European Heart Journal (2000) **21** 2042–2055
doi:10.1053/euhj.2000.2309, available online at http://www.idealibrary.com on IDEAL®

Safety and preliminary efficacy of one month glycoprotein IIb/IIIa inhibition with lefradafiban in patients with acute coronary syndromes without ST-elevation

A phase II study

K. M. Akkerhuis[1,8], K.-L. Neuhaus[2,†], R. G. Wilcox[3], A. Vahanian[4], J.-L. Boland[5], J. Hoffmann[6], T. Baardman[7], G. Nehmiz[6], U. Roth[6], A. P. J. Klootwijk[1], J. W. Deckers[1,8], M. L. Simoons[1], for the Fibrinogen Receptor Occupancy STudy (FROST) Investigators*

[1]*Thoraxcenter, Erasmus University and University Hospital Rotterdam, The Netherlands;* [2]*Städtische Kliniken Kassel, Germany;* [3]*Queen's Medical Centre, University Hospital Nottingham, United Kingdom;* [4]*Hopital Tenon, Paris, France;* [5]*Hopital de la Citadelle, Liege, Belgium;* [6]*Boehringer Ingelheim Pharma KG, Germany;* [7]*Boehringer Ingelheim BV, The Netherlands;* [8]*Cardialysis BV, Clinical Research Management and Core Laboratories, Rotterdam, The Netherlands*

Aims Oral glycoprotein IIb/IIIa inhibitors might enhance the early benefit of an intravenous agent and prevent subsequent cardiac events in patients with acute coronary syndromes. We assessed the safety and preliminary efficacy of 1 month treatment with three dose levels of the oral GP IIb/IIIa blocker lefradafiban in patients with unstable angina or myocardial infarction without persistent ST elevation.

Methods The Fibrinogen Receptor Occupancy STudy (FROST) was designed as a dose-escalation trial with 20, 30 and 45 mg lefradafiban t.i.d. or placebo. Five hundred and thirty-one patients were randomized in a 3:1 ratio to lefradafiban or placebo in a double-blind manner. Efficacy was assessed by the incidence of death, myocardial infarction, coronary revascularization and recurrent angina. Safety was evaluated by the occurrence of bleeding classified according to the TIMI criteria and by measuring clinical laboratory parameters.

Results There was a trend towards a reduction in cardiac events with lefradafiban 30 mg when compared with placebo and lefradafiban 20 mg. The benefit was particularly apparent in patients with a positive ($\geq 0{\cdot}1$ ng . ml^{-1}) troponin I test at baseline and less so in those with a negative test result. In patients receiving lefradafiban, the cardiac event rate decreased with increasing minimal levels of fibrinogen receptor occupancy. There was a dose-dependent increase in the incidence of bleeding: the composite of major or minor bleeding occurred in 1% of placebo patients, 5% of patients receiving lefradafiban 20 mg and in 7% of patients receiving 30 mg, with an excessive risk (15%) in the 45 mg group which resulted in early discontinuation of this dose level. Gingival and arterial or venous puncture site bleedings were most common and accounted for more than 60% of all haemorrhagic events. There was an increased incidence of neutropenia (neutrophils $<1{\cdot}5 \times 10^9$/l) in the lefradafiban groups (5·2% vs 1·5% in the placebo group), which did not result from bone marrow depression but rather from a reversible redistribution of neutrophils by margination or clustering.

Conclusion One month's treatment with the oral glycoprotein IIb/IIIa inhibitor lefradafiban in patients with unstable angina and myocardial infarction without persistent ST elevation resulted in a decrease in cardiac events with lefradafiban 30 mg and a dose-dependent increase in haemorrhagic events. The observed favourable trend towards a reduction in cardiac events in patients with elevated troponin levels requires confirmation in a large clinical trial.
(Eur Heart J 2042–2055, doi:10.1053/euhj.2000.2309)

Key Words: Unstable angina, myocardial infarction, lefradafiban, glycoprotein IIb/IIIa blockers, platelet aggregation inhibitors.

See page 1992 for the Editorial comment on this article

Revision submitted 26 June 2000, and accepted 28 June 2000.

Correspondence: K. Martijn Akkerhuis, MD, Cardialysis BV, P.O. Box 2125, 3000 CC Rotterdam, The Netherlands.

*Investigators and study organization of the Fibrinogen Receptor Occupancy STudy are listed in the Appendix.

†Professor Neuhaus has died since the acceptance of this paper.

0195-668X/00/212042+14 $35.00/0

Introduction

Coronary thrombosis is a pivotal event in the pathogenesis of acute coronary syndromes and ischaemic complications resulting from coronary interventions[1–3]. The final common pathway to coronary thrombus formation involves aggregation of platelets via their glycoprotein (GP) IIb/IIIa receptors[4]. Intravenous inhibitors of GP IIb/IIIa receptors have demonstrated efficacy in reducing ischaemic complications in patients undergoing percutaneous coronary intervention and in those with unstable angina or myocardial infarction without persistent ST elevation[5–13]. Studies with these IIb/IIIa inhibitors have shown early clinical benefit during the short-term period of intravenous administration with no additional benefit after the infusions were stopped[12–15]. Despite intensive medical therapy, including short-acting GP IIb/IIIa inhibitors during the acute phase, outcomes among patients hospitalized with acute coronary syndromes remain unsatisfactory with a continuous increase in ischaemic events after discontinuation of initial therapy, such that the risk of death or myocardial infarction within the first month after development of the acute coronary syndrome is as high as 10–15%[12,13,16,17]. This may reflect incomplete healing of the vessel wall or the continuance of an activated haemostatic system for several weeks or months after the acute event[18,19]. These data suggest a need for prolonged and profound inhibition of platelet aggregation, which might be afforded by oral GP IIb/IIIa receptor blockers, in order to enhance the early benefit achieved by intravenous agents and prevent subsequent events.

Lefradafiban is an orally active prodrug which is metabolized in two steps to fradafiban, a non-peptide GP IIb/IIIa receptor inhibitor[20]. A recently conducted first phase II study confirmed that lefradafiban causes a dose-dependent inhibition of platelet aggregation which was safe when administered for 48 h in dosages up to 45 mg t.i.d. in patients with stable coronary artery disease undergoing percutaneous coronary intervention[20]. The present FROST study (Fibrinogen Receptor Occupancy STudy) was designed to assess the safety and preliminary efficacy of 1 month's treatment with different dose levels of lefradafiban in patients admitted with unstable angina or myocardial infarction without persistent ST elevation.

Methods

Study population

In 41 European centres, patients aged between 18 and 80 years with either unstable angina or non-ST-segment elevation myocardial infarction were eligible for enrolment if they presented within 24 h of the onset of chest pain and had ECG evidence of myocardial ischaemia (ST-segment depression, transient ST-segment elevation or T-wave changes). Criteria for exclusion included concomitant serious illness (active cancer or significant liver or renal disease), history of cerebrovascular accident or epilepsy, history of cranial or intraspinal surgery, active bleeding, peptic ulcer disease, past or present haemorrhagic diathesis or gastrointestinal bleeding within the preceding 3 months, recent major surgery or organ biopsy, puncture of a non-compressible vessel within the preceding 3 weeks, uncontrolled hypertension (systolic blood pressure above 200 mmHg or diastolic blood pressure above 100 mmHg), history of thrombocytopenia or platelet count $<100\,000$ per μl within the preceding 24 h, concurrent use of or anticipated need for oral anticoagulation, recent myocardial infarction or receipt of thrombolytic therapy, ECG abnormalities interfering with a reliable interpretation of the ST-segment (e.g. left ventricular hypertrophy with major repolarization changes or left bundle branch block), planned percutaneous coronary intervention or coronary bypass surgery within 24 h following enrolment, child-bearing potential, unwillingness to accept blood products, planned administration of a GP IIb/IIIa inhibitor or receipt of such agent within the preceding 30 days, or use of an investigational device or drug in the preceding 30 days. The protocol was approved by the institutional review board at each study centre and all patients gave written informed consent to participate.

Concomitant therapy

All patients were treated with aspirin and either unfractionated or low-molecular-weight heparin, according to local preference. Aspirin was administered orally in a dose of 150–250 mg immediately following the first intake of study drug and subsequently in a dose of 100 mg daily. Intravenous heparin was to be given as a bolus of 70 U . kg^{-1} (maximum 5000 U), followed by an infusion at a rate of 15 U . kg^{-1} per hour (maximum 1000 U per hour) for 2–5 days to achieve and maintain an activated partial thromboplastin time between 1·5 and 2·0 times the local control value. No recommendations were made with respect to the dosing of low-molecular-weight heparin which was given for 2–5 days. Other medications were given at the discretion of the treating physician.

Study design

The study was designed as a dose-escalation trial with 20, 30 and 45 mg lefradafiban t.i.d. or placebo. Within each dose level, patients were randomized in a 3:1 ratio to receive lefradafiban or placebo in a double-blind manner. These dose levels were selected based on results from previous studies with lefradafiban, to achieve and maintain mean values of platelet GP IIb/IIIa receptor inhibition (FRO = fibrinogen receptor occupancy) of 56%, 67% and 75%, respectively. Study medication was administered as an oral solution three times a day

and was to be continued for 30 days. It was not to be taken within 2 h after or 1 h prior to a meal. The first dose was given immediately after enrolment in the study while subsequent doses were given at 8-h intervals. An additional loading dose was administered 3·5 h following the first dose.

A Data and Safety Monitoring Board was established to continuously monitor the data on safety and efficacy and to provide continued surveillance as necessary in case of untoward bleeding complications or other adverse events. The decision to proceed to a higher dose level was made after this Board had reviewed the safety profile of the preceding dose level. The protocol did not prespecify statistical rules for stopping the study. If the Data and Safety Monitoring Board recommended adjustment in the study design or early cessation of the trial or a certain dose level, the Steering Committee reviewed the recommendation and made the final decision.

Clinical and laboratory monitoring

Patients underwent physical examination and extensive laboratory evaluation for haematology, coagulation and biochemistry at baseline and at regular intervals during hospitalization and subsequent 30-day follow-up. Qualitative determination of cardiac troponin-I status was performed at baseline. The assessment was performed by the local hospital staff using the Cardiac STATus™ rapid format troponin-I bedside assay (Spectral Diagnostics Inc, threshold 0·1 ng . ml^{-1}). ECGs were obtained before enrolment and both during as well as 30 min after episodes of chest pain. Additional ECGs were recorded 2, 3 and 30 days after enrolment. Patients were continually assessed for the occurrence of bleeding complications and other adverse events. After hospital discharge, patients returned for a follow-up visit every 7 days during the first 5 weeks and then at 2 and 6 months after enrolment.

During enrolment and follow-up in the 20 mg dose level, it was observed that patients receiving lefradafiban more frequently exhibited a clinically relevant drop in leukocyte count as compared with placebo. To obtain a more complete overview on the occurrence, time course and severity of the leukopenia and to provide an increased surveillance on the safety of study patients, a protocol amendment was made which increased the frequency of the haematology evaluations and added blood samples to be taken for central analysis to further investigate the potential underlying pathophysiological mechanisms. In patients in whom a drop in leukocyte count was observed (i.e. value below the lower limit of normal and/or decrease in leukocytes of at least 30% relative to the baseline value), measurements were to be continued until leukocyte count had normalized or returned to otherwise medically acceptable values. From these samples, the incidence of neutropenia was re-estimated using absolute cell count cut-off values similar to the ranges defined for ticlopidin. This method accommodates for the relative decline in leukocyte count which occurs in patients with acute myocardial infarction and unstable angina after hospital treatment. In two patients, puncture of bone marrow was performed. Intake of trial medication was discontinued if leukocyte count dropped below 50% of the lower limit of normal.

Pharmacokinetics and pharmacodynamics

In all lefradafiban dose groups, blood samples were drawn at baseline and day 2, as well as during the weekly follow-up visits for determination of the fradafiban plasma concentration and associated pharmacokinetic parameters. Levels of fibrinogen receptor occupancy were calculated from the fradafiban plasma concentration using a pharmacokinetic model whose parameters were established from preceding studies[20].

ECG core laboratory

Computer-assisted continuous 12-lead ECG-ischaemia monitoring (ELI ST-100, Mortara Instruments, Milwaukee, U.S.A.) was performed, starting immediately after the intake of the first study medication and continuing for 24 h to detect and quantify recurrent ischaemia. All continuous ECG recordings were analysed at the ECG core laboratory (Cardialysis BV) by independent reviewers unaware of treatment assignment. The procedures of editing and analysis of the continuous ECG recording data, as developed and applied by the core laboratory, have been described in detail elsewhere[22,23]. The onset of an ischaemic episode was defined as either ST depression or ST elevation of at least 100 μV in one or more of the 12 ECG leads developing within a 10-min period and persisting for at least 1 min. The number of patients with recurrent ischaemia, the number of ischaemic episodes and the ischaemic burden in patients with recurrent ischaemia were determined.

Study end-points

The primary safety end-point in this trial was the occurrence of bleeding complications classified as major, minor, or insignificant according to the criteria of the Thrombolysis in Myocardial Infarction (TIMI) Study Group[24]. Major bleeding was defined as intracranial haemorrhage or bleeding associated with a drop of 3·1 mmol . l^{-1} (5 g . dl^{-1}) or more in the haemoglobin concentration or of 15 percentage points or more in the haematocrit. Bleeding was defined as minor if it was spontaneous and observed as gross haematuria or haematemesis, or if blood loss was observed with a drop of 1·9 mmol . l^{-1} (3 g . dl^{-1}) or more in haemoglobin or of 10 percentage points or more in the haematocrit. If no

bleeding site was identifiable, a drop of 2·5 mmol . l^{-1} (4 g . dl^{-1}) or more in haemoglobin or of 12 percentage points or more in the hematocrit was considered to indicate minor bleeding. Blood loss insufficient to meet criteria for minor bleeding was classified as insignificant. To account for transfusion, haemoglobin and hematocrit values were adjusted if patients received packed red blood cells or whole blood within 48 h prior to the measurement by using the method of Landefeld *et al.*[25].

The efficacy end-point was the composite of any of the following events during the 1 month treatment period: death from any cause, non-fatal myocardial infarction, any percutaneous coronary intervention or coronary artery bypass grafting. Myocardial infarction was considered to have occurred if there was an elevation of creatine kinase (CK)-MB or CK above the upper limit of normal in at least two samples with one value above twice the upper limit of normal. Following percutaneous and surgical revascularization, the elevation of cardiac enzyme levels had to be at least 3 and 5 times above the upper limit of normal, respectively. Suspected infarctions were assessed by a Clinical Events Committee blinded to treatment assignment. Secondary end-points included the recurrence of unstable angina during study treatment (defined as chest pain with concomitant ischaemic ECG changes) as well as the number of patients exhibiting recurrent ischaemia and the ischaemic burden in patients with ischaemia as detected and quantified by continuous 12-lead ECG-ischaemia monitoring within 24 h after the start of study medication.

Statistical analysis

Based on an expected rate of study drug discontinuation of 25%, the number of patients to be enrolled in each lefradafiban group to evaluate the primary safety outcomes was approximately 100. Therefore, approximately 132 patients were to be enrolled in each dose level (99 lefradafiban and 33 placebo). Continuous variables are presented as means with standard deviation, and dichotomous variables as percentages. Data from the three placebo groups were combined to provide more stable outcome estimates. Bleeding incidences were determined at 72 h after the last intake of study medication in order to provide the most conservative estimate of the safety of lefradafiban. The times from start of study treatment to the first bleeding event are displayed as Kaplan–Meier curves censored at 72 h after the last intake. A multiple logistic regression analysis for predictors of bleeding was performed, which also included the fibrinogen receptor occupancy, duration of heparin use and median on-treatment aPTT level. Frequencies of the clinical efficacy end-points were determined on an intention-to-treat basis at 30 days after randomization, as well as from randomization until 72 h after the last intake of study medication to account for the high number of patients discontinuing study treatment. The efficacy parameters were evaluated among all patients as well as among patients with elevated troponin-I levels at baseline vs those with normal levels.

Fibrinogen receptor occupancy-efficacy outcome analysis

The relationship between the level of fibrinogen receptor occupancy and the composite of death, myocardial infarction and recurrent unstable angina was investigated in patients who received lefradafiban and had at least one sample for determination of fradafiban plasma concentration within 8 h of last study drug administration. The fibrinogen receptor occupancy was calculated from up to four plasma concentrations. Per patient, the minimum of the fibrinogen receptor occupancy values was investigated in relation to cardiac outcome. As this was a mechanistic type of analysis, the frequency of the composite end-point was determined while patients were still receiving treatment.

Results

A total of 531 patients were enrolled between April 1997 and October 1998: 218 patients received lefradafiban 20 mg t.i.d., 136 lefradafiban 30 mg t.i.d., 47 lefradafiban 45 mg t.i.d. and 130 placebo. In the initial study design, approximately 132 patients were to be enrolled in each dose level (99 lefradafiban and 33 placebo). During enrolment in the first (20 mg) dose level, however, it became apparent that more patients than anticipated discontinued trial medication due to revascularization procedures (percutaneous coronary intervention with subsequent ticlopidin and CABG). The use of ticlopidin together with lefradafiban was excluded by the protocol because no interaction data were available at that time. To obtain more safety data on this dose level, the Data and Safety Monitoring Board advised increasing the group size of this dose level to 300 patients before proceeding to the next (30 mg) dose level. The FROST trial was discontinued in October 1998 after 61 patients had been enrolled in the 45 mg dose level (47 assigned lefradafiban and 14 placebo). At that time, the Data and Safety Monitoring Board recommended cessation of recruitment and discontinuation of treatment in this dose group since five (11%) of 47 patients receiving lefradafiban 45 mg had had a major bleeding complication.

There were no substantial differences in baseline characteristics among the treatment groups (Table 1): 69% were male, 33% had had a previous myocardial infarction, 14% had undergone a percutaneous coronary intervention and 11% coronary artery bypass surgery. In all treatment groups, study drug was discontinued prematurely in more than 50% of patients (Fig. 1 and Table 2). Early discontinuation was highest among patients treated with lefradafiban 45 mg (60%, excluding

Table 1 Baseline characteristics

	Placebo n=130	Lefradafiban		
		20 mg t.i.d. n=218	30 mg t.i.d. n=136	45 mg t.i.d. n=47
Age (years)	63 (10)	62 (10)	63 (10)	63 (12)
Gender (% male)	74	67	68	72
Weight (kg)	80 (15)	78 (13)	78 (15)	80 (15)
Height (cm)	171 (9)	170 (9)	171 (9)	171 (10)
SBP (mmHg)	128 (19)	131 (21)	131 (22)	121 (19)
DBP (mmHg)	74 (12)	76 (12)	75 (14)	70 (11)
Heart rate (beats . min^{-1})	72 (11)	71 (13)	72 (13)	69 (14)
Diabetes (%)	15	15	21	17
Current smoker (%)	28	30	26	30
Previous MI (%)	32	31	38	34
Previous PCI (%)	12	15	15	11
Previous CABG (%)	13	11	12	4
CHF (%)	6	3	4	6

For continuous variables, the mean values are provided with the standard deviation in parentheses. CABG=coronary artery bypass grafting; CHF=congestive heart failure; DBP=diastolic blood pressure; MI=myocardial infarction; PCI=percutaneous coronary intervention; SBP=systolic blood pressure.

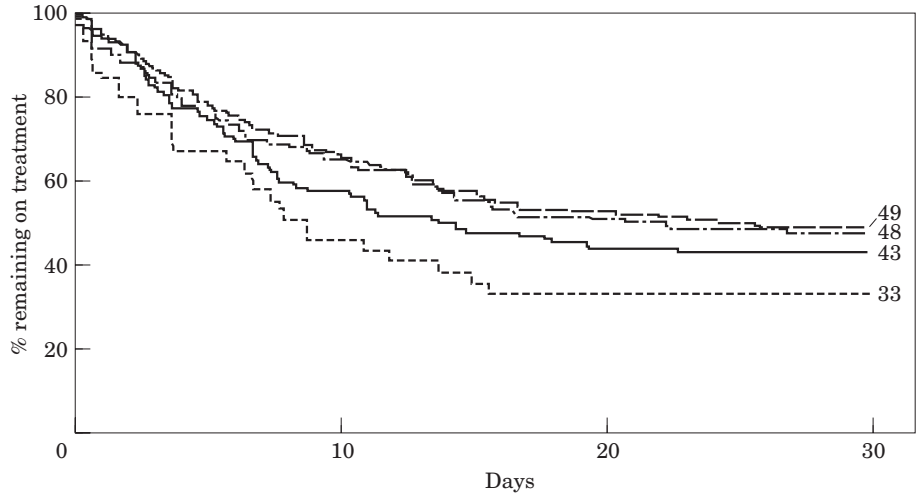

Figure 1 **Kaplan–Meier estimates of the probability of continuing the study drug during the 30-day treatment period. — — —=20 mg; — . —=placebo; ——=30 mg; – – – –=45 mg.**

those discontinued prematurely due to the early cessation of the trial), followed by patients in the 30 mg group (58%), with slightly lower rates in the placebo and 20 mg treatment arms (55% and 51%, respectively). Discontinuation occurred mainly within the first 2 weeks of study drug administration. The majority of the patients who did not withdraw from study drug during this period completed the intended 30-day treatment period. Among all treatment groups, major reasons for study drug discontinuation were the occurrence of an adverse event (mostly bleeding), planned coronary artery bypass surgery and the use of other antiplatelet agents (abciximab, ticlopidin) during or following percutaneous coronary intervention (Table 2).

Efficacy results

Cardiovascular events

Event rates determined from start of treatment up to 72 h of study drug discontinuation showed a trend towards a beneficial effect of lefradafiban 30 mg when compared with placebo and lefradafiban 20 mg on the incidence of death or myocardial (re)infarction according to the Clinical Events Committee. When compared with placebo, there was a 30% relative reduction in the composite end-point of death, myocardial (re)infarction, percutaneous coronary intervention or CABG in the lefradafiban 30 mg group (Table 3). Also, the composite outcome of death, myocardial (re)infarction or recurrent

Table 2 Reasons for early discontinuation from study drug (%)

	Placebo n=130	Lefradafiban		
		20 mg t.i.d. n=218	30 mg t.i.d. n=136	45 mg t.i.d. n=47
Total of patients	55%	51%	58%	77%
Reasons				
Early stop 45 mg group	2			17
Adverse event	12	15	23	21
PCI (ticlopidin/abciximab)	15	12	12	19
CABG	12	11	10	6
Consent withdrawn	0	2	4	2
Normal angiography	4	2	4	0
Other	10	10	6	11

Abbreviations, see legend to Table 1.

Table 3 Efficacy outcomes (%)

	Placebo n=130	Lefradafiban			*P*
		20 mg t.i.d. n=218	30 mg t.i.d. n=136	45 mg t.i.d. n=47	
At 72 h following last study drug administration					
Death/MI	3·1%	3·2%	2·2%	4·3%	0·72
Death/MI/PCI/CABG	31·5	30·7	22·1	25·5	0·096
Death/MI/AP-rehosp	7·7	6·9	2·9	6·4	0·10
Death/MI/AP-any	17·7	17·0	4·4	12·8	<0·001
At 30-day follow-up					
Death/MI	3·1	4·1	4·4	4·3	0·75
Death/MI/PCI/CABG	43·1	35·9	32·6	40·4	0·077

Percentages refer to total number of patients in each treatment group. *P*-value (2-sided) provided according to Fisher's Exact Test for comparison between placebo and lefradafiban 30 mg. AP-any=any recurrent unstable angina pectoris; AP-rehosp=recurrent angina pectoris leading to rehospitalization; CABG=coronary artery bypass grafting; MI=myocardial infarction as adjudicated by the Clinical Events Committee; PCI=percutaneous coronary intervention.

angina leading to rehospitalization determined at 72 h after study drug discontinuation was reduced in patients receiving lefradafiban 30 mg (Table 3). A similar pattern was apparent when the incidence of death, myocardial (re)infarction or any recurrent unstable angina was evaluated among the four treatment groups. At 30 days, the incidence of death or myocardial (re)infarction was low and comparable among all treatment groups, while the reduction in the 30-day composite of death, myocardial (re)infarction, percutaneous coronary intervention or CABG was less pronounced (relative reduction 24% when compared with placebo, Table 3).

Efficacy by troponin-I status

Of the 531 patients entered, 455 (86%) had troponin-I assay results available. The test result at baseline was positive ($\geq 0{\cdot}1$ ng . ml^{-1}) in 118 (26%) of these patients and was negative in 337 (74%) patients. The proportion of patients with positive vs negative troponin-I assay results were comparable among the four treatment arms. In each treatment group, the incidence of the 30-day composite end-point of death, myocardial (re)infarction, percutaneous coronary intervention or CABG was higher among patients with a positive troponin-I test result at baseline when compared with those with a negative test result (Fig. 2). In patients with a positive troponin-I as well as in those with a negative test result, the composite end-point occurred most frequently in patients receiving placebo, while the event rate decreased with increasing doses of lefradafiban, with the exception of the 45 mg dose (very small number of patients). The dose-dependent reduction in event rate associated with lefradafiban 20 mg and 30 mg appeared greater in patients with a positive troponin-I at baseline (ca. 15% and 30%, respectively) than in those with a negative test result (10% and 13%).

Efficacy by fibrinogen receptor occupancy level

Three hundred and fifty six patients received lefradafiban and had at least one evaluable fradafiban plasma concentration value for determination of the fibrinogen receptor occupancy level. There was a clear relationship between the minimum fibrinogen receptor occupancy level and the observed incidence of the

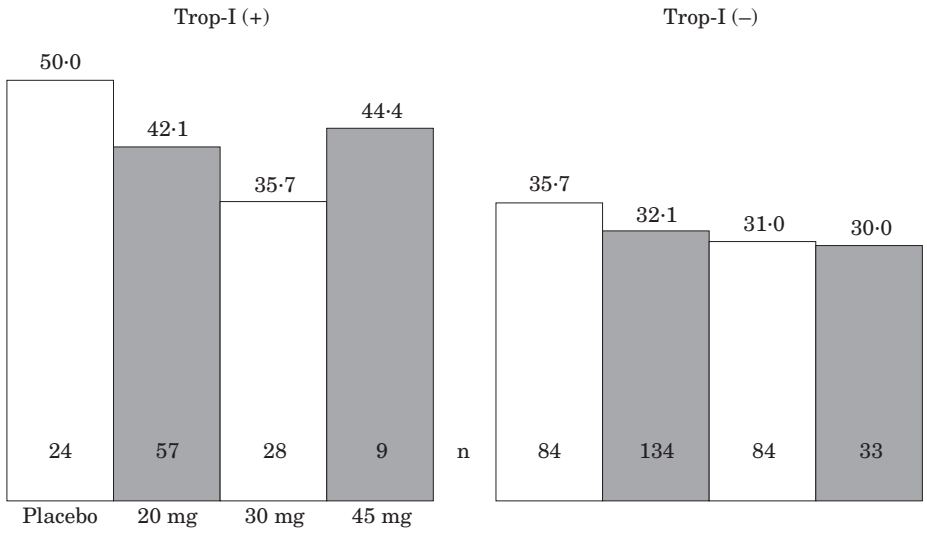

Figure 2 **Incidence of the composite end-point of death, MI, PCI or CABG at 30-day follow-up among patients with a positive troponin-I test result at baseline (left panel) vs those with a negative test result (right panel). CABG = coronary artery bypass grafting; MI = myocardial infarction as adjudicated by the Clinical Events Committee; PCI = percutaneous coronary intervention.**

composite of death, myocardial infarction and any recurrent unstable angina (Fig. 3). The event rate decreased with increasing minimal fibrinogen receptor occupancy levels. The lowest event rate was found in patients with a minimal fibrinogen receptor occupancy level of at least 70%. In contrast, in patients with a minimal fibrinogen receptor occupancy level below 50%, the cardiac event rate appeared higher than in those receiving placebo (Fig. 3).

ECG–ischaemia monitoring results

Four hundred and thirteen patients (78%) had continuous ECG recordings suitable for analysis. During the 24-h monitoring period, ischaemic episodes were detected in 33 (33%) of the 99 placebo patients, 56 (31%) of the 178 lefradafiban 20 mg patients, 25 (25%) of the 101 lefradafiban 30 mg patients and 16 (46%) of the 35 lefradafiban 45 mg patients. There were no differences between groups in the number of recurrent ischaemic episodes or the amount of ischaemic burden.

Haemorrhagic events

A dose-dependent increase in bleeding incidence was observed with the majority of the bleedings occurring during the first 2 weeks of study drug administration (Fig. 4). The incidence of major or minor bleeding complications was low in patients receiving placebo (1%) while it gradually increased with higher doses of lefradafiban, up to 15% in patients treated with 45 mg t.i.d. (Table 4). Intracranial haemorrhage occurred in a single patient who was treated with thrombolysis for acute myocardial infarction while receiving lefradafiban 20 mg (Table 5). The percentage of patients who required a blood transfusion ranged from 1% in the placebo group to 9% in the 45 mg group with intermediate figures for the 20 mg and 30 mg groups. A dose-related increase for bleeding complications leading to discontinuation from study drug was observed. A similar dose-related increase was apparent for insignificant bleeding events; the percentage of patients in the placebo group experiencing any bleeding was 19% as compared with 39% in the 20 mg group, 57% in the 30 mg group and 67% in the 45 mg group (Table 4). Among all treatment groups, gingival and arterial or venous puncture site bleedings were most common and accounted for more than 60% of all haemorrhagic events (Table 5).

By multiple logistic regression analysis (model terms: fibrinogen receptor occupancy, age, weight and estimated creatinine clearance as continuous measurements, as well as gender), higher levels of fibrinogen receptor occupancy and a higher age were found to be significantly related to an increased bleeding incidence. The odds for bleeding increased with a factor of 1·023 for every increase in fibrinogen receptor occupancy level by 1% point (95% confidence interval (CI), 1·016–1·030) and 1·022 for 1 year increase in age (95% CI, 1·003–1·044). In addition, gender proved to be a significant predictor of bleeding (female vs male, odds ratio 1·54 with 95% CI, 1·03–2·46). In separate analyses, no statistically significant association was found between the occurrence of bleeding and the duration of heparin therapy or the median on-treatment aPTT level.

Haematological changes

Treatment with lefradafiban was associated with an increased incidence of leukopenia, or, more precisely,

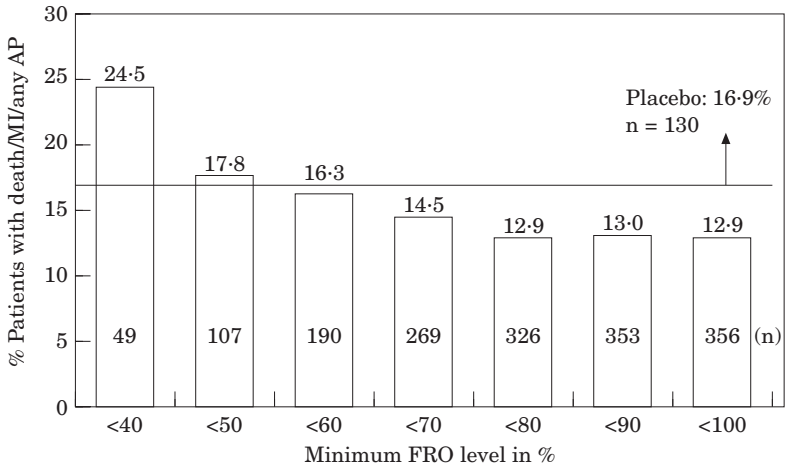

Figure 3 **Incidence of the composite end-point of death, myocardial infarction or any recurrent angina in relation to the minimum fibrinogen receptor occupancy level. Event rates are determined while patients were still receiving study treatment. AP = any recurrent angina; FRO = fibrinogen receptor occupancy; MI = myocardial infarction as adjudicated by the Clinical Events Committee.**

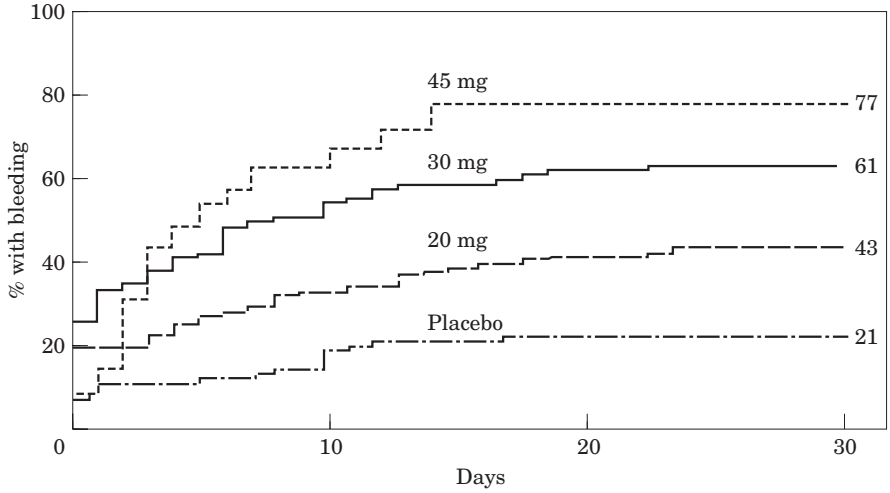

Figure 4 **Kaplan–Meier estimates of the occurrence of any bleeding complication. Bleeding episodes are included up to 72 h following last study drug administration.**

neutropenia. The incidence of leukopenia, defined as leukocyte count below 4×10^9/l, was estimated at 5·7% in the lefradafiban groups compared with 2·3% in the placebo group. Neutropenia (neutrophils below $1{\cdot}5 \times 10^9$/l) occurred in 5·2% of the lefradafiban groups and 1·5% of the placebo group. In the patients with observed neutropenia, the decrease in neutrophils was characterized by an early onset (immediately after first study drug administration or within the next 2 days) and a fast recovery after discontinuation of lefradafiban (Fig. 5). Although the clinical symptoms of chills and fever occurred in patients with neutropenia, none developed a serious infection or permanent neutrophil deficiency. Based on central analysis of blood and bone marrow samples obtained in patients with severe neutropenia by independent expert haematologists, it was concluded that the observed neutropenia most likely resulted from a reversible redistribution of neutrophils by margination or clustering rather than from bone marrow depression. All bone marrow samples showed continued cellularity with a deficiency of later-stage cells of the granulocyte series. There was no evidence of aplastic anaemia or changes associated with agranulocytosis. In addition, patients with neutropenia responded to treatment with G-CSF or steroids with prompt normalization of their neutrophil counts. In patients with a simultaneous decrease in other blood cell populations the pattern was consistent with a generalized redistribution but not with impaired production by or release of cells from the bone marrow.

Table 4 Incidence and severity of bleeding events until 72 h after study drug discontinuation (%)

	Placebo n=130	Lefradafiban		
		20 mg t.i.d. n=218	30 mg t.i.d. n=136	45 mg t.i.d. n=47
Bleeding event				
TIMI — major	1%	3%	3%	11%
TIMI — minor	0	1	4	6
TIMI — major or minor	1	5	7	15
Requiring transfusion	1	2	4	9
Leading to discontinuation	2	5	10	15
Any	19	39	57	67

Percentages refer to total number of patients in each treatment group.
TIMI=Thrombolysis in Myocardial Infarction.

Table 5 Location of bleeding (%)

	Placebo n=130	Lefradafiban		
		20 mg t.i.d. n=218	30 mg t.i.d. n=136	45 mg t.i.d. n=47
Bleeding event				
Intracranial	0%	1%	0%	0%
Retroperitoneal	0	1	0	0
Gastrointestinal	0	7	10	11
Genitourinary	3	5	10	4
Haematoma	5	9	9	11
Oral/gingival/epistaxis	3	15	31	28
Puncture site	12	19	29	34

Percentages refer to total number of bleedings located at each site.

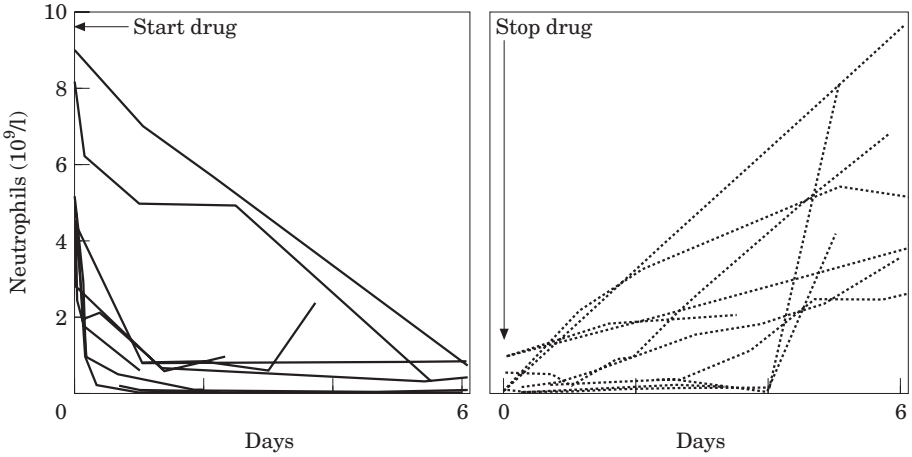

Figure 5 **Neutrophil counts in individual patients experiencing neutropenia, in relation to study drug start and discontinuation.**

Thrombocytopenia (platelets below 90×10^9/l) occurred in only two (0·5%) of 401 patients treated with lefradafiban. No placebo recipient had thrombocytopenia.

Discussion

In this double-blind, randomized, dose-escalation trial of 1 month GP IIb/IIIa inhibition with lefradafiban in

patients with acute coronary syndromes, we observed a decrease in cardiovascular events with lefradafiban 30 mg and a dose-dependent increase in haemorrhagic events.

Safety

A close relationship between the dose of lefradafiban administered, the plasma concentration of fradafiban, the fibrinogen receptor occupancy, and the degree of platelet inhibition have been established in previous studies with lefradafiban and fradafiban, both in healthy volunteers and in patients with coronary artery disease[20,21].

Bleeding

As in other studies with long-term treatment with oral GP IIb/IIIa receptor blockers, bleeding occurred frequently and was dose-dependent. However, the majority of the bleedings were mild or clinically insignificant. The incidences of bleeding complications in the lefradafiban 20 mg and 30 mg groups were similar to the rates observed with other oral GP IIb/IIIa receptor blockers at similar levels of platelet inhibition[26–30], while the very high risk of major or minor bleeding in the 45 mg group resulted in cessation of recruitment and discontinuation of treatment in this dose group. The results of the multivariable logistic regression analysis in the present study support earlier observations with an increase in the risk of bleeding of 2·3% for every increase in fibrinogen receptor occupancy level by 1% point[26,27]. The magnitude of this association is similar to that found in a previous study of lefradafiban in patients with stable coronary artery disease undergoing elective percutaneous coronary intervention[21].

The clinical pattern of bleeding was largely mucocutaneous: epistaxis, gingival bleeding, gastro-intestinal and genito-urinary bleeding, or bruising. No excess strokes were observed with lefradafiban. This pattern has also been observed with other GP IIb/IIIa receptor blockers[6,13,26–30], and is similar to that seen with thrombocytopenia and in Glanzmann's thrombasthenia[31]. Arterial and venous puncture sites were the second most common location of bleeding. Other studies have demonstrated that bleeding at vascular puncture sites can be reduced by the use of low-dose, weight-adjusted heparin regimens, early femoral arterial sheath removal and careful access site management[7,10,32]. Although the protocol recommended a low-dose, weight-adjusted heparin regimen during the early phase of medical treatment, no specific heparin dosing regimen during percutaneous coronary intervention was provided.

Given the inter-patient variability in drug level and degree of platelet inhibition observed with oral GP IIb/IIIa receptor antagonists, another potential strategy for reducing bleeding complications could be to monitor the degree of platelet inhibition achieved in individual patients and to adjust the dose to a target level, as is done with anticoagulant therapy[33,34].

Neutropenia and thrombocytopenia

Treatment with lefradafiban was associated with an increased incidence of neutropenia[21]. Whereas leukopenia due to bone marrow depression has been reported for other antiplatelet agents (ticlopidin)[35], expert analysis of blood and bone marrow samples obtained in FROST patients revealed that the observed neutropenia most likely did not result from bone marrow depression but rather from a reversible redistribution of neutrophils by margination or clustering. It was reassuring that all patients showed a fast recovery of neutrophil count after discontinuation of lefradafiban, and that none developed infection or permanent neutrophil deficiency. However, more investigations are needed to further elucidate the exact mechanisms involved, as well as to determine the optimal duration of surveillance and the possible clinical associations.

The incidence of thrombocytopenia associated with lefradafiban (0·5%) was low and similar to that reported for other oral GP IIb/IIIa receptor blockers[26–30].

Efficacy

The 30-day incidence of death or non-fatal myocardial infarction was low in this study. The study was not designed to detect differences in clinical outcomes between the treatment groups, yet there was a trend towards a reduction in cardiovascular events in the lefradafiban 30 mg group compared with placebo and lefradafiban 20 mg. The sample size for the 45 mg cohort of patients was too small to detect a meaningful trend. The reduction in the composite of death, myocardial infarction, percutaneous coronary intervention or CABG was greater in the on-treatment analysis than when the end-point was determined at 30 days. This suggests that the treatment benefit may be enhanced in patients who continue on medical therapy[36]. In patients undergoing percutaneous coronary intervention or CABG, the post-procedural event rates are very low and no benefit is to be expected[28,37].

Patients with a positive troponin-I bedside assay at baseline were at increased risk of unfavourable outcome. This observation is concordant with the results of previous studies which have shown that among patients with unstable angina, elevated serum troponin-T and troponin-I levels are independent predictors of short- and long-term risk of adverse cardiac events[38–41]. Troponin-T and troponin-I reflect minimal or larger myocardial injury due to occlusion of the culprit vessel or distal embolization of platelet thrombi originating from the culprit lesion[42,43]. As glycoprotein IIb/IIIa receptor blockers inhibit thrombus formation at the culprit lesion and facilitate the resolution of thrombi[44], they are expected to be particularly effective in patients with elevated troponin levels[45,46]. Indeed, treatment benefit among FROST patients appeared greatest in

those with positive troponin-I at baseline. This observation parallels those of the FRISC and FRISC II trials, and the recently reported troponin substudies of the CAPTURE and PRISM trials[36,38,45,46]. The data from the present study therefore support the therapeutic concept that elevated troponin-T or troponin-I levels identify the group of patients with acute coronary syndromes and active thrombosis who are at high risk for cardiac events and who will benefit most from a more intensive treatment strategy including the administration of GP IIb/IIIa receptor blockers and low-molecular-weight heparin.

The trend towards a reduction in clinical events with lefradafiban 30 mg in this study contrasts with the results of recent, large-scale clinical trials of long-term oral GP IIb/IIIa inhibition with xemilofiban, orbofiban and sibrafiban in patients after percutaneous coronary intervention[28] and acute coronary syndromes[29,30]. In this respect, several points deserve consideration. First, the risk of recurrent ischaemic events in the patient populations included in these trials may have been too low to detect benefit of long-term GP IIb/IIIa receptor blockade. In the EXCITE trial, xemilofiban or placebo was administered prior to and for 6 months after percutaneous coronary intervention[28]. In accordance with the results of previous trials of intravenous GP IIb/IIIa receptor blockers, xemilofiban protected against procedure-related complications. However, the risk of subsequent events following percutaneous coronary intervention was very low, and no further benefit was observed. In OPUS-TIMI-16, patients were included for up to 72 h following an episode of chest pain, while ischaemic ECG changes or positive cardiac enzymes were not mandatory for enrolment[29]. Long-term treatment with orbofiban in this trial resulted in a modest 11% relative reduction in cardiac events at 30 days, primarily due to a reduction in urgent coronary intervention. In the SYMPHONY trial, patients could be included for up to 7 days after the onset of the acute coronary syndrome, and had to be stabilized for more than 12 h from the initial presentation[30]. Almost 25% of all patients underwent a percutaneous coronary intervention between the qualifying episode and randomization. No benefit was observed with sibrafiban after 90 days of treatment. All three trials showed a trend towards increased mortality in the GP IIb/IIIa inhibitor treatment groups.

The pharmacokinetic and pharmacodynamic profile of these agents is characterized by a steep dose-response relationship and by a short half-life relative to the dosing interval[26,27,29]. Thus, the twice-daily dosing regimen in the orbofiban and sibrafiban trials may have caused widely fluctuating inhibition of platelet aggregation[29,30]. This may have allowed complete recovery in platelet function between doses in some patients, with exposure of activated GP IIb/IIIa receptors on the platelet's surface[47]. Whereas it has been reported that high peak blood levels of oral GP IIb/IIIa receptor blockers increase the risk of bleeding[26,27], recent studies of platelet activation have also raised the possibility that platelet receptor antagonists may, at low concentrations, alter the steric conformation of the GP IIb/IIIa receptor sites, and paradoxically, enhance the thrombogenicity of these sites[47,48].

In this study, the cardiac event rate decreased with increasing minimal fibrinogen receptor occupancy levels, with the lowest event rate found in patients with a minimal fibrinogen receptor occupancy level of at least 70%. In contrast, patients with a minimal fibrinogen receptor occupancy level below 50% appeared to have a higher cardiac event rate than patients receiving placebo. These findings suggest that an effective treatment with the GP IIb/IIIa receptor antagonist lefradafiban can be anticipated with marked levels of inhibition of platelet aggregation (i.e. fibrinogen receptor occupancy levels of at least 70%).

It should be emphasized that the present study was not designed and powered to definitively evaluate differences in clinical outcomes between treatment groups. The observed favourable trend in the lefradafiban 20 mg and 30 mg groups with a reduction in clinical events requires confirmation in an adequately-powered clinical trial.

Conclusion

It is a challenge to exploit the potential beneficial antithrombotic effect of oral GP IIb/IIIa inhibitors in relation to the associated risk of haemorrhage[26,49]. Dose-adjustment on the basis of patient characteristics that influence drug levels, such as renal function and body weight[26,29,30], as well as dose-titration to a target level of platelet inhibition, measured with a rapid platelet-function assay[33,34], may improve the overall safety and efficacy profile. To increase the treatment benefit of (oral) GP IIb/IIIa receptor blockers, one may choose to treat only patients who are at high risk of adverse cardiac events, such as those who present with elevated troponin levels or who exhibit recurrent ischaemia and continue on medical therapy[23,38,45,46]. These patients may particularly benefit from a more aggressive therapeutic approach[38,45,46].

Although data from this study may suggest that an effective treatment with the GP IIb/IIIa receptor antagonist lefradafiban can only be anticipated with marked levels of inhibition of platelet aggregation (i.e. fibrinogen receptor occupancy levels of at least 70%), the level of platelet inhibition needed to prevent recurrent ischaemic cardiac events, as well as the optimal duration of treatment after an acute coronary syndrome require further investigation. A potential treatment strategy for patients admitted with acute coronary syndromes would include the administration of a rapid-acting intravenous GP IIb/IIIa receptor blocker during the acute phase, followed by conversion to an orally active preparation of the same compound for prolonged outpatient secondary prevention[49]. If a patient undergoes percutaneous coronary intervention, it may be advisable to continue

the oral drug, or to convert to the intravenous preparation during and for 12–24 h following the intervention[5–10]. As the risk of subsequent events following percutaneous coronary intervention is low, no further treatment with oral GP IIb/IIIa receptor blockers seems required[28,37]. The need for concomitant anticoagulant therapy should be evaluated.

The intravenous GP IIb/IIIa receptor blocker fradafiban and its orally active prodrug lefradafiban, as two complementary preparations of the same compound[20], are suited to be used in such strategy, and might be evaluated for their efficacy in reducing ischaemic cardiac events among patients with acute coronary syndromes in a large clinical trial.

The FROST study was supported by Boehringer Ingelheim.

References

[1] Davies MJ, Thomas AC. Plaque fissuring — the cause of acute myocardial infarction, sudden ischemic death, and crescendo angina. Br Heart J 1985; 53: 363–73.

[2] Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992; 326: 242–50, 310–8.

[3] Ambrose JA, Weinrauch M. Thrombosis in ischemic heart disease. Arch Intern Med 1996; 156: 1382–94.

[4] Lefkovits J, Plow EF, Topol EJ. Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine. N Engl J Med 1995; 332: 1553–9.

[5] The EPIC Investigators. Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. N Engl J Med 1994; 330: 956–61.

[6] The CAPTURE Investigators. Randomised placebo-controlled trial of abciximab before and during coronary intervention in refractory unstable angina: the CAPTURE study. Lancet 1997; 349: 1429–35.

[7] The EPILOG Investigators. Platelet glycoprotein IIb/IIIa receptor blockade and low-dose heparin during percutaneous coronary revascularization. N Engl J Med 1997; 336: 1689–96.

[8] The IMPACT-II Investigators. Randomised placebo-controlled trial of effect of eptifibatide on complications of percutaneous coronary intervention: IMPACT-II. Lancet 1997; 349: 1422–8.

[9] The RESTORE Investigators. Effects of platelet glycoprotein IIb/IIIa blockade with tirofiban on adverse cardiac events in patients with unstable angina or acute myocardial infarction undergoing coronary angioplasty. Circulation 1997; 96: 1445–53.

[10] The EPISTENT Investigators. Randomised placebo-controlled and balloon-angioplasty-controlled trial to assess safety of coronary stenting with use of platelet glycoprotein-IIb/IIIa blockade. Lancet 1998; 352: 87–92.

[11] The PRISM Study Investigators. A comparison of aspirin plus tirofiban with aspirin plus heparin for unstable angina. N Engl J Med 1998; 338: 1498–505.

[12] The PRISM-PLUS Study Investigators. Inhibition of the platelet glycoprotein IIb/IIIa receptor with tirofiban in unstable angina and non-Q-wave myocardial infarction. N Engl J Med 1998; 338: 1488–97.

[13] The PURSUIT Trial Investigators. Inhibition of platelet glycoprotein IIb/IIIa with eptifibatide in patients with acute coronary syndromes. N Engl J Med 1998; 339: 436–43.

[14] Tanguay J-F. Do differences in pharmacology of platelet glycoprotein IIb/IIIa inhibitors affect clinical outcomes. Eur Heart J Supplements 1999; 1 (Suppl E): E27–35.

[15] Ronner E, Dykun Y, Van den Brand MJBM, Van der Wieken LR, Simoons ML. Platelet glycoprotein IIb/IIIa receptor antagonists. An asset for treatment of unstable coronary syndromes and coronary intervention. Eur Heart J 1998; 19: 1608–16.

[16] Fragmin During Instability in Coronary Artery Disease (FRISC) Study Group. Low-molecular-weight heparin during instability in coronary artery disease. Lancet 1996; 347: 561–8.

[17] The TIMI IIIB Investigators. Effects of tissue plasminogen activator and a comparison of early invasive and conservative strategies in unstable angina and non-Q-wave myocardial infarction. Results of the TIMI IIIB trial. Circulation 1994; 89: 1545–56.

[18] Merlini PA, Bauer KA, Oltrona L *et al*. Persistent activation of the coagulation system in unstable angina and myocardial infarction. Circulation 1994; 90: 61–8.

[19] Ault KA, Cannon CP, Mitchell J *et al*. Platelet activation in patients after an acute coronary syndrome: results from the TIMI-12 trial. Thrombolysis in Myocardial Infarction. J Am Coll Cardiol 1999; 33: 634–9.

[20] Müller TH, Weisenberger H, Brickl R, Narjes H, Himmelsbach F, Krause J. Profound and sustained inhibition of platelet aggregation by fradafiban, a nonpeptide platelet glycoprotein IIb/IIIa antagonist, and its orally active prodrug, lefradafiban, in men. Circulation 1997; 96: 1130–8.

[21] Van den Brand MJBM, Baardman T, Suryapranata H *et al*. Initial experience with BIBU 104XX; a new, oral glycoprotein IIb/IIIa receptor blocker. Eur Heart J 1997; 18 (Abstr Suppl): 244.

[22] Klootwijk P, Meij S, van Es Ga *et al*. Comparison of usefulness of computer assisted continuous 48-h 3-lead with 12-lead ECG ischaemia monitoring for detection and quantitation of ischaemia in patients with unstable angina. Eur Heart J 1997; 18: 931–40.

[23] Klootwijk P, Meij S, Melkert R, Lenderink T, Simoons ML. Reduction of recurrent ischemia with abciximab during continuous ECG-ischemia monitoring in patients with unstable angina refractory to standard treatment (CAPTURE). Circulation 1998; 98: 1358–64.

[24] Rao AK, Pratt C, Berke A *et al*. Thrombolysis in Myocardial Infarction (TIMI) Trial-phase I: hemorrhagic manifestations and changes in plasma fibrinogen and the fibrinolytic system in patients treated with recombinant tissue plasminogen activator and streptokinase. J Am Coll Cardiol 1988; 11: 1–11.

[25] Landefeld CS, McGuire E, Rosenblatt MW. A bleeding risk index for estimating the probability of major bleeding in hospitalized patients starting anticoagulant therapy. Am J Med 1990; 89: 569–78.

[26] Cannon CP, McCabe CH, Borzak S *et al*. for the TIMI 12 Investigators. Randomized trial of an oral platelet glycoprotein IIb/IIIa antagonist, sibrafiban, in patients after an acute coronary syndrome: results of the TIMI 12 trial. Circulation 1998; 97: 340–9.

[27] Kereiakes DJ, Kleiman NS, Ferguson JJ *et al*. for the ORBIT Trial Investigators. Pharmacodynamic efficacy, clinical safety, and outcomes after prolonged platelet glycoprotein IIb/IIIa receptor blockade with oral xemilofiban: results of a multicenter, placebo-controlled, randomized trial. Circulation 1998; 98: 1268–78.

[28] O'Neill WW, Serruys P, Knudtson M *et al.* Long-term treatment with a platelet glycoprotein-receptor antagonist after percutaneous coronary revascularization. N Engl J Med, 2000, 342: 1316–1324.

[29] Cannon CP, McCabe CH, Wilcox RG *et al.* for the OPUS-TIMI-16 Investigators. Oral glycoprotein IIb/IIIa inhibition with orbofiban in patients with unstable coronary syndromes (OPUS-TIMI 16) trial. Circulation 2000; 102: 149–156.

[30] The SYMPHONY Investigators. Comparison of sibrafiban with aspirin for prevention of cardiovascular events after acute coronary syndromes: a randomized trial. Lancet 2000; 355: 337–45.

[31] George JN, Caen JP, Nurden AT. Glansmann's thrombasthenia: the spectrum of clinical disease. Blood 1990; 75: 1383–95.
[32] Lincoff AM, Tcheng JE, Califf RM *et al.* for the PROLOG Investigators. Standard versus low-dose weight-adjusted heparin in patients treated with the platelet glycoprotein IIb/IIIa receptor antibody fragment abciximab (c7E3 Fab) during percutaneous coronary revascularization. Am J Cardiol 1997; 79: 286–91.
[33] Coller BS. Monitoring platelet GP IIb/IIIa antagonist therapy. Circulation 1998; 97: 5–9.
[34] Smith JW, Steinhubl SR, Lincoff AM *et al.* Rapid platelet-function assay: an automated and quantitative cartridge-based method. Circulation 1999; 99: 620–5.
[35] Yeh SP, Hsueh EJ, Wu H, Wang YC. Ticlopidine-associated aplastic anemia. A case report and review of literature. Ann Hematol 1998; 76: 87–90.
[36] The FRISC II Investigators. Long-term low-molecular-mass heparin in unstable coronary–artery disease: FRISC II prospective randomised multicentre study. Lancet 1999; 354: 701–7.
[37] FRISC II Investigators. Invasive compared with non-invasive treatment in unstable coronary-artery disease: FRISC II prospective randomised multicentre study. Lancet 1999; 354: 708–15.
[38] Lindahl B, Venge P, Wallentin L. Troponin T identifies patients with unstable coronary artery disease who benefit from long-term antithrombotic protection. FRISC study group. J Am Coll Cardiol 1997; 29: 43–8.
[39] Ohman EM, Armstrong PW, Christenson RH *et al.* for the GUSTO-IIa Investigators. Cardiac Troponin T levels for risk stratification in acute myocardial ischemia. N Engl J Med 1996; 335: 1333–41.
[40] Antman EM, Tanasijevic MJ, Thompson B *et al.* Cardiac-specific Troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. N Engl J Med 1996; 335: 1342–9.
[41] Hamm CW, Meinertz T, Berger J, Kreymann G, Heeschen C, Goldmann BU. Emergency room triage of patients with acute chest pain by means of rapid testing for cardiac troponin T or troponin I. N Engl J Med 1997; 337: 1648–53.
[42] Falk E. Unstable angina with fatal outcome: dynamic coronary thrombosis leading to infarction and/or sudden death: autopsy evidence of recurrent mural thrombosis with peripheral embolization culminating in total vascular occlusion. Circulation 1985; 71: 699–708.
[43] Davies MJ, Thomas AC, Knapman PA, Hangartner JR. Intramyocardial platelet aggregation in patients with unstable angina suffering sudden ischemic cardiac death. Circulation 1986; 73: 418–27.
[44] Heeschen C, Van den Brand MJ, Hamm CW, Simoons ML. Angiographic findings in patients with refractory unstable angina according to troponin T status. Circulation 1999; 100:1509–14.
[45] Hamm CW, Heeschen C, Goldmann B *et al.* for the CAPTURE Investigators. Benefit of abciximab in patients with refractory unstable angina in relation to serum troponin T levels. N Engl J Med 1999; 340: 1623–9.
[46] Hamm CW, Heeschen C, Goldmann BU, White HD. Benefit of tirofiban in high-risk patients with unstable angina identified by troponins in the PRISM trial. Circulation 1999; 18 I-775: 4092.
[47] Peter K, Straub A, Kohler B *et al.* Platelet activation as a potential mechanism of GP IIb/IIIa inhibitor-induced thrombocytopenia. Am J Cardiol 1999; 84: 519–24.
[48] Peter K, Schwarz M, Ylanne J *et al.* Induction of fibrinogen binding and platelet aggregation as a potential intrinsic property of various glycoprotein IIb/IIIa ($\alpha_{IIB}\beta_3$) inhibitors. Blood 1998; 92: 3240–9.
[49] Vorchheimer DA, Fuster V. Oral platelet glycoprotein IIb/IIIa receptor antagonists: the present challenge is safety. Circulation 1998; 97: 312–4.

Appendix

FROST study organization

Steering Committee
ML Simoons (Study Chairman, Rotterdam, The Netherlands); K-L Neuhaus (Kassel, Germany); RG Wilcox (Nottingham, United Kingdom); A Vahanian (Paris, France); J-L Boland (Liège, Belgium); J Hoffmann, A Barner (Boehringer Ingelheim, Germany); T Baardman (Boehringer Ingelheim, The Netherlands)

Data and Safety Monitoring Board
HR Büller (Amsterdam, The Netherlands), JGP Tijssen (Statistician, Amsterdam, The Netherlands)

Coordinating centres
The Netherlands: Boehringer Ingelheim NL: T Baardman, M Hendriks; Cardialysis BV: KM Akkerhuis, JW Deckers, ML Simoons; *Germany*: Boehringer Ingelheim Pharma KG: J Hoffmann, A Riedel, G Nehmiz; *United Kingdom*: Boehringer Ingelheim Limited: J Kaye, G Temple; Nottingham Clinical Trial Data Centre: I Borland; *France*: Boehringer Ingelheim France: C Courseau, I Assier, B Riquet; *Belgium*: Boehringer Ingelheim Belgium: L Oostvogels

Core laboratory for continuous ECG monitoring
Cardialysis BV, Clinical Research Management and Core Laboratories, Rotterdam, The Netherlands: KM Akkerhuis, APJ Klootwijk, E vd Leur, JW Deckers

Expert review neutropenia data
DC Dale (Seattle, WA, U.S.A.), M Lichtman (Rochester, NY, U.S.A.), HR Büller (Amsterdam, The Netherlands), CLM Arkesteijn (Woerden, The Netherlands)

Clinical Events Committee
Cardialysis BV, Clinical Research Management and Core Laboratories, Rotterdam, The Netherlands: WAJ Bruggeling, P van der Meer, H van Meurs, EJ Müller, P Nierop, PP Kint, KM Akkerhuis

Study centres and principal investigators
The Netherlands (217 patients)
MC Alkmaar, *VAWM Umans* (42); Sint Franciscus Gasthuis, *P Nierop* (37); Tweesteden Ziekenhuis, *H Baars* (27); Spaarne Ziekenhuis, *EJ Müller* (23); Academisch Ziekenhuis Rotterdam-Dijkzigt, *C vd Zwaan, ML Simoons* (22); Ziekenhuis Hilversum, *P de Milliano* (22); MC Leeuwarden, *R Breedveld* (14); Ziekenhuis Sint Jansdal, *R Dijkgraaf* (12); Ziekenhuis De Tjongerschans, *GM Jochemsen* (7); Sint Clara Ziekenhuis, *M Scheffer* (5); Ziekenhuis De Weezenlanden, *H Suryapranata* (4); Hofpoort Ziekenhuis, *E Wajon* (2).

Germany (115 patients)
Johannes Gutenberg-Universität Mainz, *H Darius* (41); Klinikum Nürnberg Süd, *M Gottwik, W Riego* (41);

Klinikum d. Albert-Ludwigs-Universität, *C Holubarsch, H Lüdemann* (10); Universität Heidelberg, *C Bode* (7); Städtische Kliniken Kassel, *K-L Neuhaus, U Zeymer* (6); St Marien Krankenhaus Siegen, *P Schuster* (4); Städtische Krankenhaus Traunstein, *K Schlotterbeck* (4); Städtische Kliniken Offenbach, *F Praetorius* (1); Kreiskrankenhaus Lüdenscheid, *K Henrichs* (1).

United Kingdom (103 patients)
Antrim Hospital, *T Trouton* (41); University Hospital Nottingham, *RG Wilcox* (26); King's Mill Hospital, *JM Rowley* (10); Birmingham City Hospital, *RDS Watson* (6); The Royal Alexandra Hospital, *IN Findlay* (5); Nottingham City Hospital, *DC Banks* (4); Leeds General Infirmary, *G Reynolds* (3); Royal Victoria Hospital, *AAJ Adgey* (3); North Manchester General Hospital, *JW Swan* (2); Victoria Hospital, *M Francis* (2); Hartlepool General Hospital, *G Tildesley* (1).

France (92 patients)
Hôpital Tenon, *A Vahanian* (28); Hôpital Notre Dame de Bon Secours, *K Khalife* (25); Centre Hospitalier de Lorient, *P Cazaux* (18); Centre Hospitalier Cochin, *P Richard* (10); Centre Hospitalier Germon et Gauthier, *C Mycinski* (7); Centre Hospitalier de Lagny, *T Domniez* (2); Hôpital de Freyming Merlebach, *P Dambrine* (1); Centre Hospitalier Universitaire de Poitiers, *D Coisne* (1).

Belgium (4 patients)
Hôpital de la Citadelle, *J-L Boland* (4).