients with graft closure were from the Canadian study TRA-B05

The incidence rates of death, MI and graft closure for all patients from the combined US and Non-US studies randomized to high dose aprotinin regimen or placebo are summarized below:

	Treatment		
Adverse Event	Aprotinin High Dose	Placebo	p-value
Death	52/1269 (4%)	44/1267 (3%)	0.410
MI	94/1269 (7%)	74/1267 (6%)	0.113
Occluded Graft	10/1269 (1%)	1/1267 (0%)	0.007*

* p-value <0.05

The statistically significant difference for occluded grafts persisted when all aprotinin-treated patients were compared to placebo patients (13/2004 or 1% versus 1/1307 or 0%; p-value= 0.013). However, 6 of the total patients with occluded graft were from a single non-US study, the Canadian study TRA-B05.

A double-blind, multi-center clinical trial (D92-048) was initiated in January 1993 for the primary purpose of comparing the incidence rates of angiographically assessed graft closure in patients undergoing primary cardiopulmonary bypass surgery for CABG, randomly assigned to high-dose Trasylol or placebo.

Summary of Study D92-48

Thirteen centers participated in the study; ten centers were located in US, two in Israel, and one in Denmark.
A total of 870 patients undergoing primary CABG surgery were randomized: 436 received aprotinin and 434 received placebo. Randomization was stratified on the basis of whether or not the patient had received aspirin within 5 days prior to surgery.

a total of 796 patients were valid for efficacy: 401 aprotinin-treated and 395 placebo-treated.

Efficacy was assessed in terms of number of patients requiring transfusion, number of RBC transfusion required, donor blood and blood product requirement, volume of thoracic drainage, length of stay in ICU, use of other hemostatic drugs, duration of surgery, need for re-operation.

Safety was assessed in terms of rates of SVG closure (by patients and by grafts), general safety results including deaths, MI, dropouts due to adverse events and serious adverse events.

The primary variable for analysis was the percent of patients with one or more occluded SVG distal anastomoses.
Graft patency was also to be analyzed secondarily on a per-graft basis (number of occluded distal anastomoses), accounting for the potential non-independence of grafts within patients.

A second variable was the number of units of donor blood or packed RBC required per patient valid for analysis of efficacy.

Graft patency was assessed by angiography performed prior to discharge but no later than 60 days after surgery. Graft closure was defined as "non patent" anastomosis with or without visible thrombus or patent with ≥ 50% obstruction and visible thrombus.

The study was designed to test for equivalence of incidence of SVG closure in the aprotinin and placebo groups. Equivalence was determined by examining the upper 95% confidence limit on the aprotinin minus placebo difference in percents. The sample size of the study (840 patients) was calculated from the historical expected rate of graft closure per patient of approximately 15% reported in an Aspirin study (Starting aspirin therapy after operation: Effects on early graft patency. S. Goldman et al. Circulation , Vol 84, No. 2, August 1991). It was estimated that 334 patients per group would give 80% power to claim equivalence within 8 percentage points of an assumed 16% placebo rate of graft closure when in fact there was no difference between treatments. A total of 873 patients were enrolled and 744 patients underwent post-operative angiography.

After the enrollment of 748 patients, an interim blinded reading of the angiograms available from 426 study patients was performed for safety assessment. The analysis indicated an overall incidence of graft closure of 15% with ranges from 3% to 32% for the various centers. Since the overall incidence of graft closure was similar to that reported in the literature, the study was allowed to continue to completion.

Prior to unblinding the data, a CI for the percent of patients with at least one occluded graft was constructed, stratifying on center and number of anastomoses. The stratification by center was due to the large variability of results among centers observed from the blinded by-center preliminary analysis. Stratification by anastomoses was chosen as a way of adjusting for correlation within patients that may make the likelihood for at least one closure dependent on the number of anastomoses. Stratified analyses were performed by M-H weighted estimates. Analyses of covariance were used to judge the effect of covariates on graft patency. For selected variables, data were

analyzed by risk for MI or risk for bleeding.

Once all patients had been enrolled in the study, the data for all deaths, angiograms and MI were retrieved from the CRFs separately form all other clinical data and were used for preliminary analyses performed internally by the project statistician. The purpose of these preliminary analyses was to expedite the safety assessment of aprotinin in terms of risk of graft closure and its clinical consequences.

The following variables were examined for the preliminary analyses:

1. Percentage of patients with one or more occluded saphenous vein graft (SVG) distal anastomoses at angiography as read by Dr.Alderman,Stanford Univ.(graft closure rate by-patient,)
2. SVG and IMA graft closure rate. (by-anastomosis)
3. MI rates
4. Mortality rates.

The results of the preliminary analysis of graft closure, MI, and mortality (submitted on 10-26-1995) showed that the incidence rates of graft closure were significantly higher in the aprotinin- treated group than in the placebo group. There was a considerable by center variation with the overall closure rate being affected by one non-US center. No significant differences between the two treatment groups were observed for deaths and MI. At the time of the preliminary analysis, additional exploratory analyses were planned in order to assess the potential role of other factors in causing the increased rate of graft closure in the aprotinin group.

On 8-2-1996, the sponsor submitted the final study report for study D92-048, including the analysis of the rates of SVG occlusion (primary study variable), the efficacy results (secondary study variable), post-hoc regression covariate analyses and additional analyses requested by the Agency.

Summary of Analysis of Efficacy:

The two treatment groups were comparable for demographic and baseline characteristics. Treatment groups were different only

with respect to volume of cardioplegia use (aprotinin 1860 mL, placebo 1721 mL, p=0.004).

The results of the primary efficacy analysis, donor blood requirement through day 12 post-operatively, in the patient population valid for efficacy are summarized in the following table. The requirement for donor blood transfusion was also analyzed in the study population stratified for bleeding risk.

Donor Blood Requirement through day 12

Variable	Aprotinin (N=401)	Placebo (N=395)
% Requiring Blood	38%*	54%
Median (range) units transfused	0(0.7)*	1
High risk for bleeding stratum		
% Requiring Blood	40%*	56%
Median (range) units transfused	0(0.5)*	1(0-11)
Milliliters required	0	350
Low risk of bleeding stratum		
% Requiring Blood	35%*	52%*
Median (range) Units transfused	0(0.7)*	1(0-12)
Milliliters required	0	350
% Requiring Blood Products	8%*	25%*
Median (range) Units blood product	0	0
% Requiring Blood or Blood Product	40%*	58%*
Median (range) blood or blood product	0	2

*P-value <0.05, compared to placebo

The mean thoracic drainage rate in the placebo group was larger that the mean rate in the aprotinin group (P-value <0.05). There was no significant difference between the treatment groups in the days spent in the ICU, in the total hospital stay, in duration of surgery, and in requirement for re-operation.

Hemostatic agents were used as shown in the following table:

Hemostatic Agents	Aprotinin	Placebo
Antihemorrhagics	41/434 (9%)*	63/433 (15%)
Pituitary Hormones (DDAVP)	10/436 (2%)*	40/433 (9%)
Other Therapeutic Products	51/417 (12%)*	96/414 (23%)

*P-value <0.05

All patients had concomitant use of non-hemostatic agents. The treatment groups were similar with respect to heparin and protamine use. All patients were given ASA after surgery.

Summary of Analysis of Safety

Graft Closure: The results of the analysis of the angiographic data showed that the incidence rates of graft closure were significantly higher in the aprotinin-treated group than in the placebo group.

The overall incidence rates of graft closure, analyzed by-patient, by-graft, and by type of graft for the two treatment groups are summarized in the following table:

	Estimates			
Variable	Aprot	Placebo	Diff	90% CI for diff*
Graft Closure:				
% By-Patients (SVG)	15.4	10.9	5.50	1.30, 9.6
% BY-Anastomoses (SVG)	7.6	4.7	2.93	0.96, 4.9
% By-Patients (IMA)	2.0	1.0	1.00	-0.65, 2.6

*The statistical section of the report states 95% CI.

The proportion of patients with graft closure in the aprotinin group was 15.4% compared to 10.9% in the placebo group. The upper 90% CI for aprotinin minus placebo difference in closure rates was 9.6% which is greater than 50% of the placebo rate. Therefore, equivalence to within 50% of neither the expected (16%) nor the observed (10.9%) placebo rates could be claimed.

A considerable by-center variation in incidence rates of SVG closure was observed (table 1). Of all the centers with enrollment of more than 10 patients, center #2 had the highest

incidence rates of graft closure for both treatment groups and center #3 reported the widest difference in rates of graft closure between treatments.

Among the US centers, which provided 54% of all patients with angiograms, the observed incidence rates of graft closure in the aprotinin and in the placebo groups were 9.4% and 9.5% respectively.

Table 1: By-patient and by-anastomosis incidence rates of graft closure: All patients with assessable saphenous vein graft.

		APROTININ				PLACEBO			
Cntr	No. Of Patient per group	# Pts with closed graft	% Pts with closed graft	# of anasto moses	% of anasto moses closed	# Pts with closed graft	% Pts with closed graft	# of anas tomo ses	% of anasto moses closed
# 1	52/54	7/52	13.5	116	6.9	5/54	9.3%	114	4.4
# 2	71/67	19/71	26.8	172	14.0	13/67	19.4%	158	8.9
# 3	38/40	11/38	28.9	93	14.0	2/40	5.0%	92	2.2
# 6	26/23	4/26	15.4	62	6.5	3/23	13.0%	61	4.9
# 7	45/39	1/45	2.2	102	1.0	1/39	2.6%	90	1.1
# 8	45/40	8/45	17.8	101	9.9	7/40	17.5%	96	8.3
# 9	36/29	1/36	2.8	119	1.7	0/29	0.0%	96	0.0
#10	20/16	2/20	10.0	54	3.7	0/16	0.0%	48	0.0
#11	2/ 3	0/2	0.0	5	0.0	1/ 3	33.3%	7	14.3
#12	19/18	1/19	5.3	48	2.1	2/18	11.1%	43	4.7
#13	0/ 1					0/ 1	0.0%	3	0.0
#14	7/ 9	1/7	14.3	19	10.5	3/ 9	33.3%	26	15.4
#15	2/ 1	½	50.0	7	0.0	0/ 1	0.0%	3	0.0
Tot. M-H*	**363/340**	**56/363**	**15.4 16.0**	**898**	**7.6 7.6**	**37/340**	**10.9% 10.6%**	**837**	**4.8 4.7**

*Mantel-Haenszel weighting of ratio estimators, with stratification by center

The observed and the predicted graft closure rates for all sites and for the US sites are summarized in the following table:

	Observed (non-stratified) Closure Rates		Predicted (stratum-adjusted) Closure Rates		90% CI for difference
	Aprotinin	Placebo	Aprotinin	Placebo	
All Centers	15.4%	10.9%	16.0%	10.6%	1.3%,9.6%
US Centers	9.4%	9.5%	9.8%	8.8%	-3.8%,5.9%

Similar results were obtained for by-grafts analyses. The Odd Ratio for non-US sites was 2.10 for aprotinin versus placebo, compared to 1.499 for all 12 sites combined and 0.983 for the US sites only.

The reasons for the difference in graft closure rates between the US and non-US centers were evaluated and the following possible causes were uncovered. The high rate of graft closure in centers 2 and 3 could have been caused by faulty use of the Hepcon instrument to monitor heparin during the early part of the study. In center 3, during the early part of the study, the vein graft was kept in blood containing aprotinin. Indeed, the analysis of graft failure by quartile shows that the incidence of graft closure was higher in the first quarter of patients enrolled at the respective centers, particularly at center 3. Difference in patient populations among centers may also account for different rates of graft closure, as suggested by center 2 where the incidence of graft closure was highest in both aprotinin and placebo groups.

Approximately 75% of all patients had only a single anastomosis per each SVG. For this subset, 18% of aprotinin and 11% of placebo patients had at least one graft closed.

Exploratory analyses were performed to determine what covariates were related to graft closure. The analyses showed a number of factors that may have contributed to the increased rate of graft closure in the aprotinin group, including female gender, small size of graft and of the native coronary, poor VEF.

General Safety Results

Perioperative MI: No significant differences were noted between the two treatment groups for the rates of perioperative MI, as shown below and in table 2:

Variable	Aprotinin	Placebo
% Definite MI	2.9	3.8
% Definite or Probable	8.6	9.1
% Definite, Probable, or Possible MI	12.3	12.0
% No MI	87.7	88.0

Table 2: Incidence rates of MI (definite, probable, or possible) by treatment and center population: all patients.

	APROTININ			PLACEBO		
Cntr	No.of Patients	Pts with events	% of Pts with event	No. of Patients	Pts with events	% of Pts with event
# 1	57	5	8.8	61	5	8.2
# 2	79	18	22.8	80	14	17.5
# 3	50	5	10.0	50	0	0.0
# 6	31	2	6.5	32	8	25.0
# 7	43	1	2.3	46	2	4.3
# 8	47	9	19.1	47	10	21.3
# 9	33	0	0.0	35	2	5.7
#10	23	2	8.7	25	4	16.0
#11	5	0	0.0	4	0	0.0
#12	22	4	18.2	20	2	5.0
#13	1	1	0.0	1	0	0.0
#14	10	7	30.0	10	2	20.0
#15	7	6	14.3	7	2	28.6
Tot	408	50	12.3	418	50	12.0

Notably, a cross-classification of MI with by-patient SVG patency data showed that more aprotinin than placebo patients were unassessable for MI and that more placebo that aprotinin patients did not have angiography performed. The cause of this imbalance is unclear.

Cross-classification using data only from patients assessable for SVG patency and for MI showed that while there was no treatment by patency subgroup interaction and no overall effect of treatment on perioperative MI rates, there was a significant effect of patency with more MI in patients with closed grafts than with patent grafts. The data are summarized below:

	Percent with Definite MI	
Subgroup	Aprotinin	Placebo
All SVG patent	2.4	2.0
> 1 SVG closed	5.6	8.3

Deaths: a total of 13 patients died. There was no difference in death rates between aprotinin and placebo groups. The incidence of deaths by treatment and by center are summarized in table 3.

Table 3: Incidence rates of death by treatment and by center. All patients.

	APROTININ			PLACEBO		
Cntr	No.of patients	Pts with events	% of ps with event	No. of patients	Pts with events	% of pts with event
# 1	62	2	3.2	64	0	0.0
# 2	84	2	2.4	84	1	1.2
# 6	32	0	0.0	33	1	3.0
# 8	48	0	0.0	47	1	2.1
# 9	37	0	0.0	35	1	2.9
#10	28	0	0.0	28	2	7.1
#12	23	1	4.3	20	0	0.0
#14	11	1	9.1	11	0	0.0
#15	7	0	0.0	7	1	14.3
Tot	436	6	1.4	434	7	1.6

Other Adverse Events: There were no significant differences for the rates of drug-related adverse events between treatment groups for each body system.

Serious adverse events occurred in 18% of aprotinin patients, and in 17% of placebo patients. Study drug was discontinued because of adverse event in 5 aprotinin and no placebo patients.
The only individual adverse events with a significant difference between groups were hemorrhage (aprotinin 1%, placebo 5%) and cardiovascular disorders (aprotinin 1%, placebo 3%).

Laboratory Parameters: There were no differences between treatment groups for laboratory parameters including LFTs and serum creatinine levels.

CONCLUSIONS

The results of Study D92-048 show an overall greater incidence of graft closure in the aprotinin group compared to the placebo group, as documented by the most reliable method of assessment (angiography) in a large study population.
A considerable by-center variation in graft closure was noted with marked reduction of the difference between the two treatment groups when only the US centers were analyzed. The incidence rates of MI and death were not significantly different in the two treatment groups.

Concomitant factors, such as inadequate heparin anticoagulation, storage of the saphenous vein grafts in aprotinin containing blood, or individual patients' risk factors, may have contributed to the development of graft closure in the aprotinin-treated group and could account for center differences.

In study D92-048, the high-dose aprotinin regimen was used and the patient population consisted of patients undergoing primary CABG. Whether the risk of graft closure is greater for patient undergoing repeat CABG or is reduced by the use of the low dose regimen are unresolved issues at present.

REVIEW OF STUDY D92-016

Title of the Study: A multicenter, randomized, double-blind, placebo-controlled, group comparison study to investigate the efficacy and safety of aprotinin in reducing blood loss and transfusion requirement in patients undergoing primary cardiopulmonary bypass surgery for myocardial infarction (CABG).

The study was performed between October 22, 1992 - June 30, 1994

Study Objectives: To compare the efficacy and safety of aprotinin, given in three different dose regimens with that of placebo in reducing the need for donor blood transfusion in patients undergoing primary open heart surgery via a median sternotomy for myocardial revascularization.

Study Design: The study was a randomized, stratified, double-blind, placebo-controlled, parallel groups Phase III clinical trial performed at 21 centers in U.S.. A total of 704 patients were randomized and valid for analysis of safety (603 male and 101 female). Of these 704 patients, 173, 180, 173, and 178 were enrolled in the high dose (HD), low dose (LD), pump prime only (PPO), and placebo (PLA) groups, respectively.

The type and number of CABG placed was dictated by the need of the patient. Anesthesia, conduct of cardiopulmonary bypass and postoperative intensive care procedures were those routinely practiced at each study center, effort was made to adhere to the same procedures and regimens for all patients.
The heparin loading dose was at least 350 IU/kg. The amount of aditional heparin was determined using Heparin Protamine Titration Test performed with Hepcon system.

Post-CPB, blood was transfused if clinically indicated or if the Hct was below 18%. Postoperatively, blood was transfused when clinically indicated or when the Hct was less than 21%.

Selection of Subjects: Any patients who had a primary diagnosis of coronary artery disease and met the inclusion and exclusion criteria were eligible for enrollment.
Male or female patients, aged over 18, who required primary open heart surgery for isolated myocardial revascularization through a

median sternotomy were eligible for the study.
Patients with known or suspected allergy to aprotinin, with history of any bleeding diathesis or known hematologic abnormality that would have required prophylactic use of hemostatic drugs (DDAVP, epsilon aminocaproic acid), or erythropoietin, patients who refuse to receive donor blood products for religious or other reasons, who had pre-donated their own autologous blood prior to hospitalization, for administration in association with this surgery; whose pre-operative blood volume was so low that homologous (donor) blood would have to be included in the prime fluid for the CPB circuit to rnaintain an adequate Hct during CPB, irrespective of the amount of blood lost during the procedure, patients with a previous sternotomy, and patients treated with any other investigationai drug within the preceding 30 days, were excluded from the study.

Patient Assignment: Patients were randomly assigned, by center, to one of four treatment groups (high-dose-, low-dose-, pump prime only-aprotinin, placebo). The randomization was stratified according to the prceived risk for peri-operative MI or bleeding. Patients at high risk for MI were patients with MI within 30 days, thrombolytic therapy or PTCA within 30 days, those with unstable angina. Patients at high risk of bleeding were patients who received ASA or NSAID within 5 days before surgery, patients with prolonged bleeding time and patients with history of bleeding or any coagulopathy in the past.

Criteria for Evaluation of Efficacy: The primary criterion for efficacy in this study was the reduction in donor blood transfusion requirement up to and through postoperative day 12. This was assessed primarily in terms of the number of units of donor blood or packed red blood cells required per patient, and secondarily in terms of the percent of patients requiring any donor transfusions.

Other efficacy criteria to be evaluated included:
1. The number of units of donor blood or packed RBC required by patients requiring any transfusion.
2. The thoracic drainage rate in mL/hr from the operative site in the first 6 hours postop. and the total thoracic drainage volume postop. until removal of the thoracic drains.

3. The incidence of reoperation for diffuse bleeding.
4. The number of donor units of platelets, fresh-frozen plasma, and cryoprecipitate administered per patient up to and through postop. day 12.
5. The number of units of donor blood, platelets, fresh frozen plasma and cryoprecipitate administered per patient during the entire hospitalization.

Criteria for Evaluation of Safety: The following safety assessments were recorded on the patient's CRFs:

- Upon ICU arrival: complete CBC, BUN, creatinine, glucose, electrolytes; LFTs, PT, PTT, CK with MB fraction.
- Six (6), 12, 18 and 24 hours after ICU arrival and on post-op days 3, 5 and 7: CK with MB fraction
- On the day after surgery: CBC, BUN, creatinine, glucose, electrolytes, LFTs, PT, PTT, and phys.exam..
- On post-op days 3, 5 and 7, and just prior to discharge: 12-lead ECG.
- On post-op days 3 and 5: SGOT, LDH, BUN and creatinine.
- About Day 7 postop. CBC, chest x-ray, phys. exam, BUN, creatinine, glucose, electrolytes, serum protein, LFTs.

For all laboratory parameters, the variable analyzed was the change from the preoperative baseline value.

Any intra- or post-operative complication, including worsening of prior condition, occurring during or subsequent to the administration of study medication was considered to be a treatment-emergent event, regardless of whether or not it was considered to be related to the administration of study drug. All treatment emergent events were graded as mild, moderate or severe. For all treatment-emergent events, the relationship to study drug was determined by the investigators.

All ECG and SGOT, LDH, CK with MB values were evaluated blindly

to assess the incidence of peri-operative myocardial infarction. Myocardial infarction (MI) was defined by the appearance of diagnostic changes on the ECG as defined by the Minnesota code for definite or probable MI, and/or the occurrence of diagnostic elevations in CK-MB isoenzyme activity in the post-operative period.

Specifically, patients were deemed to have had a peri-operative Ml if a new 2 step Q wave change in the Minnesota code as compared to the preoperative ECG was present, or if the CK-MB was ≥ 120 U/l at 6, 12, and 18 hours post-operatively.
If a second operation was necessary, the same classification algorithm was used for the occurrence of a peri-operative Ml.
The CEL was provided with documentation of important clinical events associated with the surgery that enhanced the MI classification (such as death with autopsy findings of a new Ml).
Quantification of the area under the CK-MB curve was performed when possible, dependent upon site compliance with enzyme requirements.
In the event that a patient had a suspected Ml event >24 hours from the time of surgery, a clinical narrative was submitted to the CEL with the ECG and enzyme data.

Approximately 4-6 weeks after discharge, patients who were able to return to the study center for follow-up had serum specimens for testing of specitic antibodies to aprotinin. They also had a serum creatinine and SGPT drawn, and underwent an angina questionnaire.

Concomitant Therapy: All perioperative medication was recorded in CRFs. Other drugs thought to improve hemostasis, such as desmopressin, epsilon aminocaproic acid, etc, were not given prophylactically, and were given therapeutically only if mandated by the patient's clinical conditions.

It was emphasized to investigators that although aprotinin is known to prolong the ACT, it should not be viewed as a heparin-sparing agent. Prior to cardiopulmonary bypass, a standard loading dose of heparin (e.g,300 1U/kg) was to be employed, and during CPB additional heparin was to be administered on the basis of heparin levels measured by a method (such as protamine titration) that was not affected by the presence of aprotinin. After discontinuation of CPB, the dose of protamine was based upon the amount of heparin that had been administered. Increased ACT values at the end of the extracorporeal circulation (ECC) (e.g, over 800 seconds) was not to lead to a higher dose of protamine.

Statistical Procedures: The protocol specified that the primary efficacy variable was the number of units of donor blood or packed red blood cells required per patient through post-operative Day 12. This was further qualified prior to study completion, to be consistent with the analyses of previous studies, to exclude from consideration any blood received pre-operatively or in the CPB prime; only intent-to-treat analyses were to include blood received in these time periods. The percent of patients requiring donor blood transfusions was to be a secondary efficacy variable.
For those patients valid for analysis of efficacy, analysis of every variable was to be performed. Intent-to-treat analyses were to be performed on all patients valid for safety; however, the protocol did not state which variables were to be analyzed for the intent-to-treat population.

In all cases the primary comparison of intererest was to be that of HD aprotinin to PLA; no adjustments for multiple comparisons was considered necessary. All tests for treatment effect were to be two-tailed and performed at an alpha level of 0.05. Analysis of variance model were used for continuous variables. Categorical variables were to be analyzed with chi-square, Fisher's exact and Mantel-Haenszel tests as appropnate. Stratification was not to be included as a factor in the models used for analysis; however, by-stratum results were to be tabulated. No interim analyses were performed.

Sample size estimation for this study was based on an expected average requirement of 2.1 units of blood in the placebo group and on the clinically meaningful reduction of 1 unit of transfused blood in the HD group. A total of 160 valid patients per treatment group would provide greater than 90% power to reject the hypothesis of no treatmen difference, when in fact a clinically meaningful difference existed. The calculations assumed two-sided testing at an alpha of 0.05, with a 15% incraase in sample size to account for the multi-center nature of the study. Based on the actual sample sizes and standard deviations observed in this study, the study was adequately powered.

Numerous tests of significance were planned and carried out for the safety analyses. P-values from the analyses were used mainly

as flags to indicate possible safety issues; adjustments for the multiplicity of tests done were not made.

The primary analysis was to be carried out on patients valid for analysis of efficacy. The protocol stated the following reasons for which patients would be considered invalid for analysis of efficacy:

- treatment discontinued due to an adverse event
- death within 6 hours post-operatively
- re-exploration due to excessive bleeding of surgical origin
- requirement for intra-aortic balloon counter pulsation or left ventricular assist device, with continued heparinization
- development of major bleeding from a site unrelated to the surgical procedure
- inclusion of donor blood or blood product in the prime volume of the CPB circuit.

Modifications of Statistical Plan: Some modifications were made to the plans outlined in the protocol. It was decided prior to unblinding the study, that analysis of variance results would be based on the main effects model, with treatment by center interactions dealt with secondarily. The analyses were carried out induding all centers with at least one (the protocol specified two) observations per treatment group.
Analyses of change from baseline in laboratory variables were performed using analysis of covariance, with baseline as the continuous covariate. This was done because the effect was highly significant in nearly every analysis, and because of the substantial reduction in standard error it afforded for some of the analyses.
For selected variables, data were analyzed by strata (risk for MI and risk for bleeding).
Validity was assessed prior to unblinding the study. Some reasons for invalidating patients were not included in the protocol. These additional reasons dealt mainly with violations of the exclusion criteria: unblinding of the random code, use of another investigational drug, predonation of blood, surgery cancelled, patient withdrawn from study by the investigator, additional procedures that could affect blood loss, pre-operative blood transfusion, inadequate heparin reversal and GI bleed.

RESULTS OF THE STUDY

There were 704 patients randomized and valid for analysis of safety; 173 HD, 180 LD, 173 PPO and 178 PLA. The number of patients at 21 centers ; one center enrolled only 2 patients, 7 centers enrolled more that 10 patients/group. All patients received the test dose. Patients for whom drug was discontinued are listed in the following table.

Reasons for Discontinuations
By Treatment

Treatment	Patient	Amount Received (ml)	Reason Discontinued
High Dose	17106	Load: 200 Pump: 0 Cont. Inf: 130	Surgery cancelled; unable to cannulate
	21107	Test dose only	Patient withdrawn from study by investigator
	21121	Test dose only	Adverse event (hypotensive crisis)
Low Dose	19120	Test dose only	Surgery cancelled
Pump Prime	2208	Load: 200 Pump: 200 Cont. Inf: 178	Adverse Event (ST elevation)
	4203	Load: 200 Pump: 200 Cont. Inf: 10	Adverse Event (hypertension, tachycardia)
	6206	Test dose only	Protocol Violation (received blood pre-op)
	9115	Test dose only	Protocol Violation (IABP inserted pre-op, prophylactically)
	10202	Load: 200 Pump: 200 Cont. Inf: 200	Adverse Event (myocardial infarction)
Placebo	9209	Test dose only	Adverse Event (rash)
	9217	Load: 200 Pump: 0 Cont Inf: 12	Adverse Event (ventricular tachycardia)
	18220	Load: 200 Pump: 200 Cont. Inf: 215	Adverse Event (excessive intra-op bleeding)

Prior to unblinding the study, patients with protocol violations that could influence the interpretation of study results (additional surgical procedures, blood received pre-operatively or in the pump pnme, blood donated prior to admission and cancellation of surgery) were removed form analysis of efficacy.

Demographic and Baseline Factors: The patients demographic, baseline characteristics and stratification, are summarized in the following table. Treatment groups were similar with respect to all of these variables, except for LVEF (more severe in the LD group, overall p-value = 0.013). Results for patients valid for analysis of safety were similar.

The PLA group had a marginally significantly higher baseline platelet count than did the PPO group, baseline PT was significantly higher in the PLA group than in each of the three active dose groups (12.4" vs 12.1 - 12.2").
All patients took at least one medication in the 14 days prior to starting study medication.

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Demographic and Baseline Summary
Patients Valid for Efficacy

Variable	High Dose (n=160)	Low Dose (n=168)	Pump Prime (n=159)	Placebo (n=157)
Percent				
High risk for MI	52	58	53	57
High risk for bleeding	76	78	79	75
Male	85	87	87	85
Caucasian	87	90	90	94
NYHA ≥ III	59	58	54	57
LVEF ≥ 30%	97	96	99	98
Mean				
Age (yr)	61	61	62	62
Weight (kg)	85	84	87	86
Height (cm)	174	175	175	175
# of grafts	3	3	3	3

EFFICACY RESULTS

Number of Subjects, Actual Dosage and Duration of Treatment
Some patients had treatment prematurely discontinued. All but one of these patients were invalid for analysis of efficacy.

Treatment emergent use of hemostatic agents was 45% (77/173) in the HD group, 46% (82/180) in the LD group, 39% (68/173) in the PPO group, as compared to 58% (104/178) in the PLA group.

Analysis of Efficacy: The results of the primary efficacy analysis are summarized in the following table. The PLA group required significantly more units of donor blood than any of the three active dose groups.

Donor Blood Requirement Through Day 12
Patients Valid for Efficacy

Variable	High Dose (n=160)	LowDose (n=168)	Pump Prime (n=159)	Placebo (n=157)
% Requiring Blood	33% *	35%*	33%*	52%*
Median (Range)				
Units Required	0.0	0.0	0.0	1.0
mL Required	0	0	0	250

*p-value < 0.05, compared to placebo

The analysis of units of donor blood required indicated a significant treatment by center interaction. No baseline or demographic variables were found on exploratory analyses to explain the interaction.
At four centers (1, 8, 10, 19) the PLA group had a better response than both the HD and LD groups with respect to mean units of donor blood required and percent of patients requiring donor blood. These centers were examined together, versus all other centers pooled.

Percent of Patients Requiring Donor Blood
Examination of Treatment by Center Interaction

Subgroup	High Dose	Low Dose	Pump Prime	Placebo
Centers 1,8,10,19	12/35(34%)	13/38(34%)	4/41(10%)	10/37(27%)
All Others	41/125(33%)	46/130(35%)	48/118(41%)	72/119(60%)

The percents of patients requiring donor blood in the HD and LD group were relatively constant across the subgroups; however, the PPO and PLA groups varied greatly.
The significant treatment by center interaction may be due to a better than average placebo response as opposed to a lack of effect in the higher dose groups.

Donor blood requirement through Day 12 for patients at high risk for bleeding was similar to those described for all efficacy patients. Less than 25% of patients were considered at low risk for bleeding. In this subset of patients results were not consistently in favor of active drug over placebo, as seen in the next table.

Donor Blood Requirement Through Day 12
Efficacy Patients at Low Risk for Bleeding

Variable	High Dose (n=38)	Low Dose (n=37)	Pump Prime (n=34)	Placebo (n=39)
%Requiring Blood	24	27	29	44
Median (Range)				
Units Required	0	0(04)*	0	0
mL Required	0	0	0	0

* P-va!ue < 0.05, compared to placebo

Because of the small sample sizes in this subset of patients it is not possible to statistically evaluate the presence of treatment by center interactions. It is of note, however, that one center (14) accounted for over 25% of the patients in this subgroup.

Percent of Low Bleeding Risk Patients Requiring Donor Blood
Examination of Treatment by Center Interaction

Subgroup	High Dose	LowDose	Pump Prime	Placebo
Center 14	2/11 (18%)	1/9 (11%)	4/9 (44%)	7/11 (64%)
All others	7/27 (26%)	9/28(32%)	6/25(24%)	10/28 (36%)

The data on donor blood requirement through Day 12 by various characteristics, including those baseline and demographic parameters for which treatment group imbalance was noted are summarked in the following table. The data suggest that the baseline imbalances for LVEF, platelet counts and PT do not influence the results.

Percent of Patients Requiring Donor Blood by Various Characteristics

		High Dose	Low Dose	Pump Prime	Placebo
Sex:	Male	38/136 (28%)	44/146 (30%)	37/139 (27%)	64/133 (48%)
	Female	15/24 (63%)	15/22 (68%)	15/20 (75%)	18/24 (75%)
Race:	Caucasian	46/139 (33%)	53/152 (35%)	44/143 (31%)	74/147 (50%)
	Non-Caucas.	7/21 (33%)	6/16 (38%)	8/16 (50%)	8/10 (80%)
Age (yr):	≤ 60	22/91 (24%)	18/90 (20%)	20/82 (24%)	32/80 (40%)
	> 60	31/69 (45%)	41/78 (53%)	32/77 (42%)	50/77 (65%)
LVEF:	Fair	16/40 (40%)	24/59 (41%)	11/45 (24%)	17/33 (52%)
Platelet Count ($x10^6/mm^3$)	≤ 231	28/79 (35%)	27/86 (31%)	27/83 (33%)	32/74 (43%)
	> 231	25/79 (32%)	31/81 (38%)	25/75 (33%)	49/80 (61%)
Prothrombin Time (sec):	≤ 12	22/83 (27%)	32/86 (37%)	26/81 (32%)	35/61 (57%)
	>12	29/73 (40%)	27/73 (37%)	25/74 (34%)	42/87 (48%)

Median Units (Milliliters) of Donor Blood Required By Various Characteristics

		High Dose	Low Dose	Pump Prime	Placebo
Sex:	Male	0 (0)	0 (0)	0 (0)	0 (0)
	Female	2 (500)	1.5 (425)	2.0 (500)	2.0 (700)
Race:	Caucasian	0 (0)	0 (0)	0 (0)	1.0 (250)
	Non-Caucas.	0 (0)	0 (0)	0.5 (155)	2.0 (675)
Age (yr):	≤ 60	0 (0)	0 (0)	0 (0)	0 (0)
	> 60	0 (0)	1.0 (290)	0 (0)	2.0 (500)
LVEF:	Fair	0 (0)	0 (0)	0 (0)	1.0 (250)
Platelet Count ($x10^6/mm^3$)	≤ 231	0 (0)	0 (0)	0 (0)	0.0 (0)
	> 231	0 (0)	0 (0)	0 (0)	1.5 (452)
Prothrombin Time (sec):	≤ 12	0 (0)	0 (0)	0 (0)	1.0 (410)
	>12	0 (0)	0 (0)	0 (0)	0 (0)

The subgroup results for the high risk group were in agreemwnt with those seen for the population as a whole. Because of the small sample, inferences based on the by-subgroup tabulations for the low risk patients may not be valid..

For those efficacy patients who required donor blood through day 12, a median of 2 units was required for each dose group. Also, 64% (34 of 53) of the HD patients, 59% (35 of 59) of LD patients and 59% (31 of 52) of PPO patients compared to 51% (42 of 82) of PLA patients required at most 2 units of donor blood.

In an intent-to-treat analysis carried out on all patients valid for analysis of safety, the results were similar to those of the efficacy population.

Donor blood requirement through the end of hospitalization was analyzed for both the efficacy and safety populations. All three active treatment groups proved to be superior to placebo.

Significantly fewer HD, LD and PPO patients required donor blood product, whether measured through Day 12 or the end of hospitalization. Placebo patients used significantly more units of blood or blood praduct through Day 12 than any active treatment group. The foilowing table summarizes these results.

Donor Blood or Blood Product Requirement Through Day 12
Patients Valid for Efficacy

Variable	High Dose (n=160)	LowDose n=168)	Pump Prime (n=159)	Placebo (n=157)
% Requiring:				
Blood Product	9*	10*	9*	25
Blood/Blood Product	34*	37*	35*	55
Median (Range) Units				
Blood Product	0	0	0	0
Blood/Blood Product	0	0	0	0

* P-value < 0.05, compared to placebo

The thoracic drainage rate in mL/hr are summarized in the following table.

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		Thoracic Drainage			
		High Dose	Low Dose	Pump Prime	Placebo
Thoracic Drainage Rate 0-6 hours Post Surgery (ml/hr)	N	159	168	158	157
	Mean	47.3*	45.4*	53.0*	103.8
	SE	4.5	4.6	4.7	4.7
Thoracic Drainage Volume: 6 Hrs Post Surgery (ml)	N	159	168	158	154
	Mean	285.9*	273.7*	318.9*	587.1
	SE	24.3	24.7	25.3	25.3
Thoracic Drainage Volume: Total Post Surgery (ml)	N	157	168	156	152
	Mean	786.1*	811.0*	898.8*	1285.8
	SE	50.2	50.8	52.4	52.4

* P-value < 0.05, compared to placebo

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All active treatment groups were superior to placebo for all three variables. There were significant treatment by center interactions in the analyses of total and 6-hour thoracic drainage. These interactions were inconsequential; mean volumes in placebo patients were greater than in the active dose groups in all centers.

Results of the analyses of thoracic drainage for those patients at high rissk for bleeding were simiiar to those seen for the population as a whole. For patients at low risk for bleeding, the PPO group was not significantly different from PLA with respect to total thoracic drainage; all other comparisons to PLA were significant.

Thoracic drainage rate was tabulated by various baseline/demographic characteristics. Treatment effects were consistent across all subgroups. These tabulations were repeated for subsets of patients at high and low risk for bleeding; the PLA group had a consistently higher thoracic drainage rate then the active treatment groups.