History of Aprotinin

A Naturally Occurring Proteolytic Enzyme Inhibitor

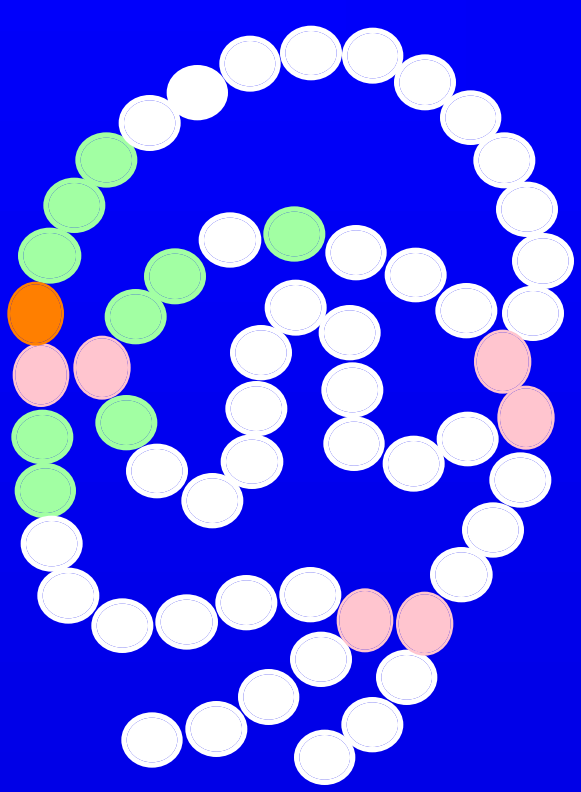
- Discovered independently in the 1930s
  - Kraut et al isolated a kallikrein inhibitor from bovine lung
  - Kunitz and Northrop described a bovine pancreatic trypsin inhibitor
- Launched as Trasylol® in Germany in 1959

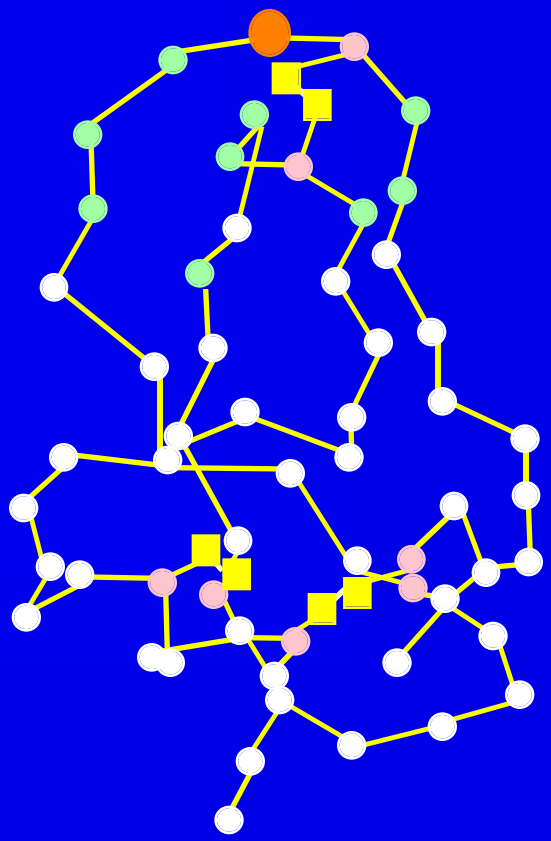
Aprotinin

A Naturally Occurring Serine Protease Inhibitor

- Consists of 58 amino acid residues
- Single-chain polypeptide: 6512 daltons
- Cross-linked by 3 disulfide bridges
- Reactive bond site is lysine-15-alanine-16
- Forms reversible stoichiometric complexes
- Reacts with serine site of enzyme

Structure of Aprotinin





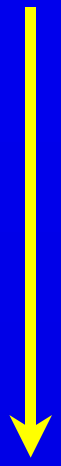
Aprotinin

A Serine Protease Inhibitor

Binds with the human serine proteases:

- Trypsin
- Plasmin
- Plasma kallikrein
- Tissue kallikrein
- Elastase
- Urokinase
- Thrombin

decreasing affinity



Serine Protease Enzyme Systems

The Potential Inhibitory Role of Aprotinin

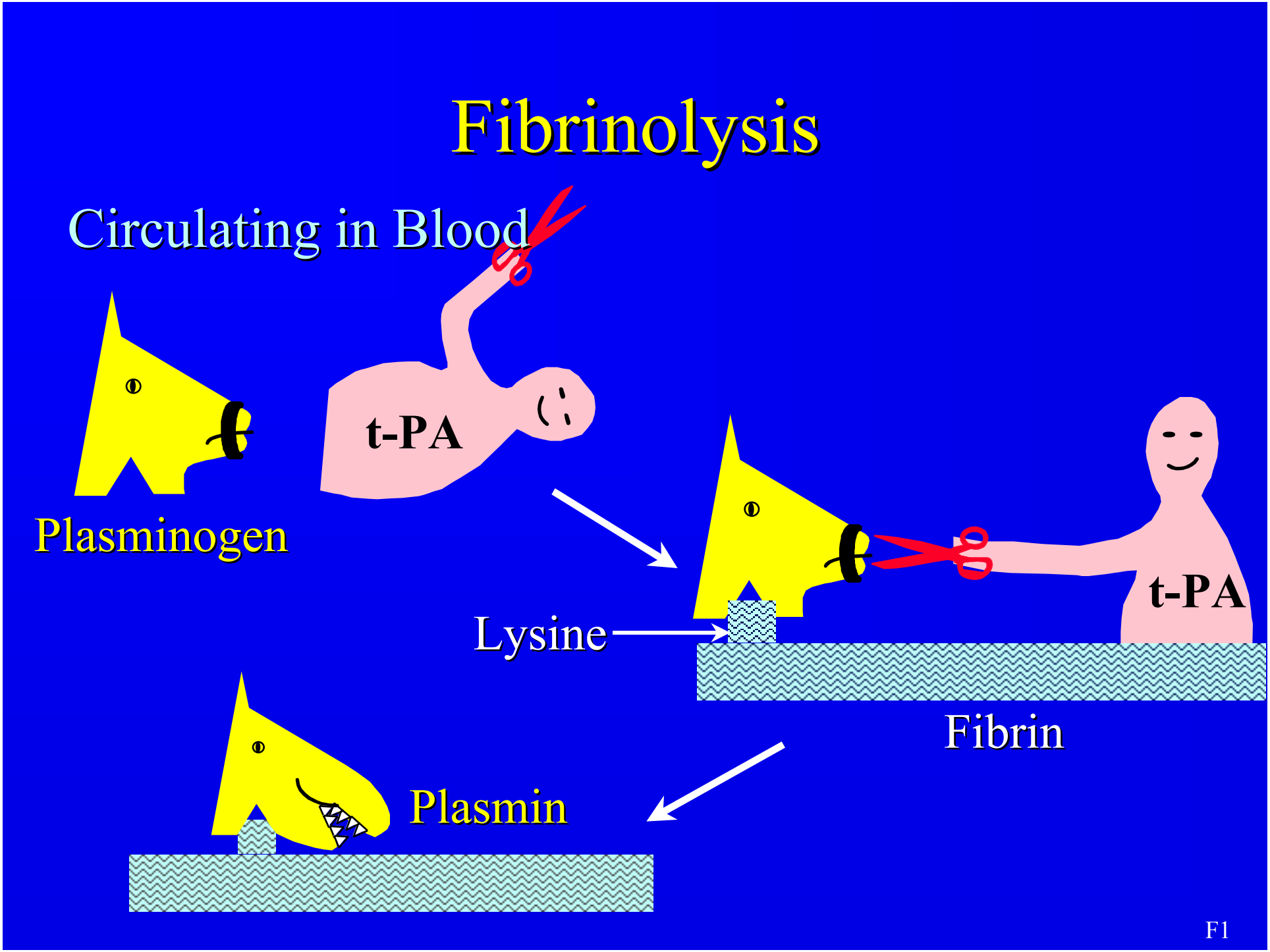
- Kallikrein-kininogen-kinin
- Complement
- Coagulation
- Fibrinolysis
- Renin-angiotensin
- Leukocyte elastase

Aprotinin

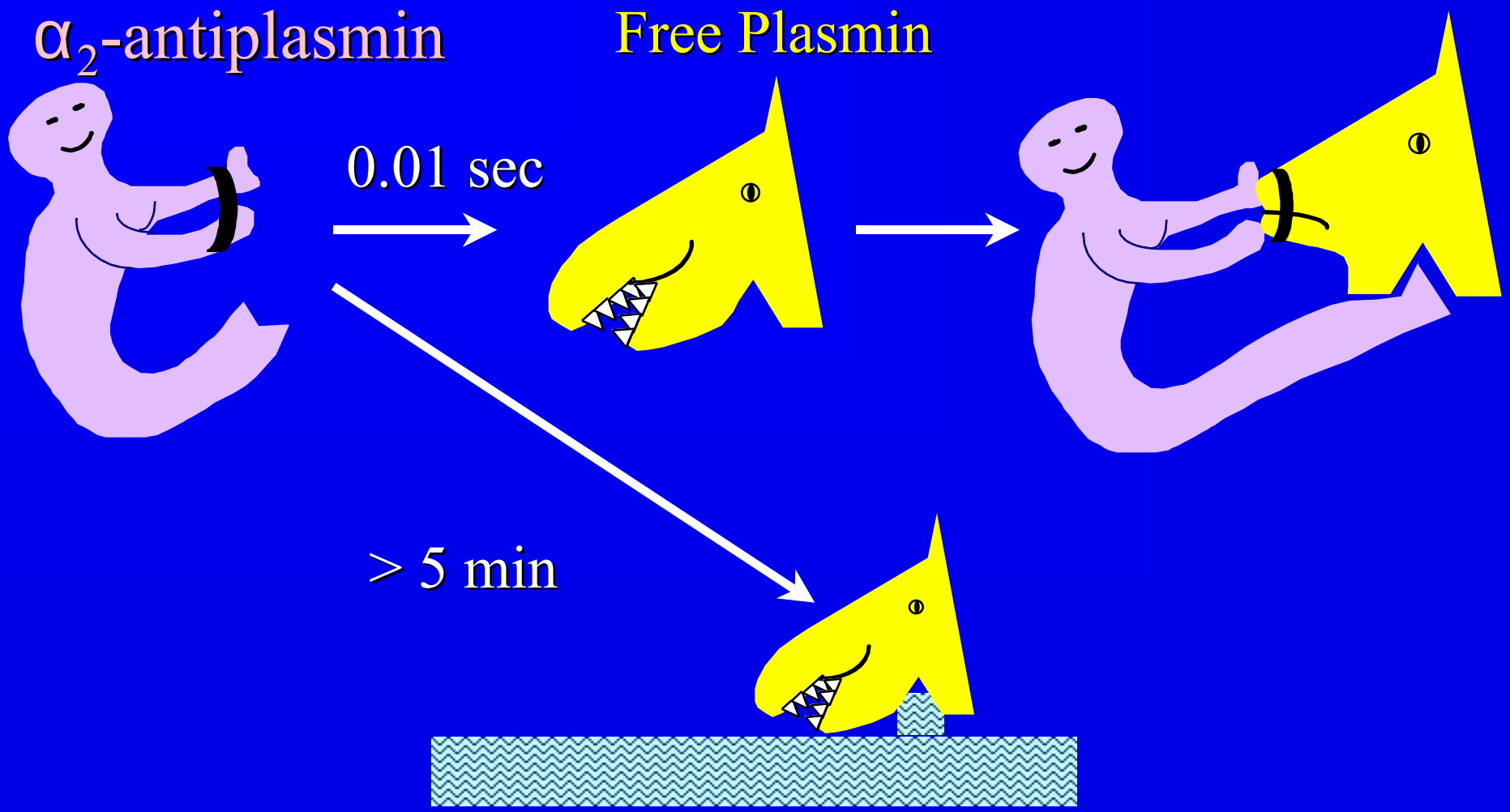
Pharmacokinetic Properties

- Inactive via oral route
- Rapid distribution into total extravascular space
- Following redistribution, plasma half-life ÷ 150 min
- Filtered by glomeruli and reabsorbed by proximal tubules
- Less than 10% excreted as unchanged drug
- Slowly degraded by lysosomal enzymes
- Terminal elimination phase half-life ÷ 10 h
- Does not cross the blood-brain barrier

Fibrinolysis

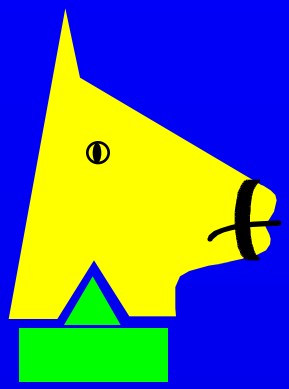


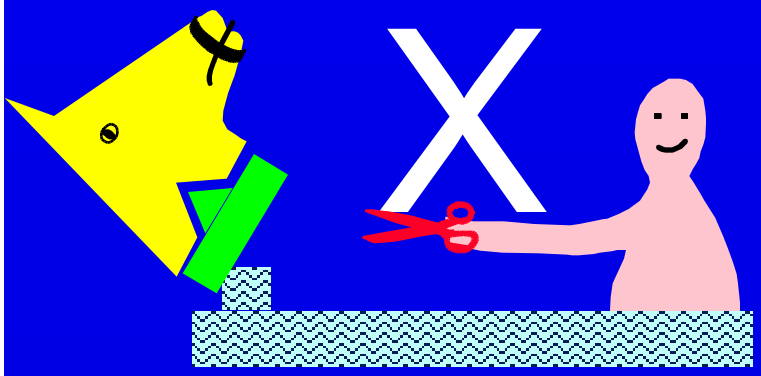
Role of α−2 Plasmin Inhibitor



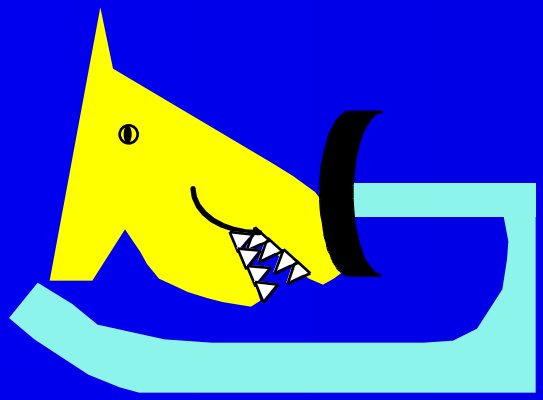
Pharmacologic Inhibition of Fibrinolysis

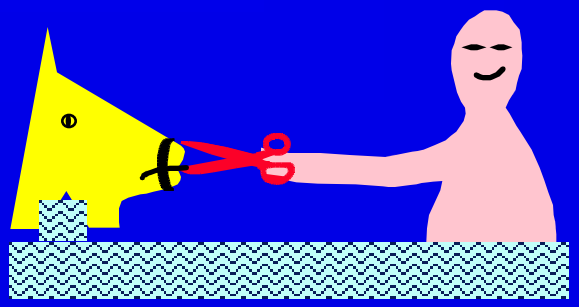
Lysine Antifibrinolytics





Serine Protease Inhibitors





Aprotinin

Approved Indication

Trasylol® is indicated for prophylactic use to reduce perioperative blood loss and the need for blood transfusion in patients undergoing cardiopulmonary bypass in the course of coronary artery bypass graft (CABG) surgery

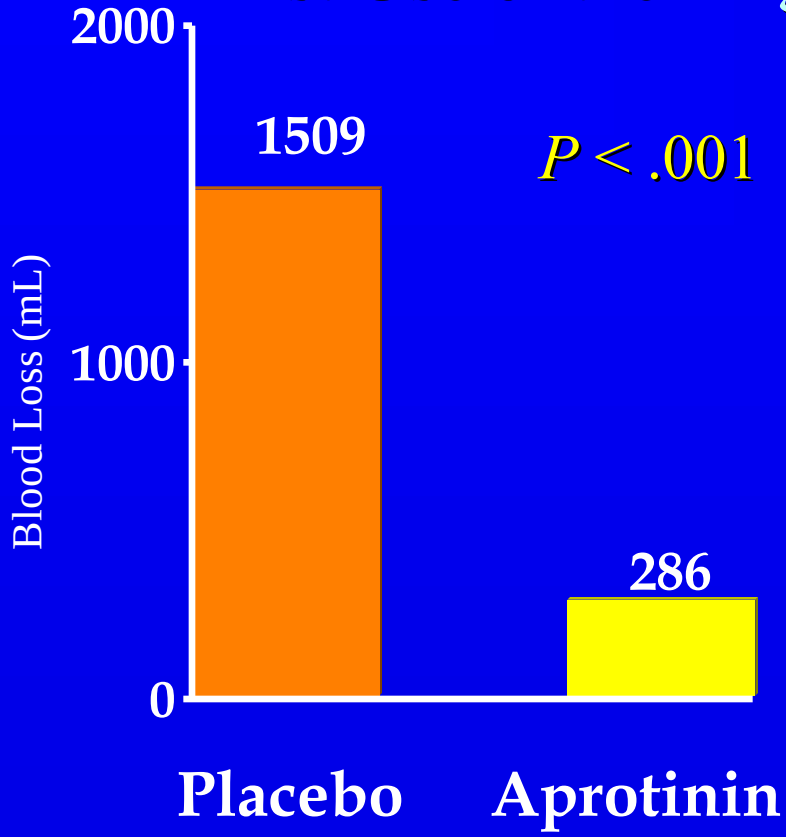
Please note important boxed warning and other safety information

Aprotinin Warning Information

Anaphylactic or anaphylactoid reactions are possible when Trasylol® is administered. Hypersensitivity reactions are rare in patients with no prior exposure to aprotinin. The risk of anaphylaxis is increased in patients who are re-exposed to aprotinin-containing products. The benefit of Trasylol® to patients undergoing primary CABG surgery should be weighed against the risk of anaphylaxis should a second exposure be required.

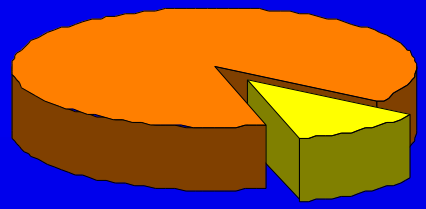
Aprotinin in Repeat Cardiac Surgery

First Use of the 'High-Dose' Regimen



$P < .001$

41 units in 11/11 patients

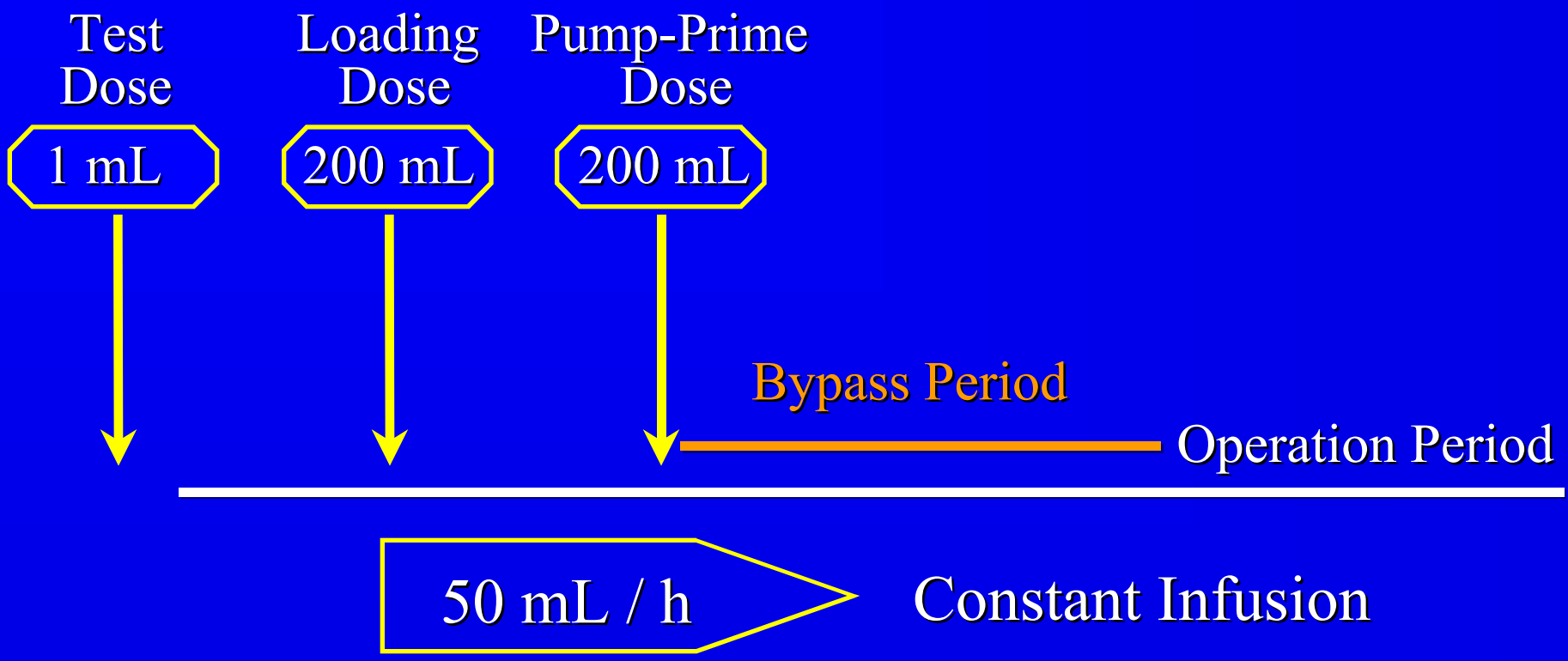


5 units in 5/11 patients

Royston et al Lancet 1987 Dec 5;2:1289-1291

High-Dose Aprotinin

Original Administration Regimen



Royston et al Lancet 1987 Dec 5;2:1289-91

Aprotinin Dosing and Administration

Test Dose	Loading Dose	Pump-Prime Dose	Constant-Infusion Dose
Regimen B (Plasmin Inhibiting)			
1 mL	**100 mL**	**100 mL**	**25 mL/h**
1.4 mg, or 10,000 KIU KIU/h	**140 mg, or 1.0 million KIU**	**140 mg, or 1.0 million KIU**	**35 mg/hr, or 250,000**

Aprotinin Dosing and Administration

Test Dose	Loading Dose	Pump-Prime Dose	Constant-Infusion Dose
Regimen A (Kallikrein Inhibiting)			
1 mL (1.4 mg, or 10,000 KIU)	200 mL (280 mg, or 2.0 million KIU)	200 mL 280 mg, or 2.0 million KIU)	50 mL/h (70 mg/h, or 500,000 KIU/h)

Dosing and Aprotinin Administration

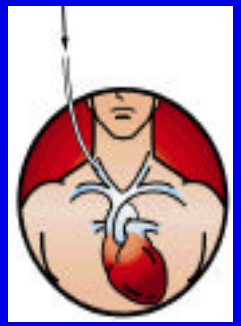
Test Dose

- All patients should first receive a test dose. Administer intravenously at least 10 minutes before loading dose.

Loading Dose

- After induction of anesthesia but prior to sternotomy, give intravenously to patient in supine position. Administer slowly over 20-30 minutes

Dosing and Aprotinin Administration



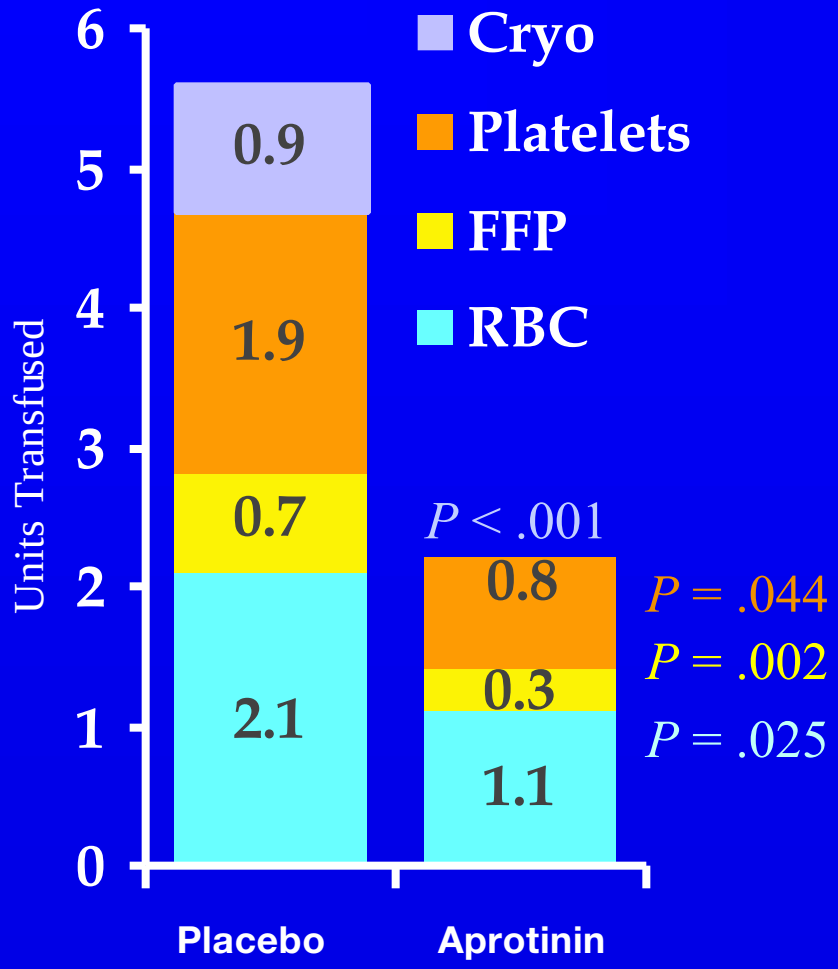
"Pump-prime" Dose

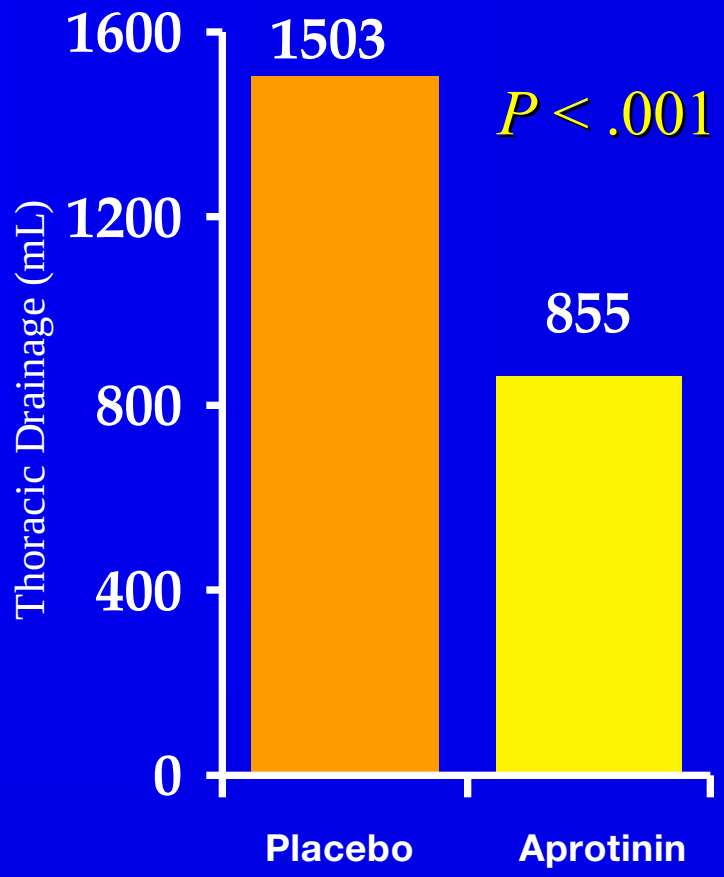
- Add to priming fluid of cardiopulmonary bypass circuit, by replacement of an aliquot of priming fluid, prior to institution of cardiopulmonary bypass.

Constant Infusion Dose

- Administer when loading dose is complete. Continue until surgery is complete and patient leaves operating room.

Aprotinin Use in Primary CABG Operations

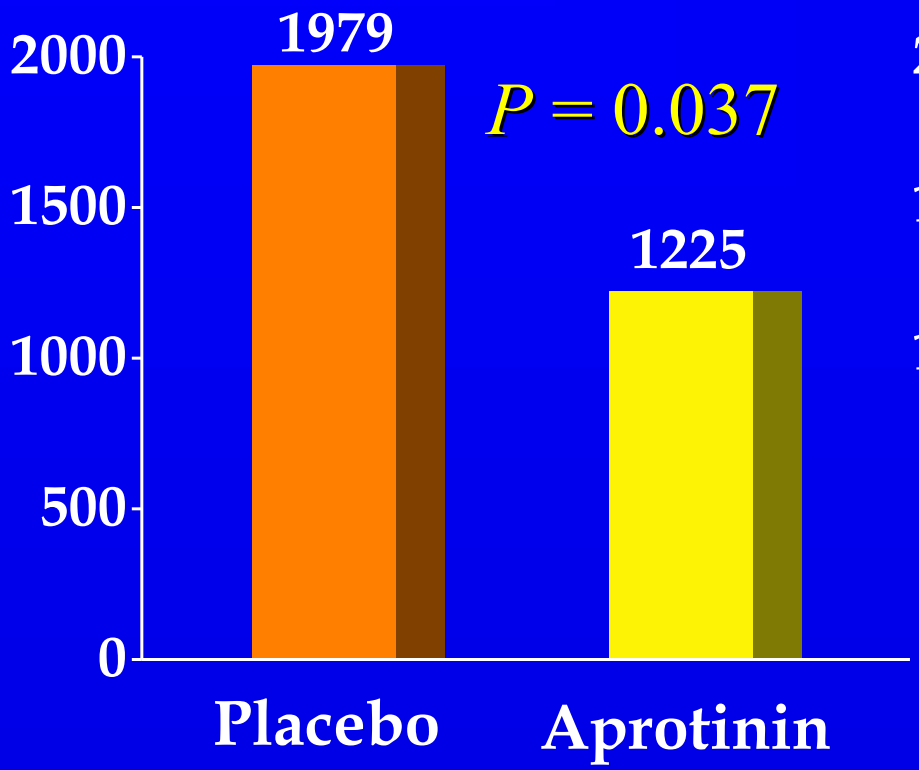




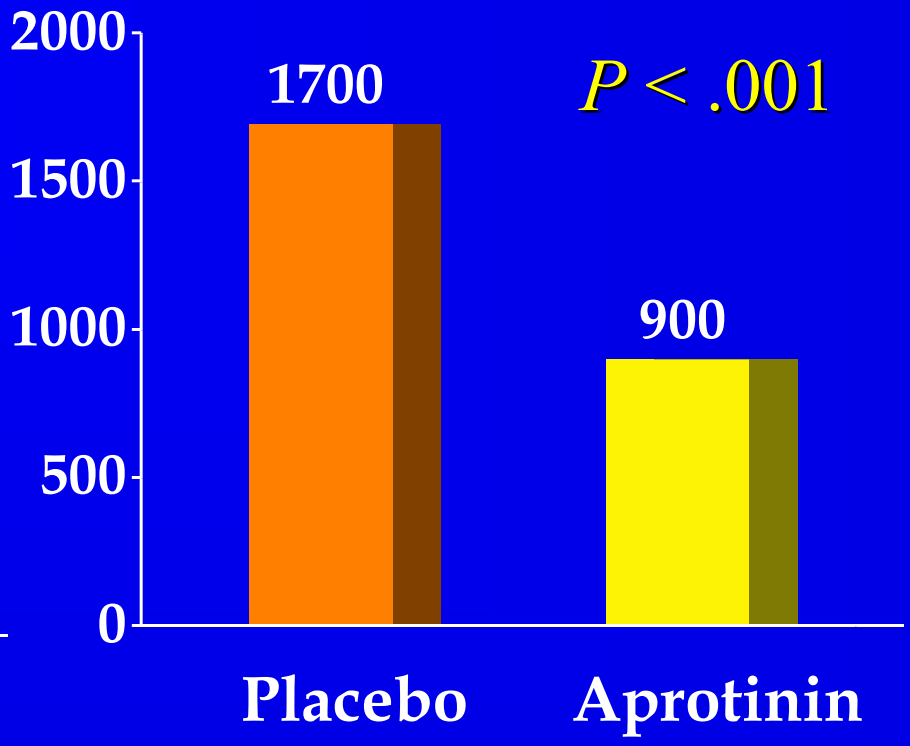
Lemmer et al J Thorac Cardiovasc Surg 1994;107:543-553

Aprotinin Use in CABG Reoperations

Total Thoracic-Drainage Volume (mL)



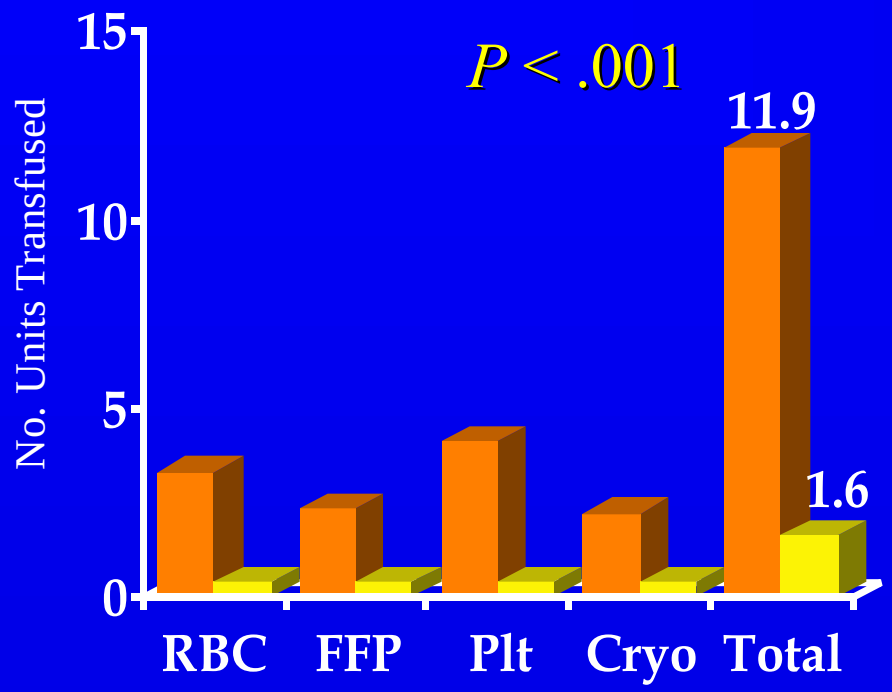
Lemmer et al
J Thorac Cardiovasc Surg 1994;107:543-53



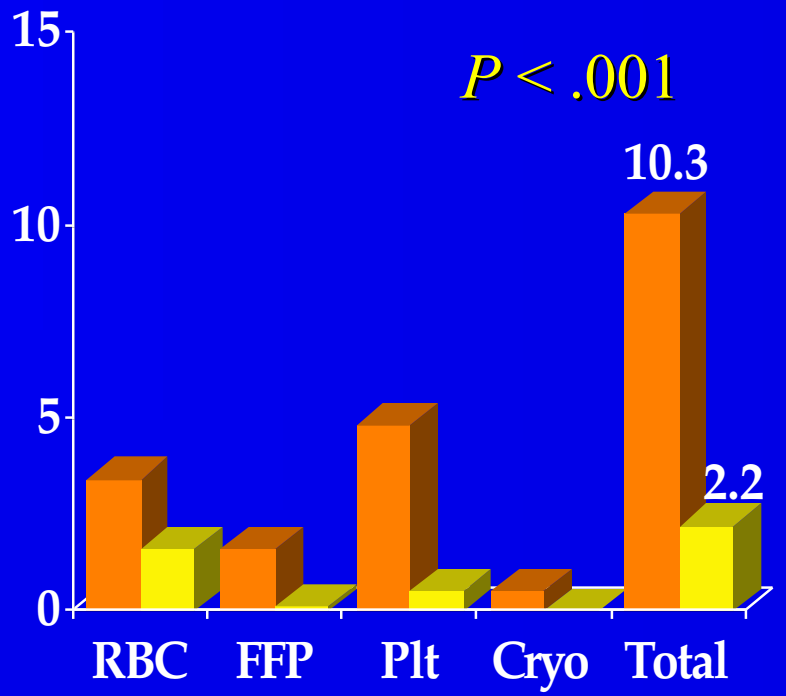
Levy et al
Circulation 1995;92:2236-44

Aprotinin Use in CABG Reoperations

Donor-Blood-Product Requirements



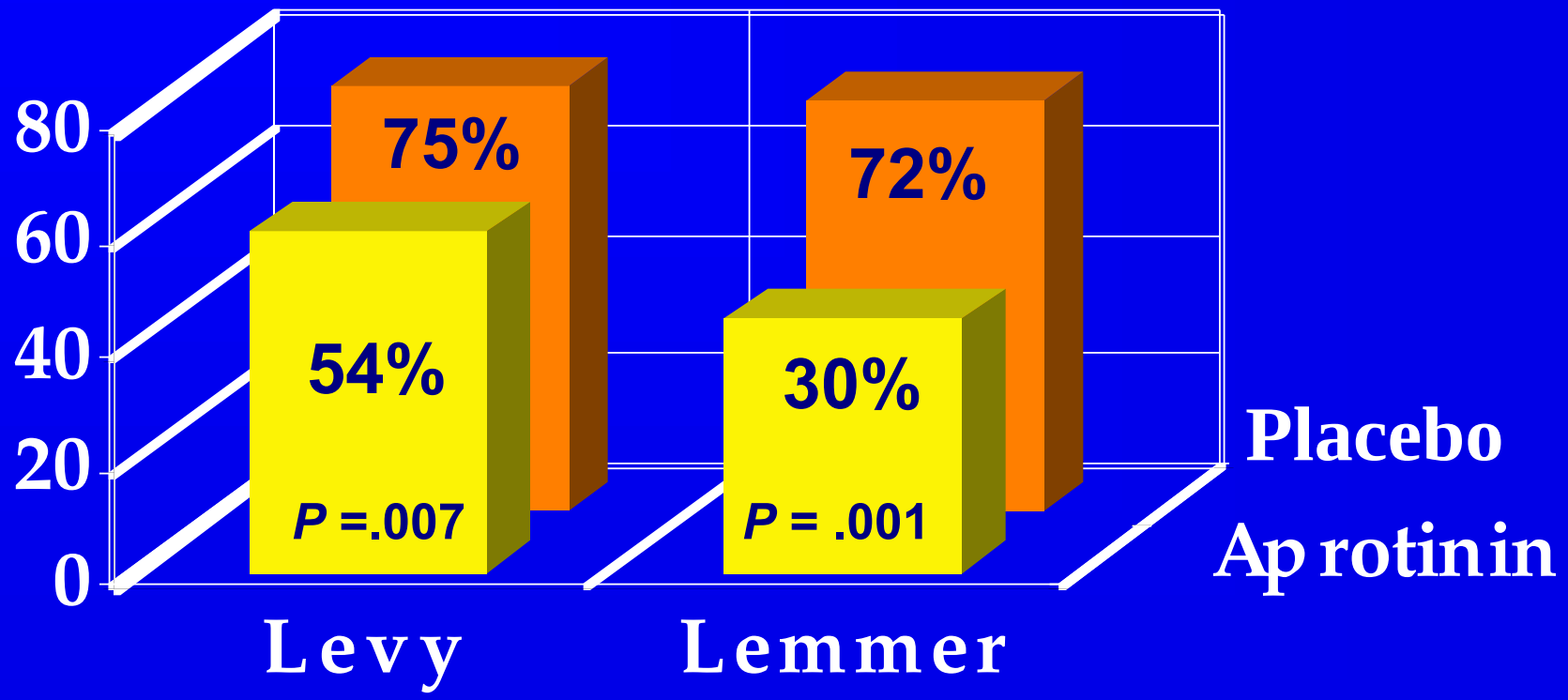
Lemmer et al
J Thorac Cardiovasc Surg 1994;107:543-53



Levy et al
Circulation 1995;92:2236-44

Aprotinin Use in CABG Reoperations

Patients Requiring Donor Red Blood Cells (%)



Levy et al Circulation 1995;92:2236-44

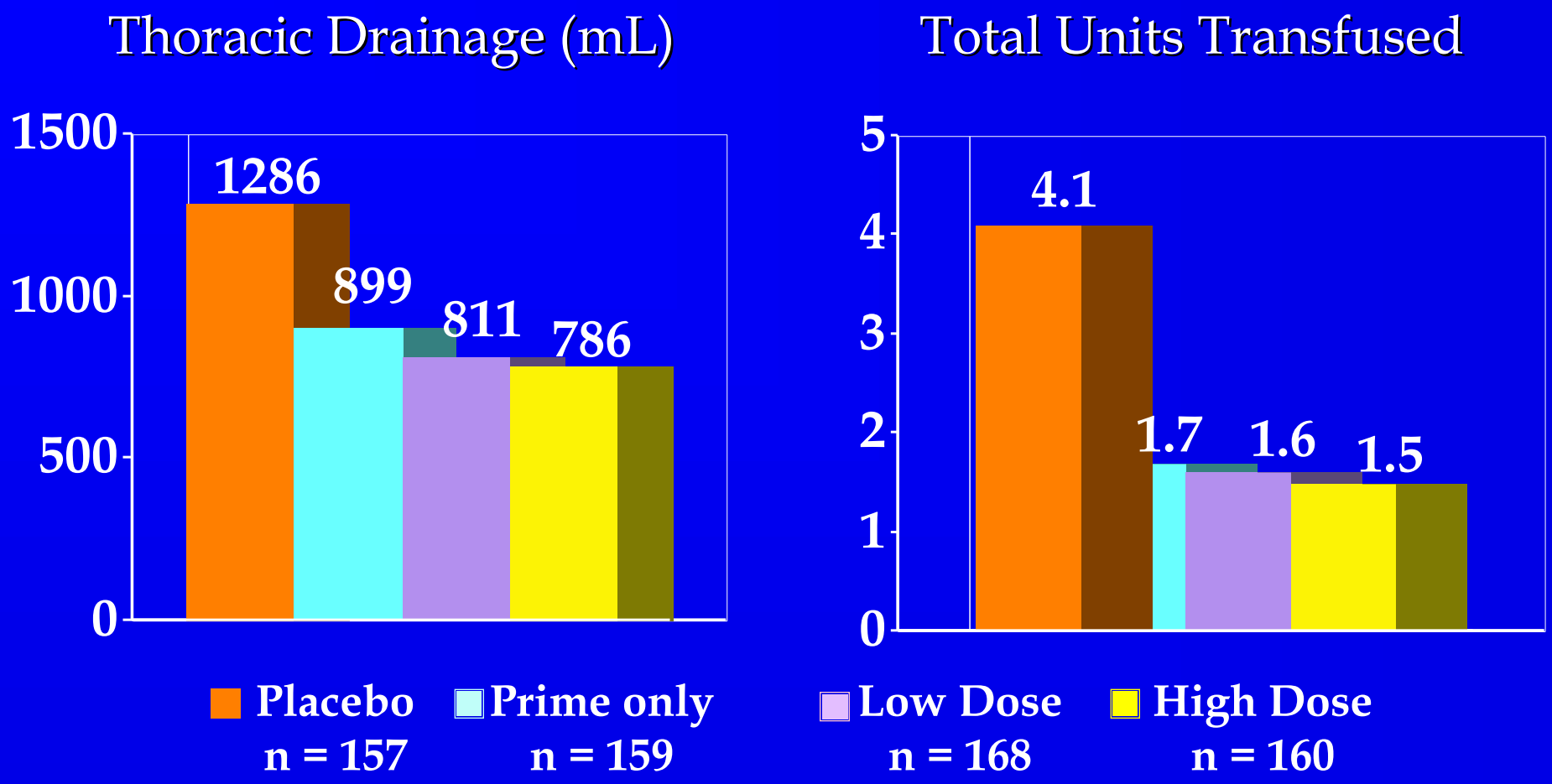
Lemmer et al J Thorac Cardiovasc Surg 1994;107:543-53

Results of Differing Dose Regimens

	Thoracic Drainage (mL)	Units Given RBC	Platelets
Placebo	1,121±683	4.1±6.2	5.4±14.6
Half Dose	866±1636	4.8±11.8	3.3±15.4
Full Dose	720±753	2.1±4.2	1.6±6.3

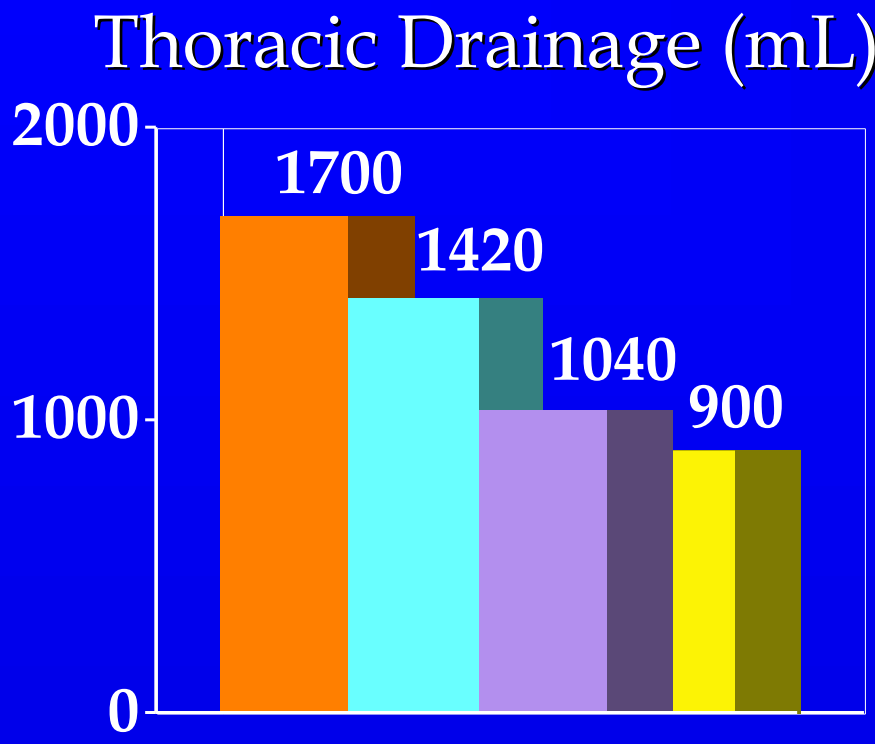
Cosgrove et al Ann Thorac Surg 1992;54:1031-38

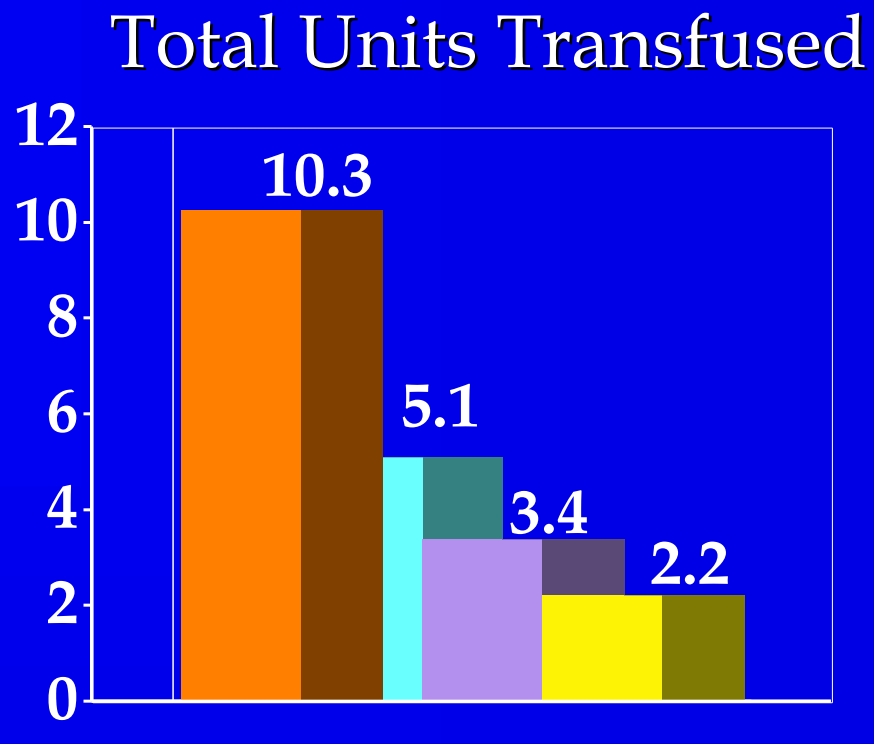
Comparative Dose Trial in Repeat CABG Surgery



Lemmer et al Ann Thorac Surg 1996;62:1659-68

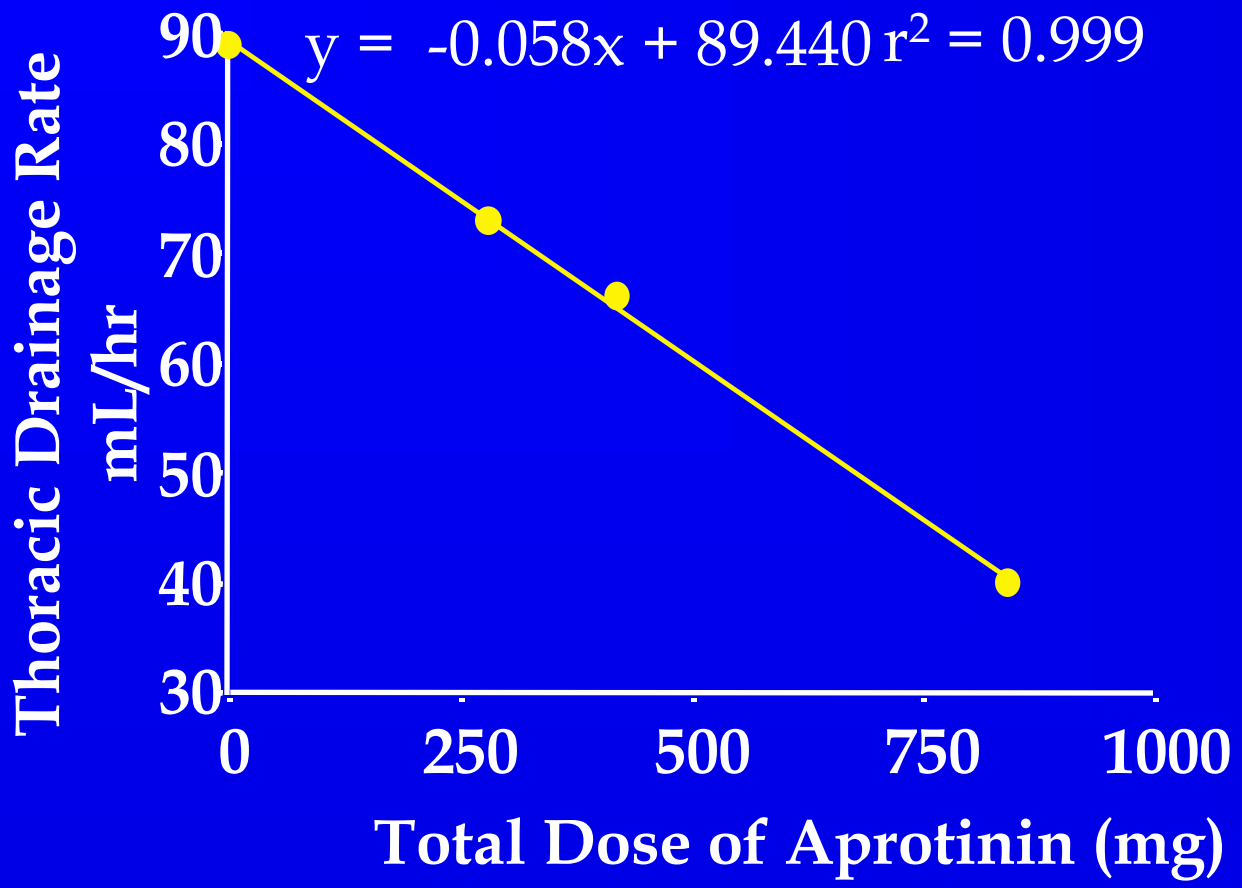
Comparative Dose Trial in Repeat CABG Surgery





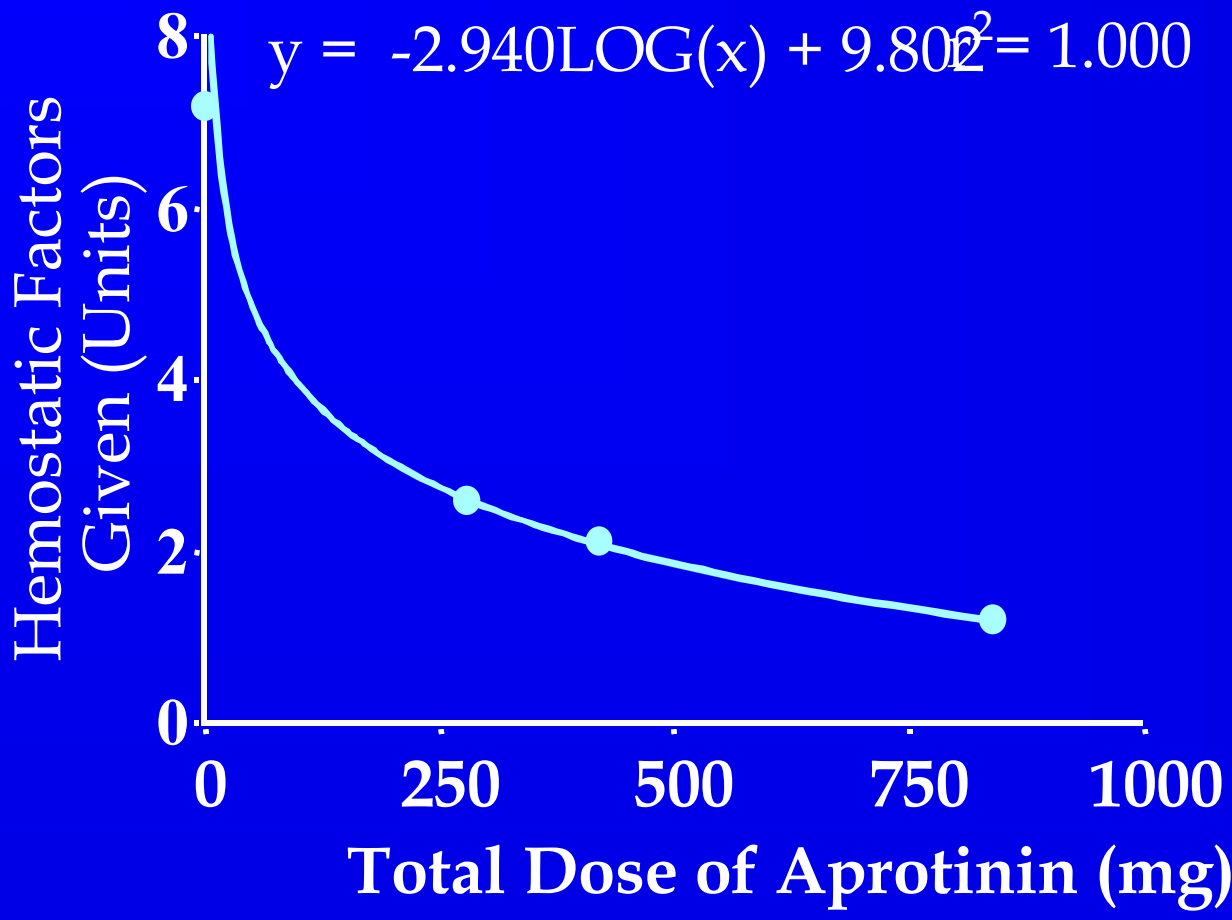
Levy et al Circulation 1995;92:2236-44

Aprotinin Dose vs Thoracic Drainage Rate



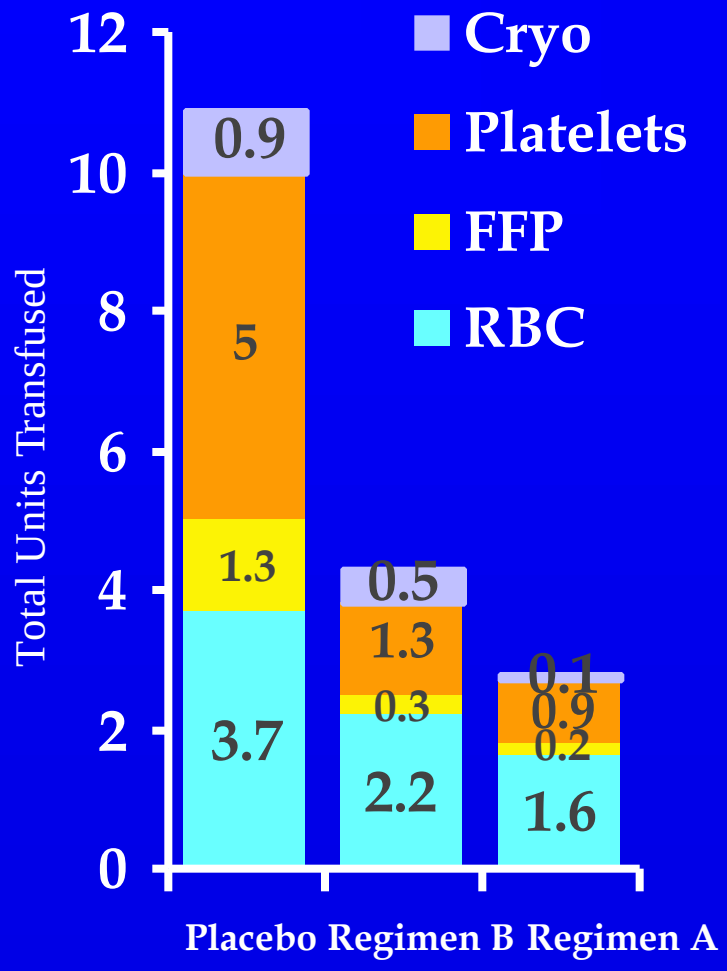
Royston D. In: Machiraju VR, ed. Redo Cardiac Surgery in Adults. New York: CME Network, 1998:10-22.

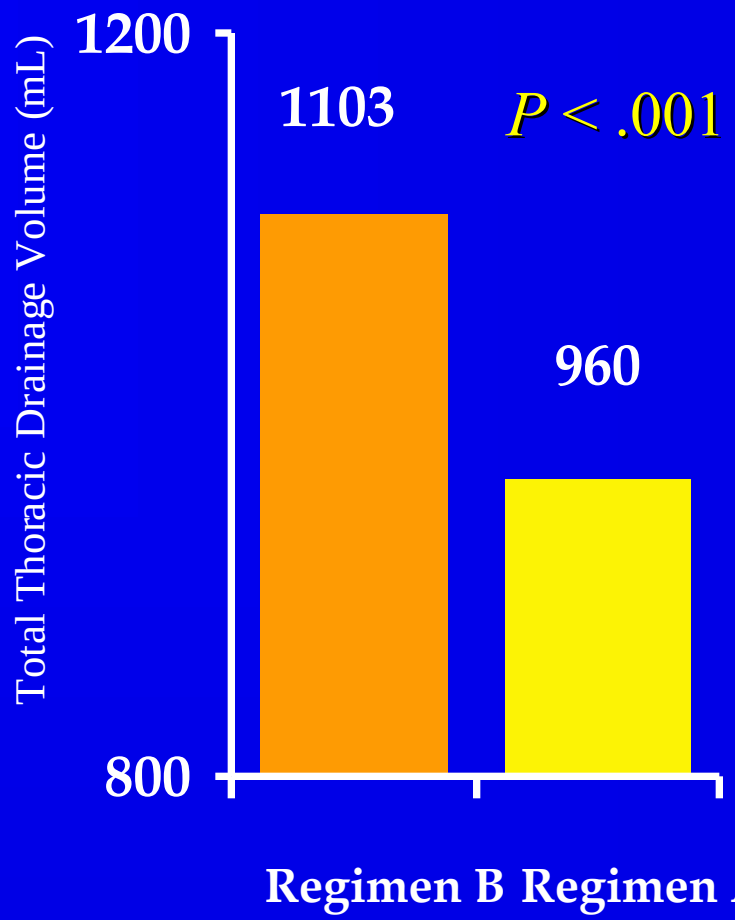
Aprotinin Dose vs Hemostatic Factors Given



Royston D. In: Machiraju VR, ed. Redo Cardiac Surgery in Adults. New York: CME Network, 1998:10-22.

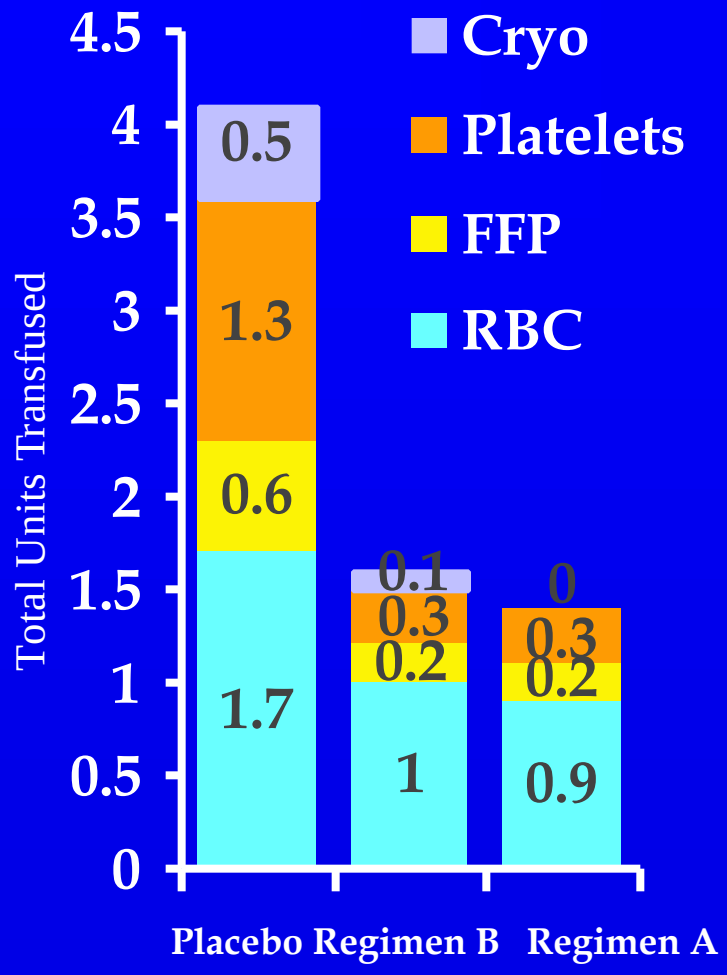
Aprotinin Use in Repeat CABG Operations

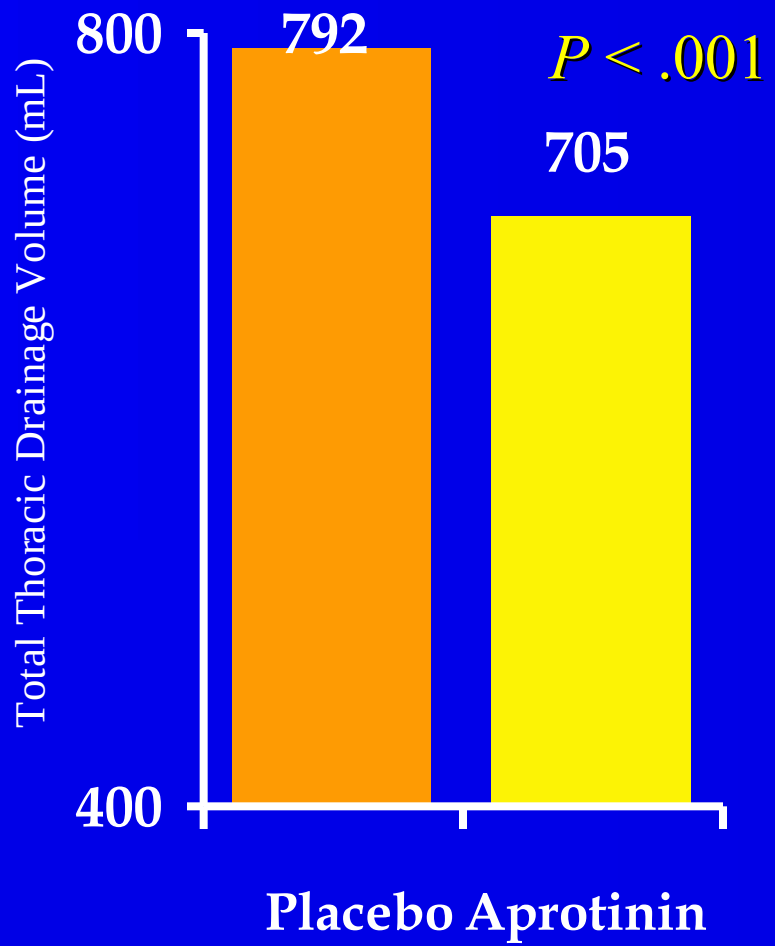




Product Information 11/98

Aprotinin Use in Primary CABG Operations





Product Information 11/98

Selected Cost Factors Related to CABG

- Drug
- Monitoring
- Blood products
- Single donor platelets
- Blood product -associated complications
- Operating room times - surgical/anesthetic
- Re-exploration
- Myocardial infarction
- Stroke
- Length of stay - ICU/hospital

Selected Cost Factors Related to CABG

- Drug
- Monitoring
- Blood products ($60-$100/unit)
- Single donor platelets ($250-$500/unit)
- Blood product associated complications
- Operating room times
  - surgical costs ($5-$15/min)
  - anesthetic costs
- Re-exploration ($3,000-$20,000)
- Myocardial infarction
- Stroke
  - in hospital ($10,000-$30,000)
  - out of hospital ($100,000-$250,000)
- LOS- ICU/Hospital ($800-$2,500/day)

Cardiac Surgery Impairs Hemostasis

Inhibition of Hemostasis

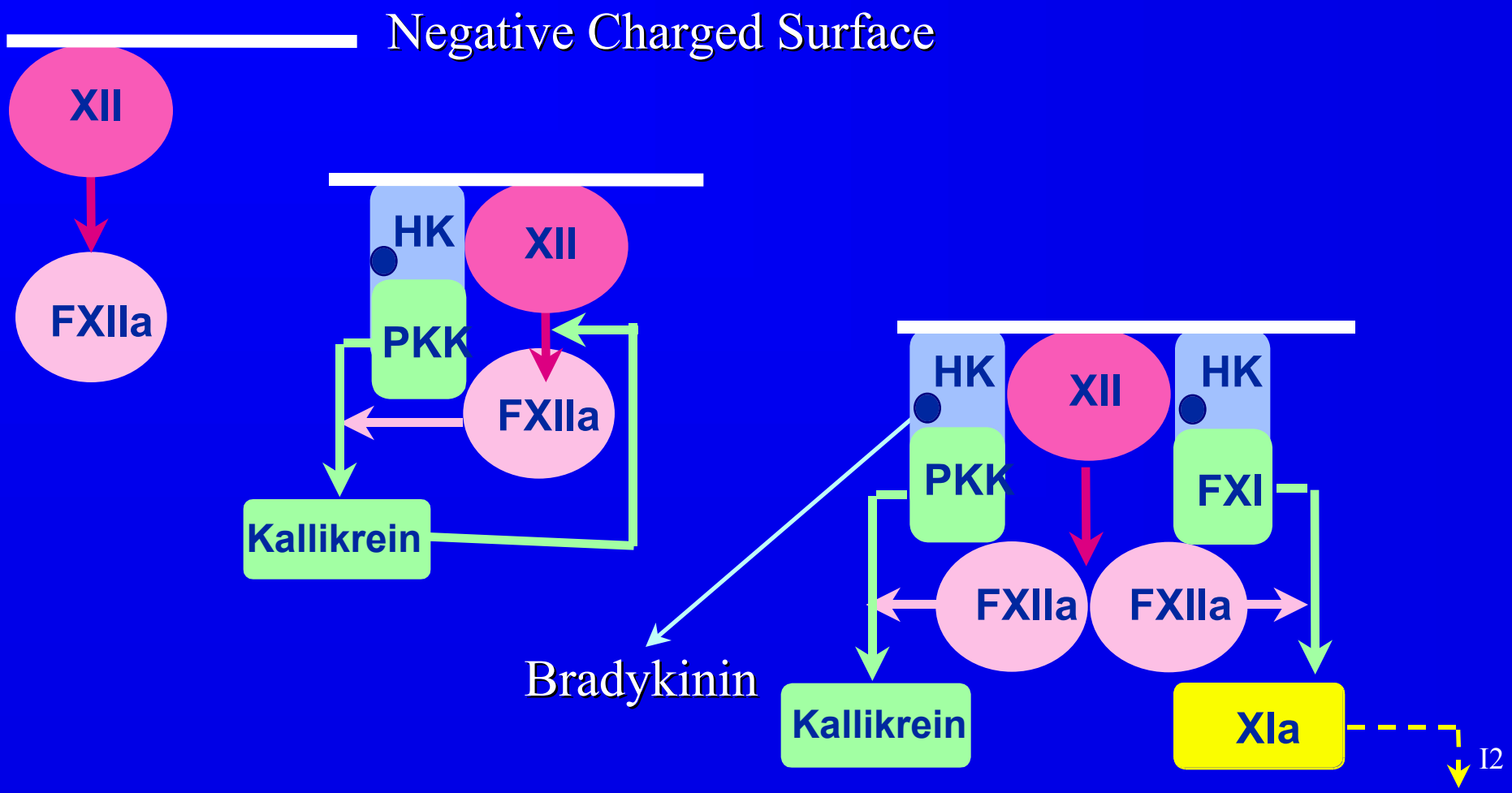
- hypothermia
- hemodilution
- heparin / protamine
- preoperative drug therapies

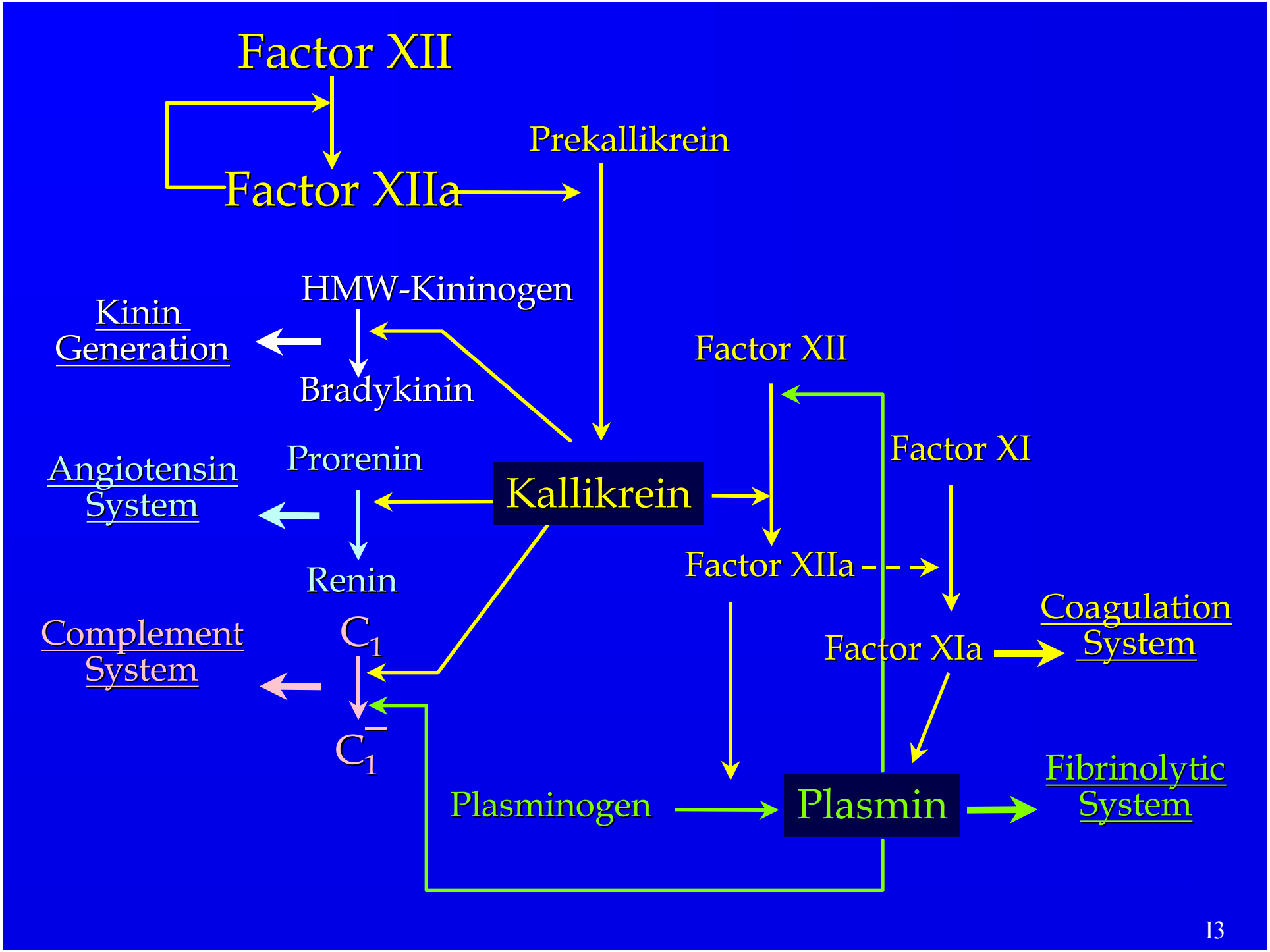
Extrinsic Pathway Activation

- pericardial shed blood
- reperfusion of heart and lungs
- local thrombin generation

Contact Activation of Factor XII

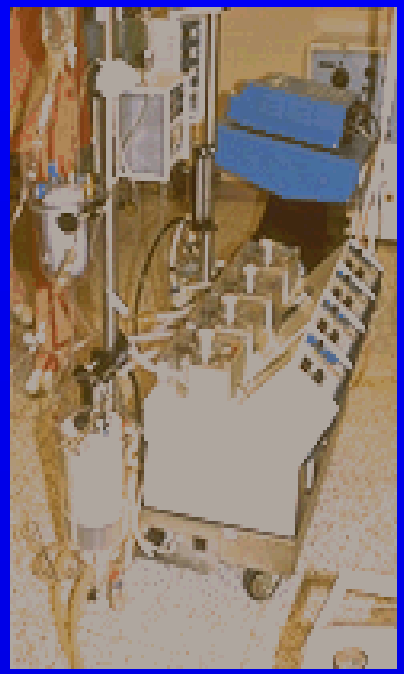
Contact Activation - The Role of Kallikrein




Factor XII
Factor XIIa
Prekallikrein
HMW-Kininogen
Kinin Generation
Bradykinin
Factor XII
Factor XI
Angiotensin System
Prorenin
Kallikrein
Renin
Factor XIIa
Coagulation System
Complement System
C_1
Factor XIa
C_1^-
Fibrinolytic System
Plasminogen
Plasmin

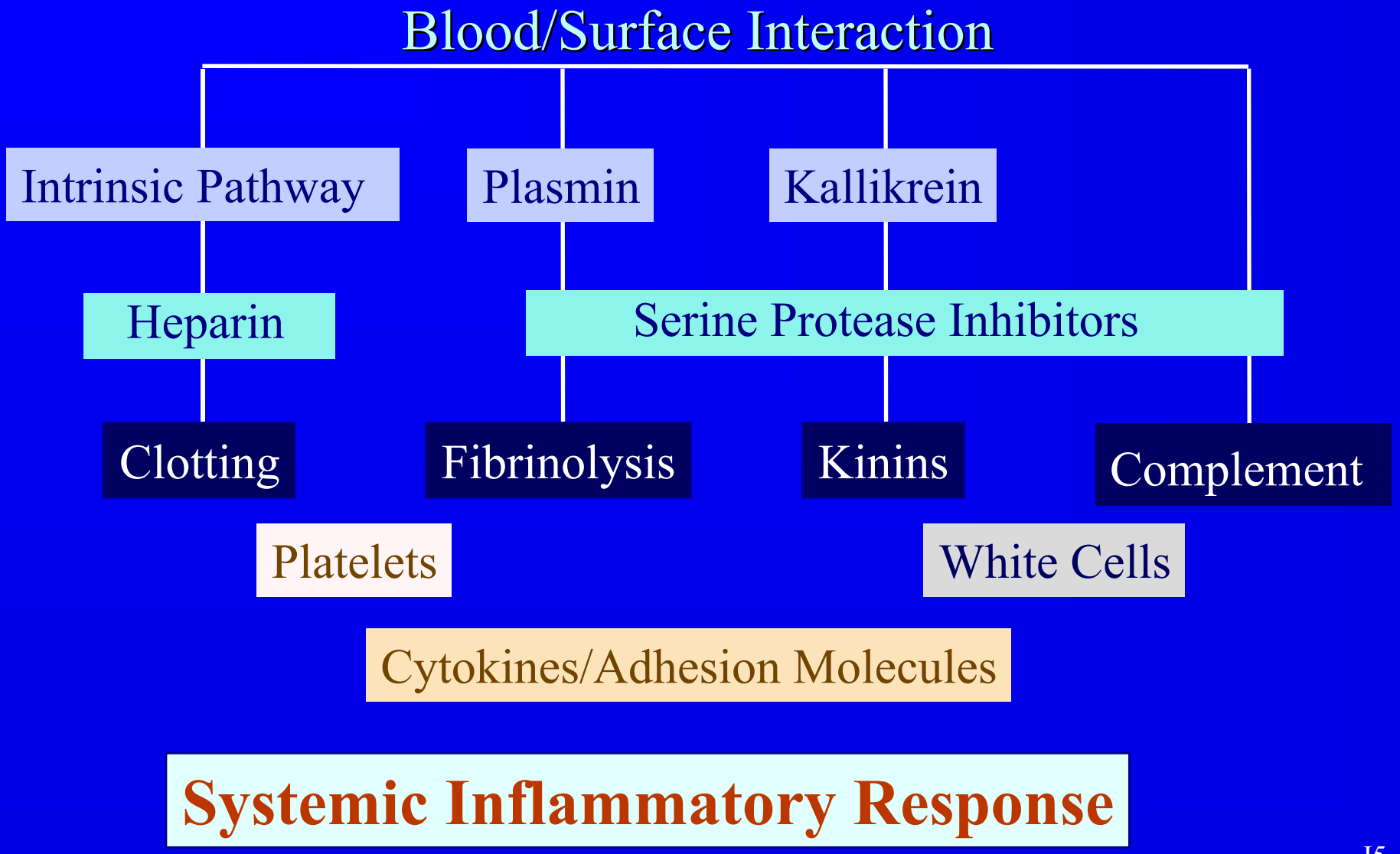
The Insult of Cardiopulmonary Bypass

Contact of Blood with the Foreign Surface of the Bypass Circuit May Activate:

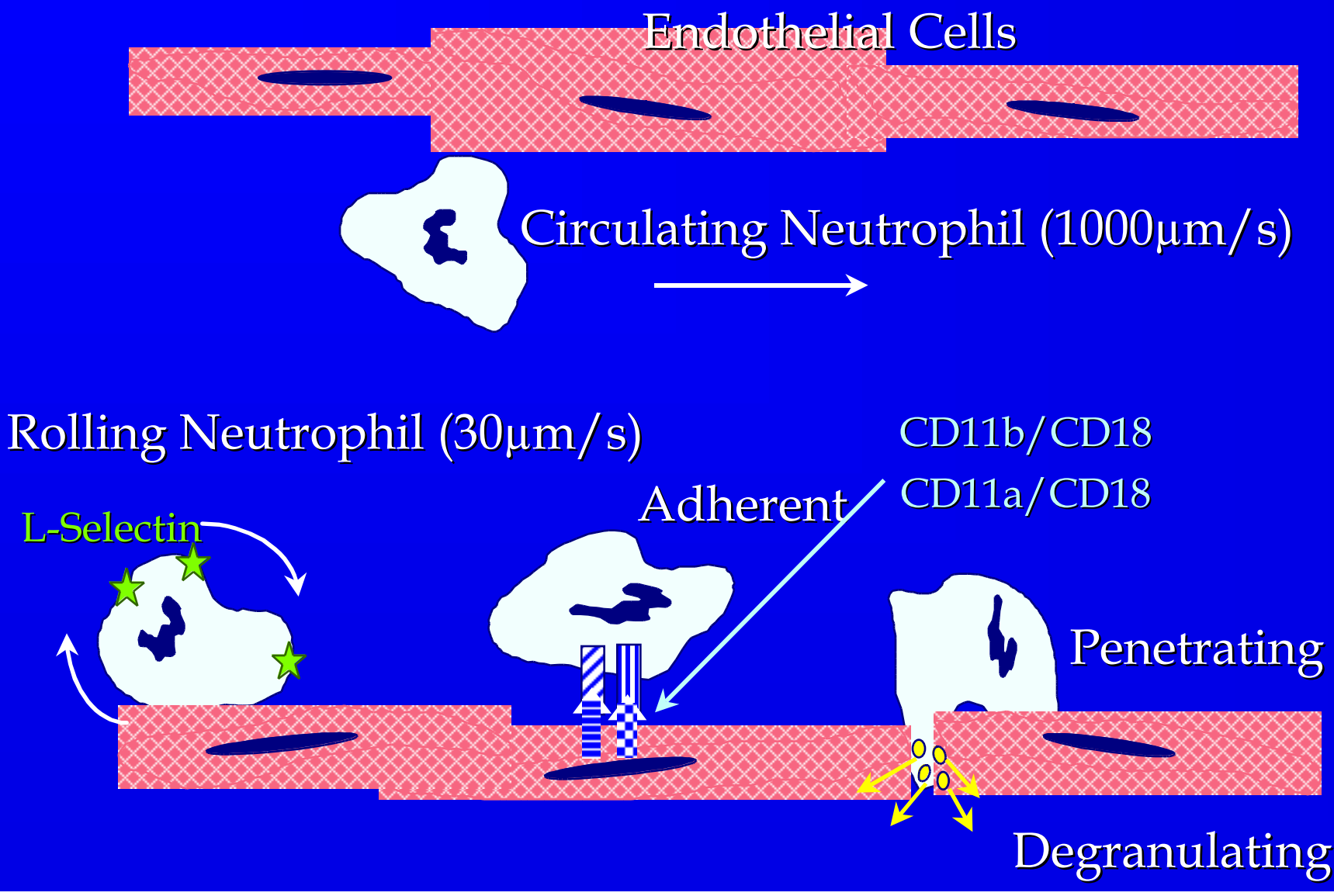


- White cells and platelets
- Complement System
- Coagulation System
- Kinin Generation
- Fibrinolytic System

Contact Activation of Blood Proteins



Neutrophil Adhesion Processes



Systemic Inflammatory Response to CPB

- The systemic response to bypass is a result of the interrelated activation of:
  - Hemostasis
  - Fibrinolysis
  - Cellular and humoral inflammatory systems
- Aprotinin's action to inhibit serine proteases (e.g., kallikrein, plasmin) attenuates:
  - Inflammatory responses
  - Fibrinolysis
  - Thrombin generation

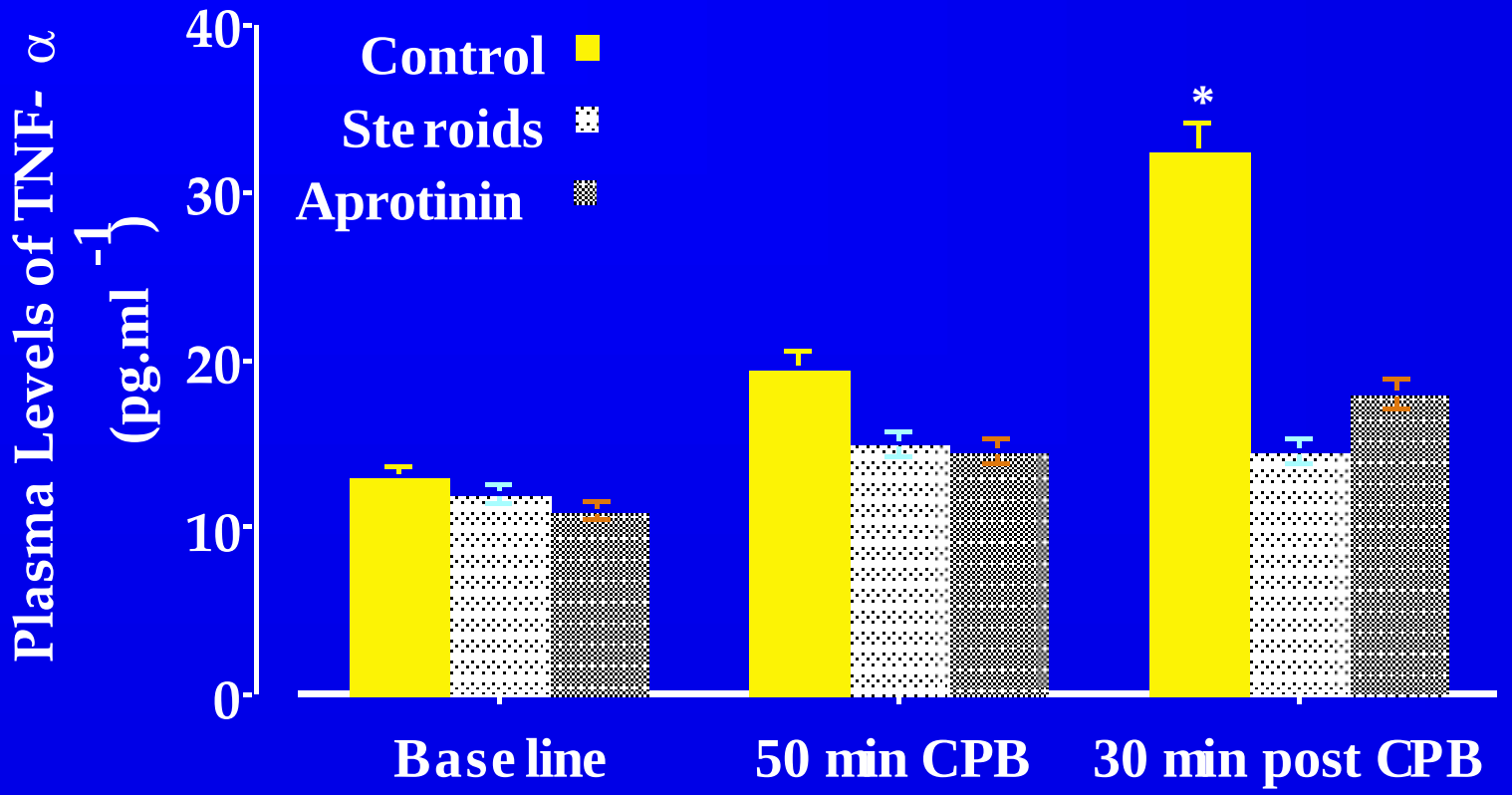
Anti-inflammatory Action of Aprotinin

Aprotinin inhibits pro-inflammatory cytokine release and maintains glycoprotein homeostasis

- Platelets - reduces glycoprotein loss (e.g., GpIb, GpIIb/IIIa)
- Granulocytes - prevents the expression of pro-inflammatory adhesive glycoproteins (e.g., CD11b)

Antiinflammatory Actions of Aprotinin

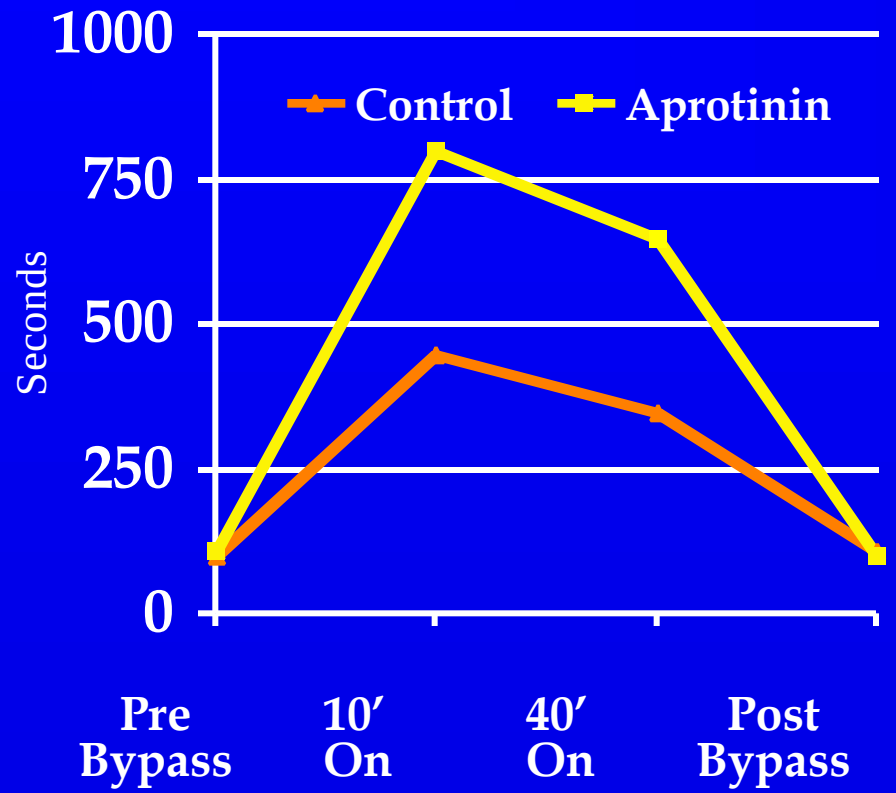
Suppression of ProInflammatory Cytokine Release



Hill et al J Thorac Cardiovasc Surg 1995;110:1658-62

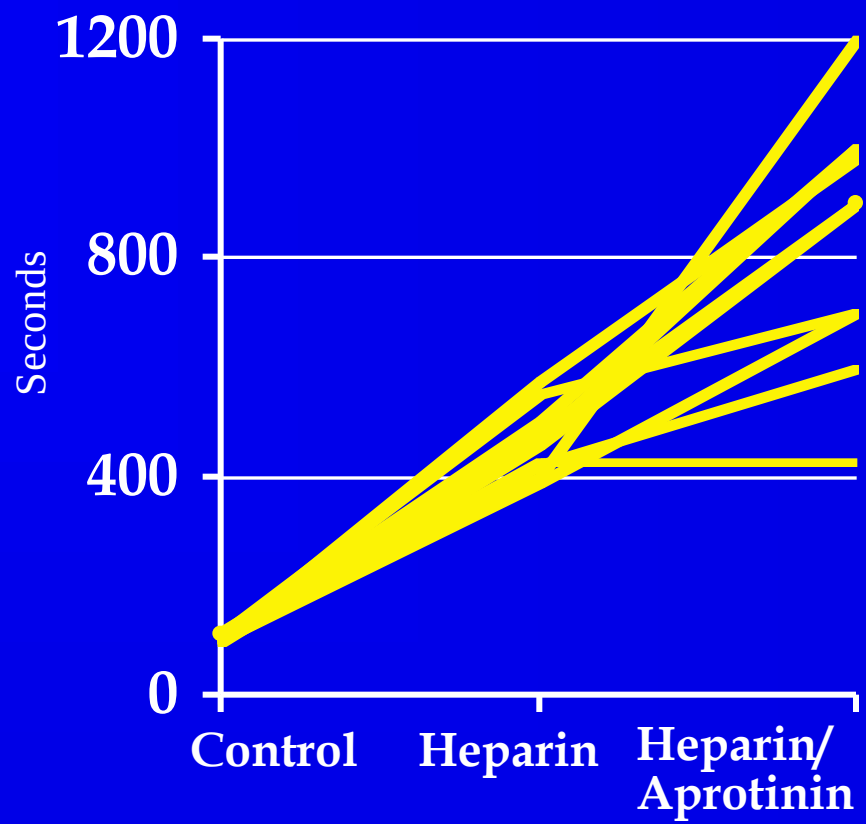
The ACT and Aprotinin

In vivo Action



Royston D
J Cardiothorac Anesth 1989;3:80

In vitro Action



Royston D
In: Pifarre R, ed. 1993

Monitoring Anticoagulation With Aprotinin

- Maintain celite-based ACT values at 750 seconds

OR

- Maintain kaolin-based ACT values at 480 seconds

OR

- Give additional heparin in a fixed-dosage regimen

OR

- Use heparin/protamine tritration, a monitoring test that does not rely on contact activation

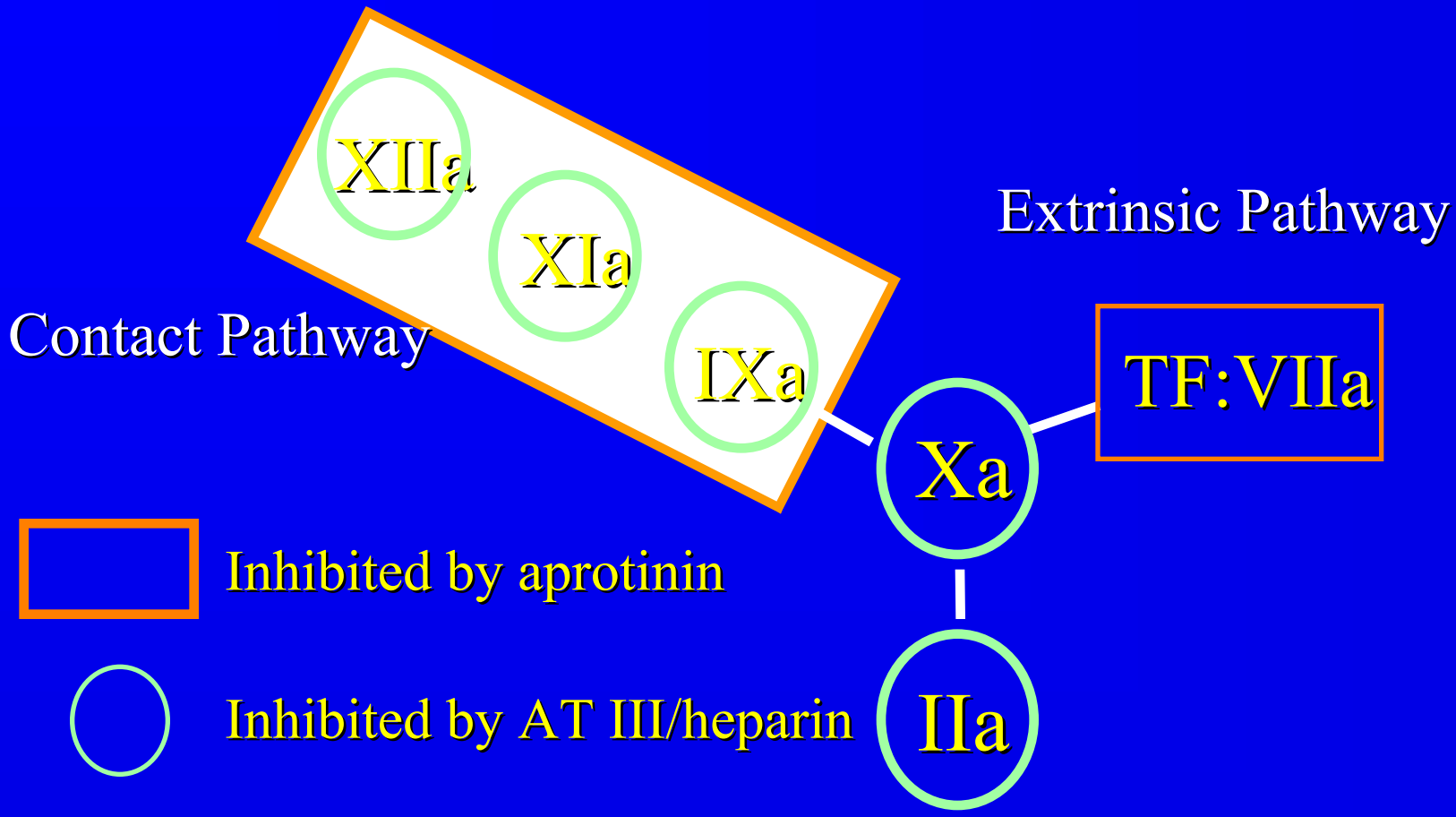
DHCA and Heparinization Management

Appropriate heparinization schedules must be used to ensure anticoagulation throughout the bypass procedure

- Activated clotting time (ACT) should be maintained at more than 1000 seconds during the procedure

- To achieve this may require a larger loading dose of heparin and an additional bolus of heparin prior to initiation of circulatory arrest

Aprotinin and Heparin Inhibition



Royston J Cardiothorac Vasc Anesth 1992;6:76-100

Coagulation Monitoring With Aprotinin

Activated Clotting Time (ACT)

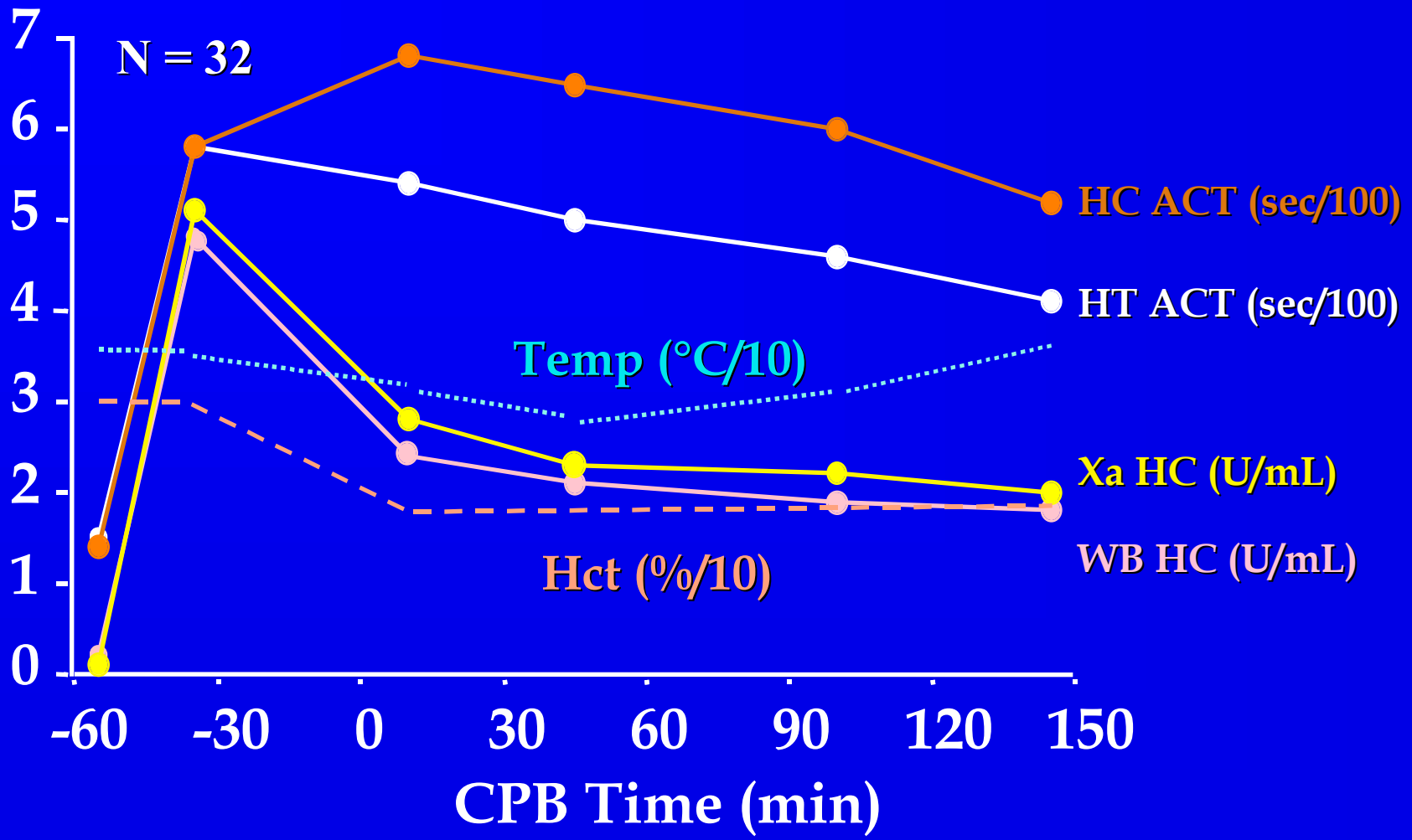
- Celite 750 seconds
- Kaolin 480 seconds

Independent of the effects of hemodilution and/or hypothermia

(Difficult to quantify during CPB)

Trasylol Prescribing Information, Bayer Corporation

Limitations of ACT in Heparin Monitoring



Despotis et al J Thorac Cardiovasc Surg 1994;108:1076-82

Coagulation Monitoring With Aprotinin

Fixed Heparin Dosing

- Loading dose + pump prime = at least 350 IU/kg
- Additional heparin should be given based on:
  - Patient weight
  - Length of CPB
- Heparin elimination
  - e.g. 1/3 initial dose in U/kg every 45 min

Trasylol Prescribing Information, Bayer Corporation

Coagulation Monitoring With Aprotinin

Heparin-Protamine Titration

- A heparin dose response, assessed by protamine titration, should be performed prior to aprotinin administration to determine heparin loading dose
- Maintain heparin levels during CPB at least above 2.7U/mL
- Maintenance of patient-specific pre-CPB reference (whole blood heparin concentration associated with kaolin ACT of approximately 480 seconds)

Trasylol Prescribing Information, Bayer Corporation

Coagulation Monitoring With Aprotinin

Heparin Reversal With Protamine

- Amount of protamine administered based on the amount of heparin given (e.g. 0.5-0.7 mg protamine:mg total heparin*) , not the ACT value

* Despotis et al J Thorac Cardiovasc Surg 1995;110:46-54
Trasylol Prescribing Information, Bayer Corporation

Aprotinin
Risk/Benefit Issues

- Hypersensitivity
- Mortality
- Myocardial Infarction
- Graft Patency
- Use with Hypothermic Circulatory Arrest
- Renal Function
- Stroke
- Miscellaneous Adverse Events

Incidence of Hypersensitivity Reactions

Including mild skin rash, bronchospasm, and anaphylaxis

Incidence
No prior exposure * <0.1%
Reexposure within six months ** 5.0%
Reexposure after six months ** 0.9%

* Bayer Corporation, Data on File

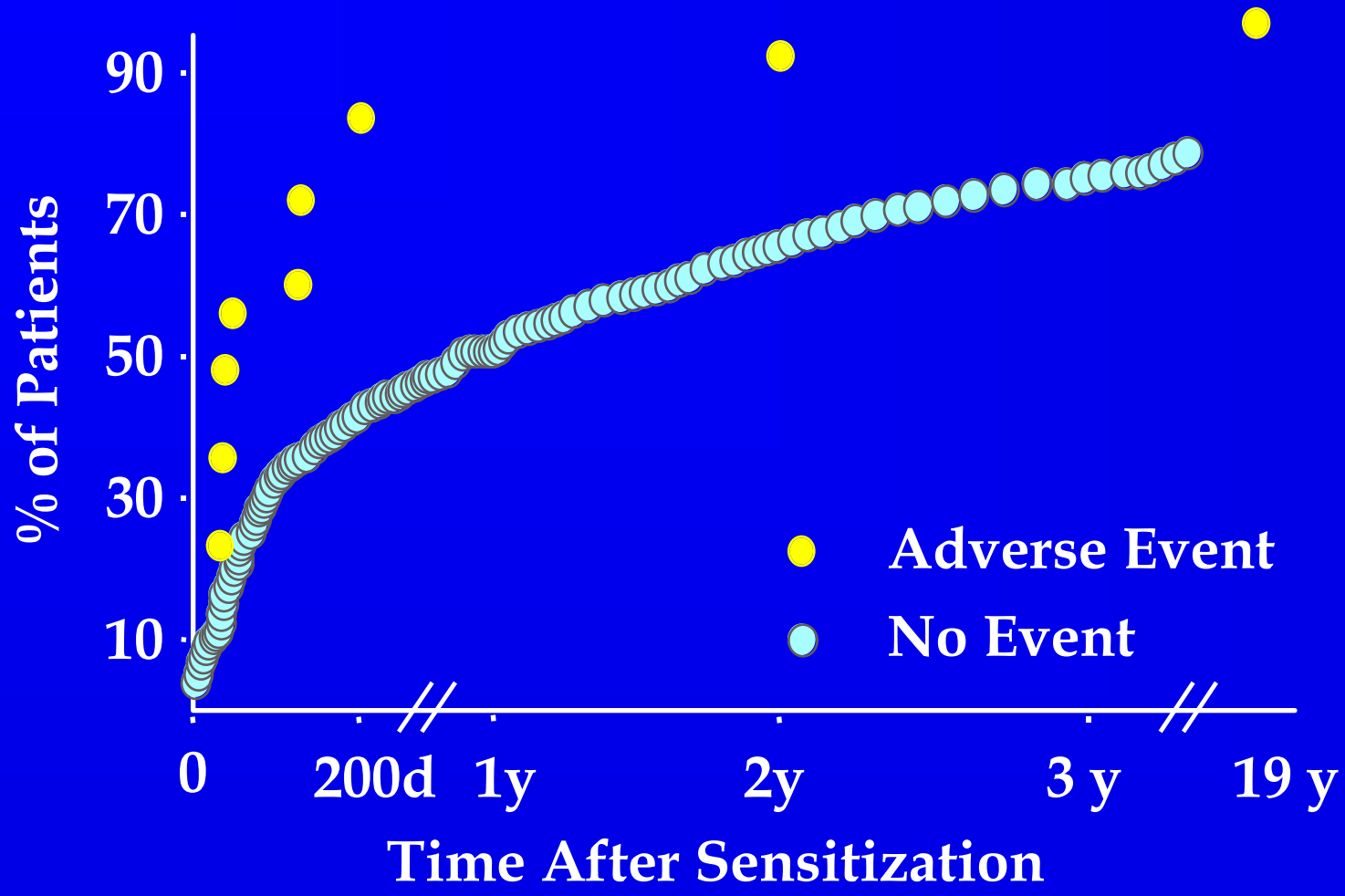
** Dietrich et al Ann Thorac Surg 1998;65:S60-4

Epidemiology of Serious Hypersensitivity Reactions

- Treated patients > 3000
- Known prior exposure ~ 200
- Major hypersensitivity 1

Ceriani et al J CardiothoracVasc Anesth 1995;9:477

Hypersensitivity Reactions to Aprotinin



Dietrich et al J Ann Thorac Surg 1998;65:S60-64

Testing for Hypersensitivity

- All patients should first receive a test dose of Trasylol to assess the potential for allergic reactions
- The 1 mL test dose should be administered intravenously at least 10 minutes before the loading dose
- Even after the uneventful administration of the test dose, the full therapeutic dose may cause anaphylaxis
- If this happens, the infusion should be stopped immediately and emergency treatment for anaphylaxis should be applied

Trasylol® Prescribing Information, Bayer Corporation

Management Recommendations

Patients With Prior Exposure

- Have standard emergency treatments for hypersensitivity or anaphylactic reactions readily available in the operating room
- Administration of the test dose and loading dose should only be done when the conditions for rapid cannulation (if necessary) are present
- Delay the addition of Trasylol® into the pump-prime solution until after the loading dose has been safely administered
- Additionally, administration of H_1 and H_2 blockers 15 minutes before the test dose may be considered

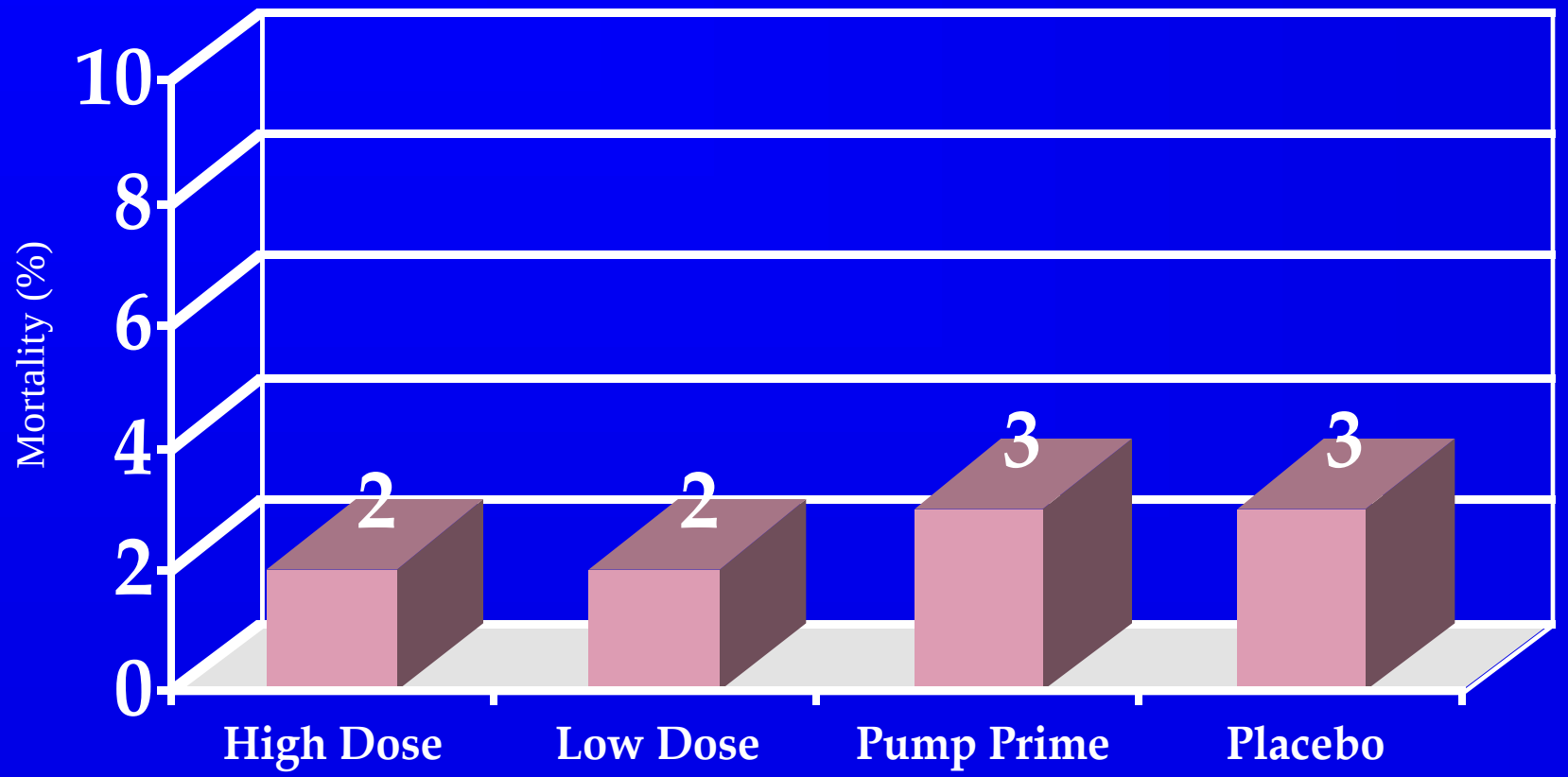
Trasylol® Prescribing Information, Bayer Corporation

Aprotinin-Drug Interactions

- Blocks the anti-hypertensive action of Captopril
- Inhibits action of thrombolytic agents *in vitro* and in animal studies
- Interferes with assays used to assess adequate heparinization during CPB

Mortality Rates

Primary CABG Surgery



Lemmer et al Ann Thorac Surg 1996;62:1659-68

Diagnosis of Myocardial Infarction

Based on Evaluation by a Blinded Core Laboratory

- Electrocardiogram
- Creatine kinase and CK-MB
- SGOT
- Lactic dehydrogenase

Definition of Myocardial Infarction

Definite MI

- Defined by a definite new Q wave on the EKG, or CK-MB levels _120 U/L at 6, 12, and 18 h postop

Definite or Probable MI

- Based on any or all of the information, including but not limited to enzyme values

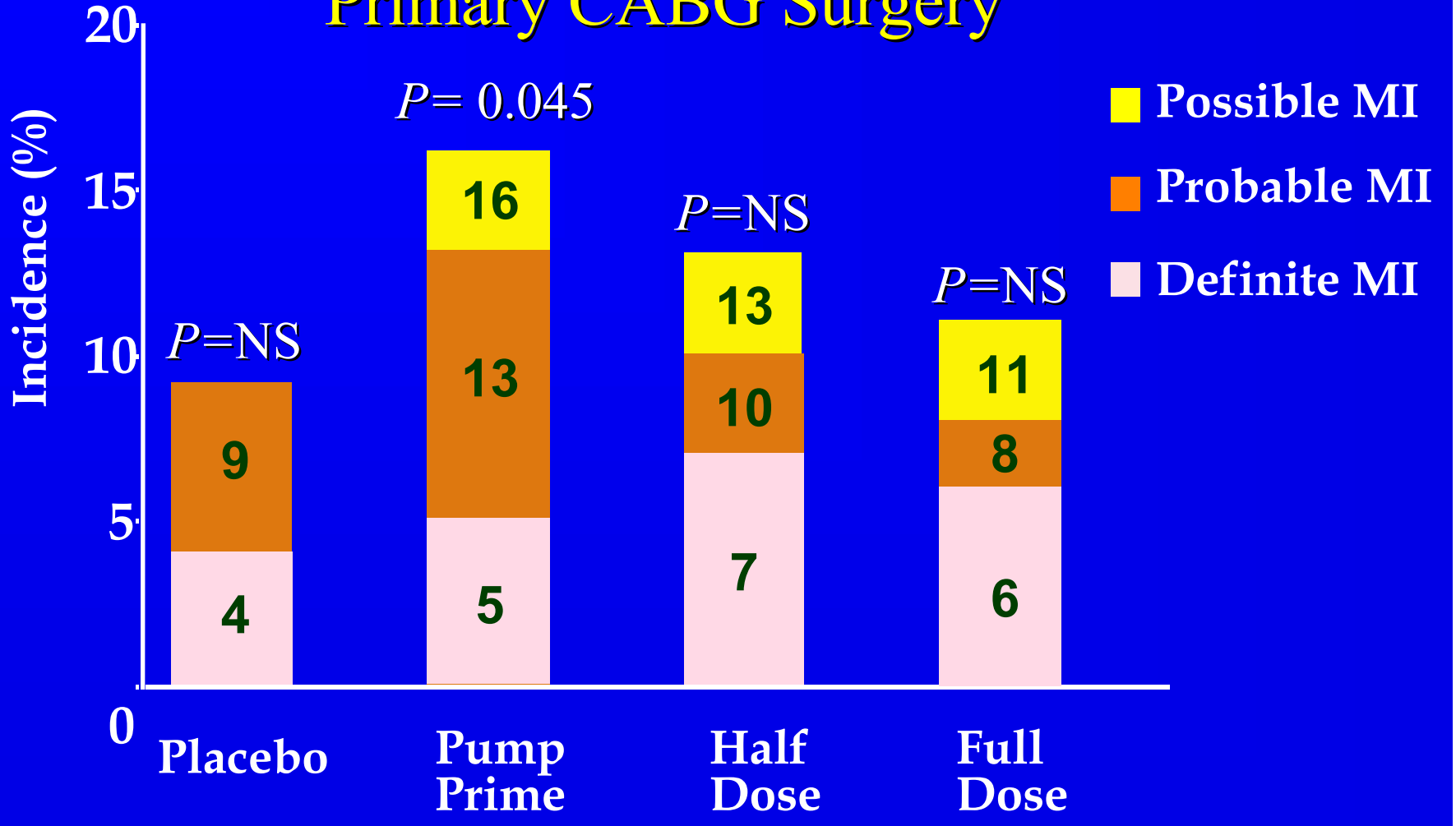
Definite, Probable, or Possible MI

- Based on any and all information

No MI

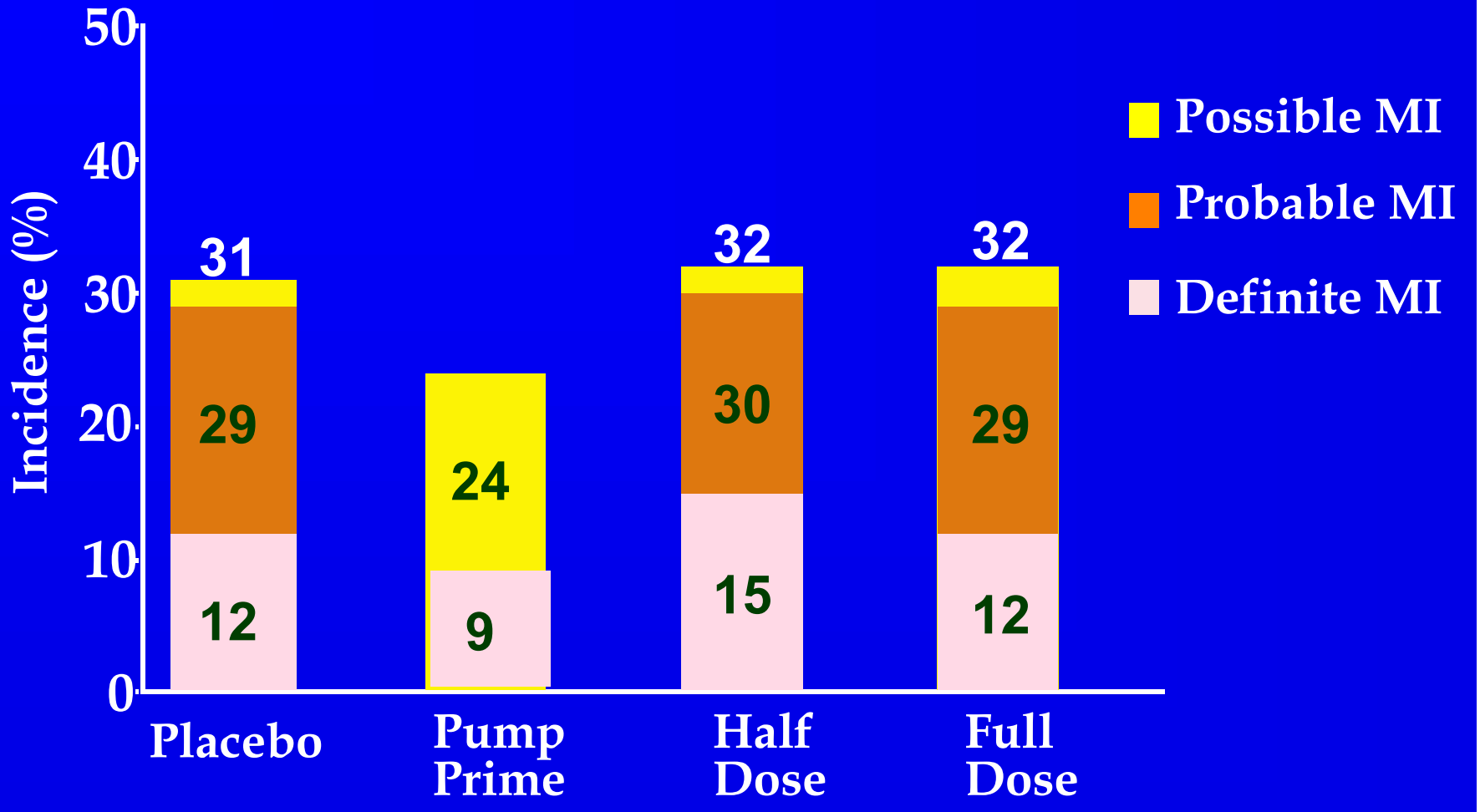
Incidence of Myocardial Infarction

Primary CABG Surgery



Lemmer et al Ann Thorac Surg 1996;62:1659-68

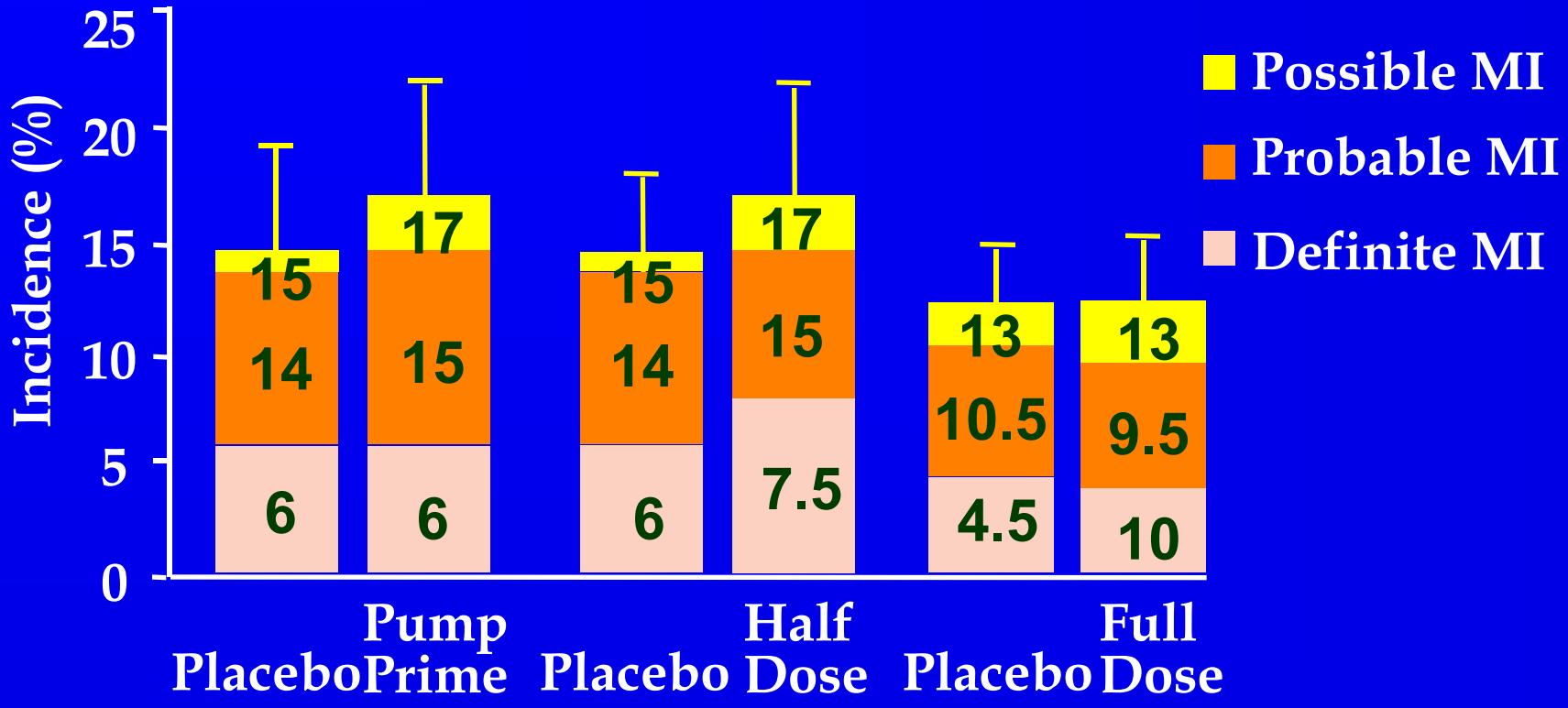
Incidence of Myocardial Infarction
Repeat CABG Surgery



Levy et al Circulation 1995;92:2236-44

Incidence of Myocardial Infarction

Effect of Aprotinin Dosing Regimens



Smith PK and Muhlbaier LH Ann Thorac Surg 1996;62:1575-7

Graft Patency Rates

	Placebo		Aprotinin	
Analysis by Graft	Occluded	Total	Occluded	Total
Bidstrup 1993 MRI	4	138	5	131
Havel 1994 Angio	2	40	2	39
Lemmer 1994 CT	8	163	14	176
Kalangos 1994 Angio	1	139	2	142
Lass 1995 Angio	13	102	13	124

Dobkowski et al Drug Safety 1998;18:21-41

Mortality Rates

Randomized Placebo Controlled Trials (2512 patients)

		High Dose (%)	Low Dose (%)	Placebo (%)
Cosgrove	1992	7	9	7
Lemmer	1994	6	-	4
Murkin	1994	6	-	0
Levy	1995	7	11	7
D'Ambra	1996	4	3	0
Lemmer	1996	2	2	3
Alderman	1998	1	-	2

Factors Affecting Graft Patency

Quality of Artery

- If > 2mm = occlusions in 17.6%
- If < 2mm = occlusions in 42.3%

Technique of Anastomosis of Distal End

- Single anastomosis failure in 9.2%
- Sequential anastomosis failure in 4.3%

Surgical Center

- Failure rate between centers showed a range of 7.1% to 57.1%

Refers to valve replacement (All NDA studies summarized-these data in Product Information)

Ollivier Arch Mal Cœur 1991;84:537-42

US Multicenter Vein Graft-Occlusion Study

Primary CABG Surgery

164 Patients at 5 Centers Evaluated by Ultrafast CT Scans

	Aprotinin	Placebo *P*
By Patient	13/83 15.7% NS	7/81 8.6%
By Graft	14/176 8.0% NS	8/163 4.9%

Lemmer et al J Thorac Cardiovasc Surg 1994;107:543-53

US Multicenter Vein Graft-Occlusion Study

Single-Center Analysis

	Aprotinin	Placebo
Occluded grafts	5/43 (11.6%)	0/38 (0%)
Poor target vessel	8/43	0/38

No differences between treatment groups in the incidence of perioperative MI assessed by enzymes or electrophysiology

Laub et al Chest 1994,106: 1370-75

US Multicenter Vein Graft-Occlusion Study

Centers	All	1 Center	4
By Patient			
Aprotinin (11.8%)	13/83	5/16	8/67
Placebo (10.8%)	7/81	0/16	7/65
By Graft			
Aprotinin (6.8%)	14/176	5/43	9/133
Placebo (6.4%)	8/163	0/38	8/125

Lemmer et al J Thorac Cardiovasc Surg 1994;107:543-53
Laub et al Chest 1994, 106: 1370-75

International Multicenter Aprotinin Graft Patency Experience (IMAGE)

Study Sites

- 10 US; 2 Israel; 1 Denmark

Patient Population

- 870 primary CABG patients randomized to receive placebo or full-dose aprotinin

Study Evaluations

- Graft patency
- Incidence of myocardial infarction
- Mortality
- Blood loss and transfusion requirements

Alderman et al J Thorac Cardiovasc Surg 1998;116:716-30

IMAGE

796 (91%) Patients assessable for blood loss/usage

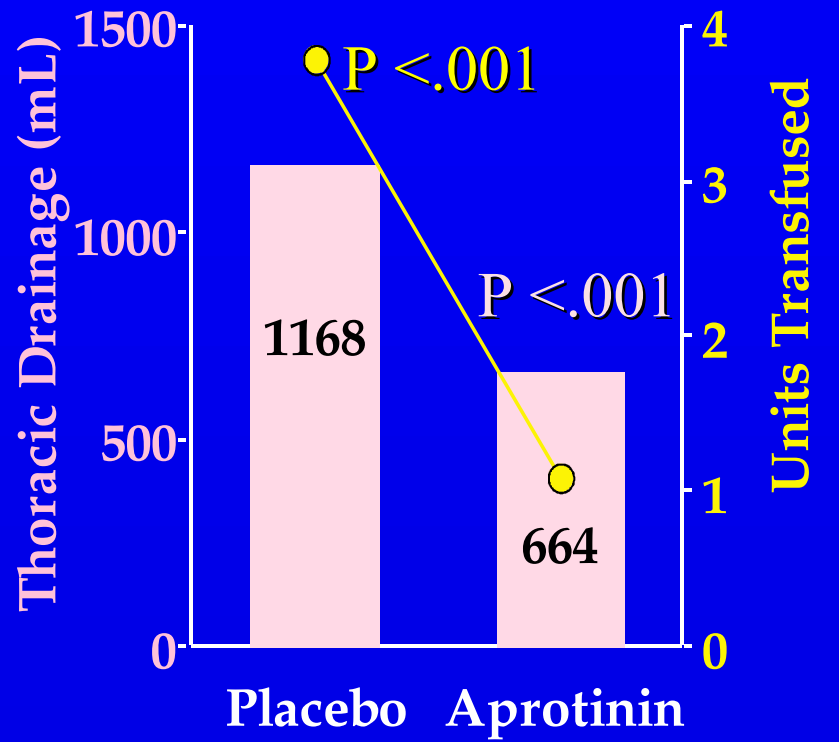
703 (81%) Patients assessable by angiography for saphenous vein-graft patency (at mean of 10.8 days postop)

831 (95%) Patients assessable for MI by ECG and cardiac enzyme evaluation

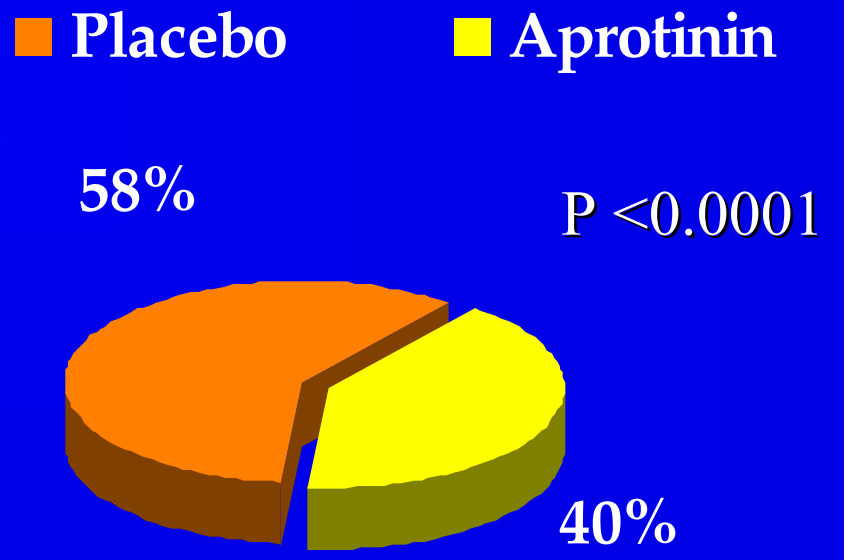
IMAGE Study

Blood Loss and Blood Product Replacement

Drainage and Transfusion



Patients Requiring Any Blood Product



Alderman et al J Thorac Cardiovasc Surg 1998;116:716-30

IMAGE Study

Internal Thoracic Artery Graft Occlusion

	Aprotinin	No Aprotinin	*P* Value
Patients Assessed	326	304	
% with Occluded Grafts	1.8	1.00	.32

Alderman et al J Thorac Cardiovasc Surg 1998;116:716-30

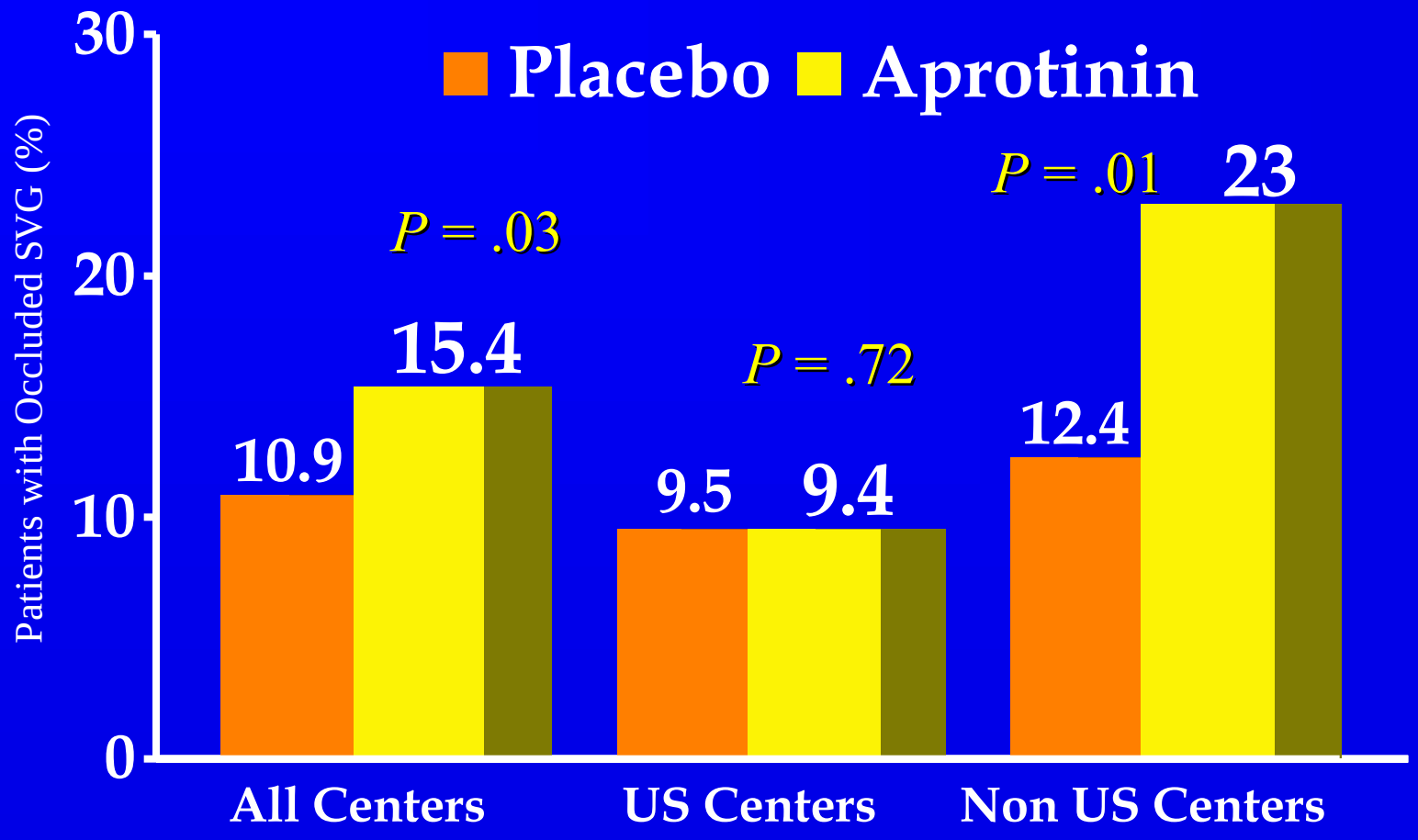
IMAGE Study

Saphenous Vein Graft Occlusion and Clinical Events

	Overall Vein Graft Closure Rates		Incidence of MI	Incidence of Death
	All Centers (n = 703) %	US Centers (n = 381) %	All Centers (n = 703) %	All Centers (n = 703) %
Aprotinin	15.4	9.4	2.9	1.4
Placebo	10.9	9.5	3.8	1.6

Alderman et al J Thorac Cardiovasc Surg 1998;116:716-30

IMAGE Study



Alderman et al J Thorac Cardiovasc Surg 1998;116:716-30

IMAGE Study

Adverse Outcome	Placebo	Aprotinin
Death	1.6% (6/434)	1.4% (5/436)
Myocardial Infarction		
Definite	3.8% (16/421)	2.9% (12/410)
Def+probable	9.1% (38/418)	8.6% (35/407)
Def+prob+possible	12.0% (50/418)	12.3% (50/408)

Alderman et al J Thorac Cardiovasc Surg 1998;116:716-30

IMAGE Study

Occluded SVG and Myocardial Infarction

	Placebo	Aprotinin
Angio + MI assessment	328	340
Occluded SVG	11% (36/328)	16% (54/340)
Occluded SVG + MI	31% (11/36)	20% (11/54)

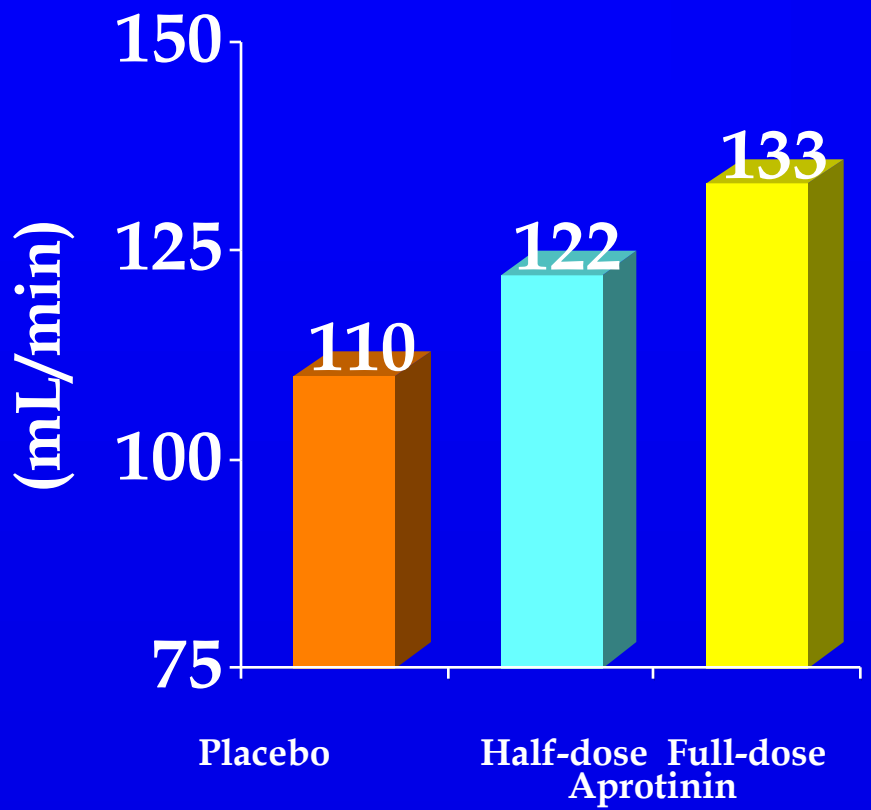
Alderman et al J Thorac Cardiovasc Surg 1998;116:716-30

Aprotinin Effects on Renal Function

- Transient and reversible effects
- May relate to:
  - Accumulation of drug in renal brush border
  - Inhibition of serine proteases associated with renal function
    - kallikrein
    - renin
  - Interaction with drug therapies
    - angiotensin-converting enzyme inhibitors

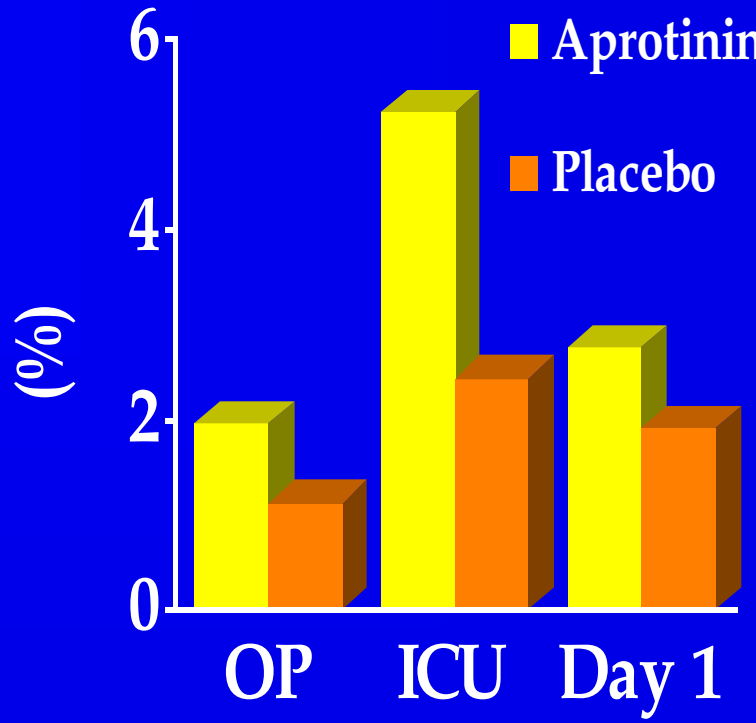
Renal Function

Creatinine Clearance



Cosgrove et al
Ann Thorac Surg 1992;54:1031

Fractional Na^+ Excretion



Blauhut et al
J Thorac Cardiovasc Surg 1991;101:958

Renal Dysfunction

Peak Increase in Serum Creatinine (mg/dL)

Value	Aprotinin	Placebo	*P*
	n = 108	n = 108	
➤ _0.5 - <1.0 mg/dL	13	9	0.37
➤ _1.0 - < 1.5 mg/dL	3	2	1.00
➤ _1.5 - < 2.0 mg/dL	1	2	1.00
➤ _ 2.0 mg/dL	3	0	0.25
Total	20	13	0.19
Dialysis	1	1	1.00

Lemmer et al Ann Thorac Surg 1995;59:132-136

Renal Dysfunction

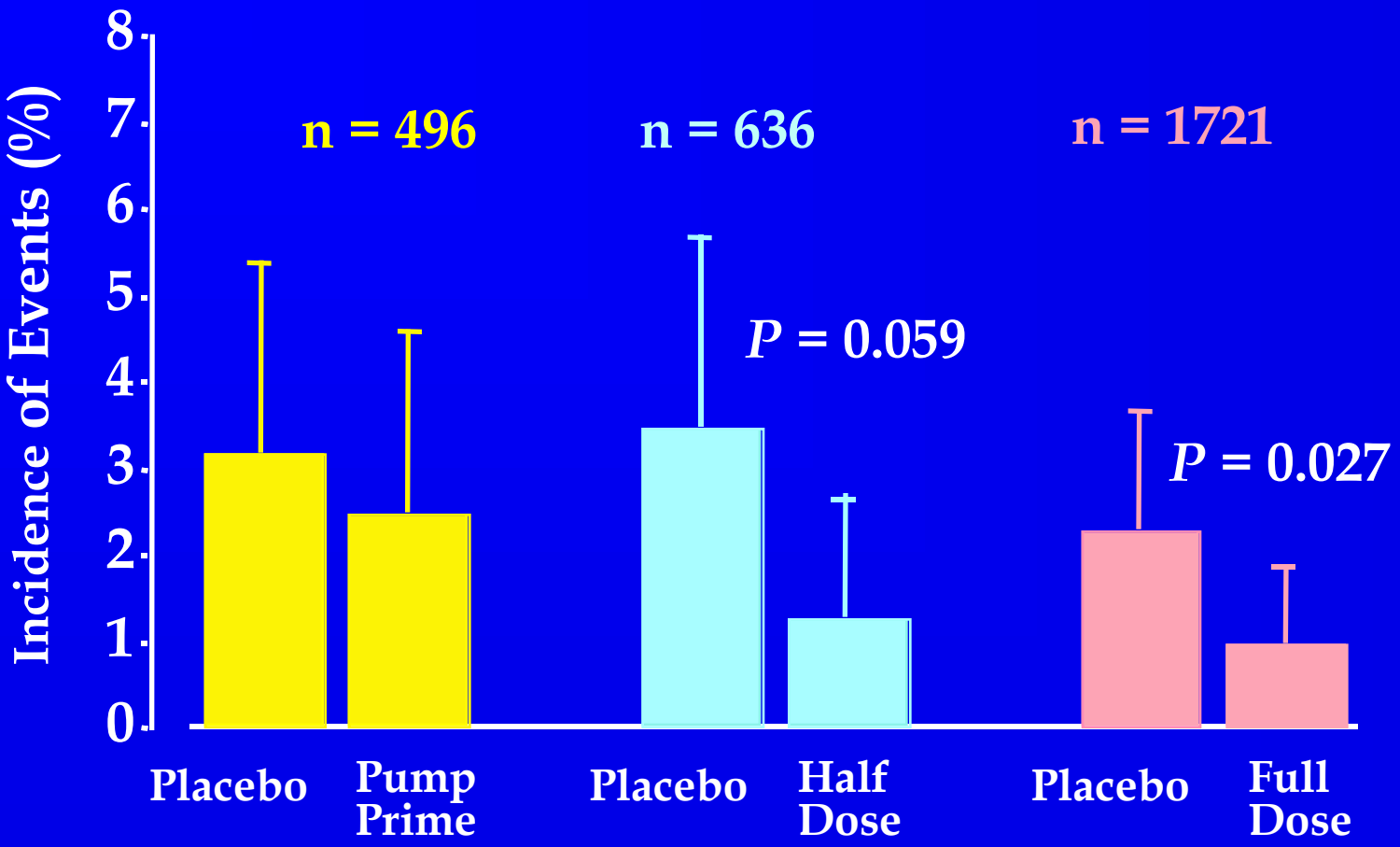
Postoperative Mean Serum Creatinine Level (mg/dL)

Treatment Group	Preop	Intensive Care Unit	Postoperative Day 1	2	3	4	7
Aprotinin	1.15	0.94	1.16	1.21	1.24	1.30	1.28*
Follow-up Visit	1.06						
Placebo	1.16	0.99	1.18	1.13	1.09	1.09	1.10
Follow-up Visit	1.07						

* $P = 0.047$

Lemmer et al Ann Thorac Surg 1995;59:132-136

Cerebrovascular Accident



Smith PK and Muhlbaier LH. Ann Thorac Surg 1996;62:1575-7

Neurologic Deficit (Stroke)

Incidence of Stroke Events in Repeat CABG Surgery

%		Number of Patients
Placebo 7		5 / 72
Aprotinin 1	Pump Prime	1 / 72
0	Low Dose	0 / 70
0	High Dose	0 / 73

$P = 0.01$

Levy et al Circulation 1995;92:2236 -44

Adverse Events

EVENT	Percentage of Patients Treated With Aprotinin n = 2002	Percentage of Patients Treated With Placebo n = 1084
Thrombosis	1.0	0.6
Shock	0.7	0.4
Cerebrovascular accident	0.7	2.1
Thrombophlebitis	0.2	0.5
Lung edema	1.3	1.5
Pulmonary embolus	0.3	0.6
Kidney failure	1.0	0.6
Acute kidney failure	0.5	0.6
Kidney tubular necrosis	0.8	0.4

Data from Trasylol® Package Insert