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

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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 (≥0.1 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 <1.5x10⁹/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.

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

See page 1992 for the Editorial comment on this article

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*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.
Introduction

Coronary thrombosis is a pivotal event in the pathogenesis of acute coronary syndromes and ischaemic complications resulting from coronary interventions\(^1\). 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\(^5\). 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\(^12\,\,13\). 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 i.v.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 presuppose 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[29]. 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 infarction or surgical revascularization procedures (percutaneous coronary intervention with subsequent ticlopidin and CABG). Myocardial infarction was considered to have occurred if there was an elevation of cardiac enzyme levels had to be at least 3 and 5 times above the upper limit of normal, respectively. Suspected infarction or surgical revascularization procedures (percutaneous coronary intervention with subsequent ticlopidin and CABG).

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). 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.

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
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
angina leading to rehospitalization determined at 72 h after study drug discontinuation was reduced in patients receiving lefrada 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 (≥ 0.1 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 lefrada, with the exception of the 45 mg dose (very small number of patients). The dose-dependent reduction in event rate associated with lefrada 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 lefrada and had at least one evaluable frada 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

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

<table>
<thead>
<tr>
<th>Reason</th>
<th>Placebo n=130</th>
<th>Lefrada 20 mg t.i.d. n=218</th>
<th>Lefrada 30 mg t.i.d. n=136</th>
<th>Lefrada 45 mg t.i.d. n=47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total of patients</td>
<td>55%</td>
<td>51%</td>
<td>58%</td>
<td>77%</td>
</tr>
<tr>
<td>Early stop 45 mg group</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse event</td>
<td>12</td>
<td>15</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>PCI (ticlopidin/abciximab)</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>CABG</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Consent withdrawn</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Normal angiography</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>11</td>
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</tbody>
</table>

**Table 3 Efficacy outcomes (%)**

<table>
<thead>
<tr>
<th>Time point</th>
<th>Placebo n=130</th>
<th>Lefrada 20 mg t.i.d. n=218</th>
<th>Lefrada 30 mg t.i.d. n=136</th>
<th>Lefrada 45 mg t.i.d. n=47</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 72 h following last study drug administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death/MI</td>
<td>3.1%</td>
<td>3.2%</td>
<td>2.2%</td>
<td>4.3%</td>
<td>0.72</td>
</tr>
<tr>
<td>Death/MI/PCI/CABG</td>
<td>31.5%</td>
<td>30.7%</td>
<td>22.1%</td>
<td>25.5%</td>
<td>0.096</td>
</tr>
<tr>
<td>Death/MI/AP-rehosp</td>
<td>77%</td>
<td>69%</td>
<td>29</td>
<td>64</td>
<td>0.10</td>
</tr>
<tr>
<td>Death/MI/AP-any</td>
<td>17.7%</td>
<td>17.0%</td>
<td>4.4</td>
<td>12.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>At 30-day follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death/MI</td>
<td>3.1%</td>
<td>4.1%</td>
<td>4.4</td>
<td>4.3</td>
<td>0.75</td>
</tr>
<tr>
<td>Death/MI/PCI/CABG</td>
<td>43.1%</td>
<td>35.9%</td>
<td>32.6</td>
<td>40.4</td>
<td>0.077</td>
</tr>
</tbody>
</table>

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 lefrada 30 mg.

Abbreviations, see legend to Table 1.
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).

A 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,
neutropenia. The incidence of leukopenia, defined as leukocyte count below $4 \times 10^9/l$, was estimated at 5.7% in the lefradafiban groups compared with 2.3% in the placebo group. Neutropenia (neutrophils below $1.5 \times 10^9/l$) 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.

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.
Thrombocytopenia (platelets below $90 \times 10^9/l$) occurred in only two (0.5%) of 401 patients treated with lefradafibran. No placebo recipient had thrombocytopenia.

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

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

Percentages refer to total number of patients in each treatment group.

**Table 5** Location of bleeding (%)

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

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.

In this double-blind, randomized, dose-escalation trial of 1 month GP IIb/IIIa inhibition with lefradafibran 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 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 (ticlopidine)[33], 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 levels 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, orbifiban and sibrafiban in patients after percutaneous coronary intervention[29] 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 orbifiban 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 the same compound for prolonged outpatient second-ary prevention[46]. If a patient undergoes percutaneous coronary intervention, it may be advisable to continue

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[46]. If a patient undergoes percutaneous coronary intervention, it may be advisable to continue

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the oral drug, or to convert to the intravenous preparation during and for 12–24 h following the intervention[7–10]. As the risk of subsequent events following percutaneous coronary intervention is low, no further treatment with oral GP IIb/IIIa receptor blockers seems required[18,37]. 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 ischemic cardiac events among patients with acute coronary syndromes in a large clinical trial.

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