Anti-platelet monoclonal antibodies for the prevention of arterial thrombosis: experience with ReoPro, a monoclonal antibody directed against the platelet GPIIb/IIIa receptor

Centocor Inc, 200 Great Valley Parkway, Malvern, PA 19355, U.S.A.

Platelets are implicated in a variety of serious vaso-occlusive and thromboembolic disorders that together constitute one of the most common causes of death throughout the world. Platelet aggregation and subsequent thrombosis at the site of an injured atherosclerotic plaque are important causative factors in the pathogenesis of unstable angina, acute myocardial infarction, reocclusion after successful coronary thrombolysis, and in thrombotic complications after percutaneous coronary transluminal angioplasty (PTCA) and coronary atherectomy. Mortality and morbidity due to vaso-occlusive and thromboembolic disorders have been reduced by thrombolytic agents, aspirin and heparin. However, these therapies are often inadequate, as evidenced by the continued incidence of treatment failures, underscoring the need for improved therapy. ReoPro (abciximab) is the Fab fragment of a genetically engineered, recombinant, monoclonal antibody (7E3) that is directed against the platelet glycoprotein (GP) IIb/IIIa receptor. The constant region of this monoclonal antibody Fab fragment has been humanized, while the variable region retains high-affinity binding to the platelet GPIIb/IIIa receptor. The binding of ReoPro to the platelet GPIIb/IIIa receptor prevents the adhesive interaction of fibrinogen, von Willebrand factor and other adhesive protein molecules with the GP IIb/IIIa receptor, interfering with platelet aggregation and thrombosis. In addition, ReoPro binds with high affinity to the x,β1, or vitronectin receptor, which is located on vascular endothelial cells. Ligand binding to x,β1 has been implicated as playing a role in arterial restenosis after coronary angioplasty.

Preclinical pharmacology studies

In vitro studies using well-defined binding and functional assays established a clear correspondence between the level of GPIIb/IIIa receptor blockade produced by ReoPro and the inhibition of platelet aggregation [1]. Maximal inhibition of platelet aggregation was observed when ≥80% of GPIIb/IIIa receptors were blocked by ReoPro. The same binding and functional assays were used to characterize the in vivo effects after administration of ReoPro to animals. In vivo studies were greatly facilitated by the ability to gain direct access to the site of action of ReoPro, the circulating platelet. In animals, bolus doses of ReoPro were defined that achieved a post-treatment blockade of at least 80% of platelet receptors and that fully inhibited platelet aggregation [1]. A large percentage of the injected dose of ReoPro becomes bound to platelets and levels of free ReoPro in the plasma exist for only a short time. Inhibition of platelet function is temporary, and recovery begins within several hours following a bolus dose. However, receptor blockade can be sustained at 80% or more by continuous intravenous infusion of ReoPro. Despite the potent inhibition of platelet function, the inhibitory effects of ReoPro were readily reversed by the transfusion of platelets in monkeys. Animal toxicology testing established that ReoPro is well tolerated in various dosing regimens and can be co-administered with other cardiovascular medications, such as aspirin, recombinant tissue plasminogen activator (rtPA), streptokinase and heparin, without evidence of hypersensitivity, haemorrhage or thrombocytopenia.

Animal efficacy studies

Efficacy studies were conducted in a number of different dog and monkey vascular injury models, including models of the deep vascular injury seen with PTCA [2–15]. In these studies, doses of ReoPro that were sufficient to achieve a blockade of ≥80% of GPIIb/IIIa receptors were effective in preventing acute thrombosis, even in models in which all control animals had thrombotic arterial occlusion. The animal studies with ReoPro demonstrated potent inhibition of platelet function and consistent prevention of arterial thrombosis in situations in which other anticoagulant and antithrombotic agents were ineffective. These findings indicated that ongoing platelet deposition, which can be inhibited by ReoPro, plays an important role in thrombotic occlusion at the site of severe stenosis and arterial injury. These studies provided the basis for establishing the effective clinical dosage regi-
mens of ReoPro for prevention of arterial thrombosis.

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Early clinical studies

The clinical experience with ReoPro comprises more than 3000 patients enrolled in over 20 clinical trials. These studies have been conducted in normal healthy subjects, in patients with stable coronary artery disease who did not receive concomitant heparin therapy and in patients with coronary artery disease who were receiving concomitant heparin therapy.

Clinical pharmacology studies

The results of preclinical studies demonstrated that doses of ReoPro sufficient to block at least 80% of GPIIb/IIIa receptors significantly inhibited platelet function and consistently prevented arterial platelet thrombosis. Based on the preclinical data, phase I studies of ReoPro tested bolus injections to identify the dose required in humans to achieve at least 80% GPIIb/IIIa blockade and inhibition of platelet aggregation in response to vigorous stimulation by potent platelet agonists. These studies demonstrated that ReoPro produced dose-dependent GPIIb/IIIa receptor blockade. At doses of 0.25 mg/kg or higher, ≥80% blockade was attained in most patients, and this was accompanied by profound inhibition of platelet function as measured by ex vivo platelet aggregation and bleeding time [1].

The anti-platelet effects of single bolus doses of ReoPro were transient, lasting approximately 6–12 h. To identify dose regimens that would provide sustained periods of platelet inhibition, regimens comprising a bolus dose followed by a repeat bolus dose or by a continuous infusion were tested. Repeat bolus dose regimens were not effective in providing sustained inhibition of platelet function. On the other hand, in patients who received a bolus dose followed by a continuous infusion of 10 μg/min, high-grade inhibition of platelet aggregation was maintained for the duration of the infusion, but a lower infusion rate (5 μg/min) did not maintain receptor blockade at the desired level [1]. In the clinical pharmacology studies, inhibition of ex vivo platelet aggregation by ReoPro was demonstrated in response to a number of platelet agonists, confirming that ReoPro inhibits platelet aggregation induced by any of the known physiological pathways, including direct receptor-mediated pathways, adhesion-mediated pathways and metabolic pathways.

Efficacy results from early phase clinical studies

The preliminary clinical efficacy of ReoPro was evaluated in a series of phase I and phase II studies. Direct evidence of the ability of ReoPro to prevent platelet thrombus formation at sites of coronary injury was obtained by measuring coronary flow velocity in patients undergoing PTCA [16,17]. Cyclic coronary flow variations are associated with transient platelet thrombus formation in response to vascular injury that can eventually lead to complete thrombotic occlusion and complete cessation of blood flow. In one study, ReoPro treatment eliminated cyclic flow in patients in whom it developed after PTCA despite treatment with aspirin and high-dose intravenous heparin [16,17]. A phase II study in high-risk patients undergoing PTCA demonstrated that in patients who received ReoPro clinical events related to recurrent ischaemia (death, myocardial infarction and urgent coronary intervention) were lower than predicted after PTCA [18]. Additional evidence that ReoPro can prevent platelet thrombus formation in patients was observed in a study of an earlier form of ReoPro, employing the murine Fab fragment of 7E3. In this study, patients treated with murine 7E3 Fab had better coronary artery patency after coronary thrombolysis as demonstrated angiographically [19]. However, it should be noted that the ability to interpret the results of these trials is limited because there were no randomized control groups in these studies.

One phase II study with ReoPro was a randomized, placebo-controlled trial of 60 patients and demonstrated that a bolus plus infusion regimen of ReoPro reduced the occurrence of clinical events associated with recurrent ischaemia during and after PTCA [20]. In this study, patients with unstable angina refractory to heparin, aspirin and nitrates were treated with a 0.25 mg/kg bolus and 10 μg/min infusion of ReoPro beginning 18–24 h before PTCA and continuing until 1 h after PTCA. Recurrent myocardial ischaemia was reduced by 50% with ReoPro from the time the treatment was started until PTCA was performed 18–24 h later. Major clinical events associated with recurrent ischaemia (death, myocardial infarction and urgent coronary intervention) occurring after randomization were assessed by a blinded clinical end point committee. The number of patients who had these
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major clinical events was reduced from 7 of 30 patients (23.3%) in the placebo treatment group to 1 of 30 patients (3.3%) in the ReoPro treatment group ($P = 0.052$, two-sided).

The phase III EPIC trial
The EPIC trial was a multicentre, randomized, double-blind, placebo-controlled, three-arm trial in patients undergoing coronary balloon angioplasty or directional coronary atherectomy who were at high risk for subsequent acute ischaemic complications [21,22]. The target population comprised patients with unstable angina and/or non-Q-wave myocardial infarction, patients with acute Q-wave myocardial infarction within 12 h of onset of symptoms and patients with other high-risk clinical or morphological characteristics. A total of 2099 patients were randomized at 56 sites in the United States into three treatment arms: placebo bolus plus 12-h placebo infusion (placebo), ReoPro (0.25 mg/kg bolus plus 12-h placebo infusion (bolus) and ReoPro 0.25 mg/kg bolus plus 10 µg/min ReoPro 12-h infusion (bolus plus infusion). All patients received standard therapy with aspirin and high-dose intravenous heparin. The primary efficacy end point was any of the following within 30 days of randomization: death, myocardial infarction (MI) or urgent intervention for recurrent ischaemia [PTCA, coronary artery bypass graft (CABG) surgery, intracoronary stent placement or intra-aortic balloon pump (IABP)]. In addition to the primary end point at 30 days, a secondary end point ascertainment whether any benefit at 30 days could be maintained for 6 months. The 6-month efficacy end point was defined as death, MI or revascularization procedures. Unlike the primary end point, this secondary end point included all PTCA (both urgent and non-urgent) and all CABG procedures (both urgent and non-urgent) as revascularization procedures of interest. All potential efficacy and major safety events (bleeding and stroke) were reviewed by an independent blinded clinical end point committee.

The treatment groups were well balanced with regard to demographics, enrolment criteria and medical history. All 2099 enrolled patients were included in the principal analyses of safety and efficacy [21].

Primary (30-day) end point results
The 30-day primary end point event rates for each treatment group by intention-to-treat analysis are shown in Table 1 and Figure 1. As shown in Figure 1, the benefit of ReoPro treatment was apparent on the first day after randomization and was sustained for the entire 30-day period of follow-up [21].

In the analysis of primary end point components, the greatest dose-response effects were seen in the MI ($P = 0.013$) and urgent intervention ($P = 0.003$) event rates. In the bolus plus infusion treatment group, a 39.4% reduction in the incidence of MI (5.2% in the bolus plus infusion treatment group versus 8.6% in the placebo treatment group, $P = 0.014$, pairwise) and a 49.1% reduction in the incidence of urgent intervention (4.0% in the bolus plus infusion treatment group versus 7.8% in the placebo treatment group, $P = 0.003$, pairwise) were observed. Within the urgent intervention category, urgent PTCA and urgent CABG occurred most frequently, while stent and IABP placement occurred rarely. There was a small number of deaths during the 30-day follow-up period: 12 in the placebo group, nine in the bolus group and 12 in the bolus plus infusion group. However, when patients who were not treated were excluded from the analysis, fewer patients in the bolus plus infusion treatment group (nine patients) died than the placebo treatment group (12 patients).

Six-month results
The results from the 6-month follow-up demonstrated that the benefit of ReoPro bolus plus

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 2099)</th>
<th>Placebo (n = 696)</th>
<th>Bolus (n = 695)</th>
<th>Bolus + infusion (n = 708)</th>
<th>Dose response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with events</td>
<td>227 (10.8%)</td>
<td>89 (12.8%)</td>
<td>79 (11.5%)</td>
<td>59 (8.3%)</td>
<td>0.009</td>
</tr>
<tr>
<td>Per cent reduction versus placebo</td>
<td>10.4%</td>
<td>34.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P$-value versus placebo</td>
<td>0.428</td>
<td>0.008</td>
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</tbody>
</table>
Figure 1
Kaplan–Meier plot of the primary (30-day) end point event rates, shown as the percentage of patients who did not have a primary end point event over time.

The occurrence of events was recorded in whole days for this analysis, with events that occurred within 24 h of randomization classified as day 0 events.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Placebo</th>
<th>Bolus</th>
<th>Bolus + infusion</th>
<th>Dose response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients randomized</td>
<td>2099</td>
<td>696</td>
<td>695</td>
<td>708</td>
<td></td>
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<tr>
<td>Patients evaluated from day 0†</td>
<td>2099</td>
<td>696</td>
<td>695</td>
<td>708</td>
<td></td>
</tr>
<tr>
<td>Patients with events</td>
<td>654</td>
<td>241</td>
<td>224</td>
<td>189</td>
<td>0.001</td>
</tr>
<tr>
<td>Per cent reduction versus placebo</td>
<td>7.1%</td>
<td>22.9%</td>
<td></td>
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</tr>
<tr>
<td>P-value versus placebo</td>
<td>0.276</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Patients evaluated after day 2‡</td>
<td>1863</td>
<td>606</td>
<td>618</td>
<td>639</td>
<td></td>
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<tr>
<td>Patients with events</td>
<td>419</td>
<td>151</td>
<td>148</td>
<td>120</td>
<td>0.007</td>
</tr>
<tr>
<td>Per cent reduction versus placebo</td>
<td>4.4%</td>
<td>24.6%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P-value versus placebo</td>
<td>0.588</td>
<td>0.007</td>
<td></td>
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<td></td>
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<tr>
<td>Patients evaluated after 30-day follow-up§</td>
<td>1728</td>
<td>549</td>
<td>580</td>
<td>599</td>
<td></td>
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<tr>
<td>Patients with events</td>
<td>313</td>
<td>105</td>
<td>117</td>
<td>91</td>
<td>0.071</td>
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<tr>
<td>Per cent reduction versus placebo</td>
<td>-5.2%</td>
<td>20.6%</td>
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<tr>
<td>P-value versus placebo</td>
<td>0.650</td>
<td>0.070</td>
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</tbody>
</table>

*Revascularization includes any PTCA (urgent and non-urgent), any CABG (urgent and non-urgent), any intracoronary stent (only in the 30-day follow-up period), and any end point IABP (only in the 30-day follow-up period).
†Patients who were evaluated from day 0 through the 6-month follow-up.
‡Patients who were evaluated from day 3 through the 6-month follow-up. Excludes patients experiencing death, MI or revascularization from day 0 to day 2.
§Patients who were evaluated after 30-day follow-up through 6-month follow-up. Excludes patients experiencing death, MI or revascularization from day 0 through the 30-day follow-up.
The benefits of ReoPro treatment for the primary end point and the 6-month end point were also consistent in all subgroups examined, including each of the three diagnostic categories present at study entry, and subgroups defined by baseline characteristics that were either prespecified in the analytical plan or defined post hoc. The efficacy benefit in these subgroups was present for both the primary end point and the 6-month end point.

Safety results of the EPIC trial
In the EPIC trial, patients received high doses of intravenous heparin while undergoing an invasive coronary intervention. Therefore bleeding was expected to be a consequence of ReoPro treatment because the potent platelet inhibition produced by ReoPro was being superimposed on the high level of anticoagulation produced by heparin. Importantly, in the EPIC trial, ReoPro treatment did not increase the risk of death or of stroke, either haemorrhagic or non-haemorrhagic, nor did it increase the risk of surgical intervention for bleeding.

Patients who received ReoPro and underwent urgent CABG had similar blood loss, transfusion rates and complication rates to patients in the placebo group who underwent CABG, even though the median time of CABG after onset of treatment was 3.7 h in the bolus plus infusion group, a time at which substantial inhibition of platelet function would still be expected [23].

The median decrease in haemoglobin within 36 h of treatment in the bolus plus infusion treatment group (2.1 g/dl) was only slightly greater than the median decrease in the placebo treatment group (1.7 g/dl), indicating that blood loss associated with PTCA was similar for most patients in the two groups. However, there were some patients who experienced a greater extent of blood loss, and these patients were analysed by incidence of major bleeding events (as defined by...
Major bleeding events and blood product transfusions were more often seen in ReoPro-treated patients. Of 158 patients with major bleeding not associated with CABG, 75 were in the bolus plus infusion treatment group, 60 were in the bolus treatment group and 23 were in the placebo treatment group. Approximately two-thirds of the patients in the bolus plus infusion and bolus treatment groups who experienced major bleeding had a major bleeding event within 36 h of the onset of treatment with study agent, and the majority of patients in each treatment group who had major bleeding events had bleeding from a vascular access site. Most of these were related to the femoral artery puncture site in the groin, but there were 16 patients with retroperitoneal haematomas: 12 in the bolus plus infusion treatment group, two in the bolus treatment group and two in the placebo treatment group. Bleeding from locations other than access sites occurred at a much lower rate, but gastrointestinal and gastourinary bleeding occurred in a higher percentage of patients with major bleeding in the ReoPro treatment groups than in the placebo-treated groups. Importantly, bleeding-related deaths were rare and occurred with similar frequency in the three treatment groups.

The following observations indicated that higher than necessary levels of heparin anticoagulation contributed to the higher rates of major bleeding events in patients who received ReoPro: (1) heparin dosing was not weight adjusted; (2) light patients had more blood loss in all three treatment groups; (3) lower body weight was associated with higher levels of anticoagulation (as measured by the activated clotting time), which in turn were associated with a higher rate of major bleeding events, suggesting that lower weight patients received inappropriately higher doses of heparin; and (4) higher doses of heparin on a per kg basis were associated with higher rates of major bleeding events, but had no apparent efficacy benefit.

Discussion
The EPIC trial demonstrated that potent inhibition of the GPIIb/IIIa receptor by ReoPro bolus followed by infusion for 12 h leads to a marked reduction in clinically significant ischaemic events for 6 months after PTCA in high-risk patients. In contrast, the shorter period of potent platelet inhibition achieved in the bolus treatment group was insufficient to protect against ischaemic events. These data indicate that potent platelet inhibition sustained for a period of 18–24 h by the bolus plus 12-h infusion regimen is required to prevent ischaemic events after PTCA in high-risk patients.

The therapeutic benefit of ReoPro treatment in the prevention of the major acute thrombotic complications of PTCA and later events during the 6 months after PTCA was accompanied by more major bleeding events and transfusions. However, the excess major bleeding was largely confined to the local vascular access site. Importantly, there was no increase in the incidence of intracerebral haemorrhage associated with ReoPro treatment, and spontaneous haemorrhage in other major organs was rare. Deaths associated with a major bleeding event occurred in a few patients, and there was no difference in death rates between the placebo and the bolus plus infusion treatment groups. In addition, surgical intervention for bleeding was rarely performed and there were no differences in rates among treatment groups. Most surgical intervention for bleeding was at the femoral access site. Thus, although the bleeding events associated with ReoPro treatment resulted in a higher rate of transfusions, these events were usually transient, not associated with long-term consequences and may be potentially reduced by modification of concomitant heparin dosing. In contrast, the acute ischaemic events during hospitalization, and later events during 6-month follow-up, that were prevented by ReoPro treatment were serious in nature and associated with irreversible consequences, including death, MI and repeat revascularization procedures.

Because the placebo group in the EPIC trial received standard anti-thrombotic treatment (aspirin plus heparin) for patients undergoing PTCA, the reduction in complications after PTCA by the bolus plus infusion regimen of ReoPro is direct evidence of the additional therapeutic value of ReoPro beyond standard anti-thrombotic therapies.

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**Clinical trials with CAMPATH-I and other monoclonal antibodies**

G. Hale* and J. M. Phillips

Department of Pathology, University of Cambridge, Cambridge, U.K.

**Introduction**

New technologies in immunology and molecular biology will have a major effect on the design of the next generation of therapies. Monoclonal antibodies, recombinant cytokines, synthetic replacements for deficient proteins, *in vitro*-stimulated immune cells and replacement genes are all being actively investigated. Their huge diversity, exquisite specificity and technical complexity pose new problems for the pharmaceutical industry. Monoclonal antibodies are the most diverse and best-studied set of biological drugs. Many have been proposed for treatment of a wide range of diseases, including cancer, autoimmunity, transplant rejection or viral infection, but their therapeutic use is still largely experimental.

The costs of pharmaceutical development, including the need to meet an increasingly sophisticated panel of regulatory requirements, are so great that only a small fraction of the potential range of specificities is being developed.

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*To whom correspondence should be addressed.

Abbreviations used: ADCC, antibody-dependent cell-mediated cytotoxicity; GPI, glycosylphosphatidylinositol; TNF, tumour necrosis factor.