Phase 1b Randomized Study of Antidote-Controlled Modulation of Factor IXa Activity in Patients With Stable Coronary Artery Disease

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Background—Whether selective factor IXa inhibition produces an appropriate anticoagulant effect when combined with platelet-directed therapy in patients with stable coronary artery disease is unknown. REG1 consists of RB006 (drug), an injectable RNA aptamer that specifically binds and inhibits factor IXa, and RB007 (antidote), the complementary oligonucleotide that neutralizes its anti-IXa activity.

Methods and Results—We evaluated the safety, tolerability, and pharmacodynamic profile of REG1 in a randomized, double-blind, placebo-controlled study, assigning 50 subjects with coronary artery disease taking aspirin and/or clopidogrel to 4 dose levels of RB006 (15, 30, 50, and 75 mg) and RB007 (30, 60, 100, and 150 mg). The median age was 61 years (25th and 75th percentiles, 56 and 68 years), and 80% of patients were male. RB006 increased the activated partial thromboplastin time dose dependently; the median activated partial thromboplastin time at 10 minutes after a single intravenous bolus of 15, 30, 50, and 75 mg RB006 was 29.2 seconds (25th and 75th percentiles, 28.1 and 29.8 seconds), 34.6 seconds (25th and 75th percentiles, 30.9 and 40.0 seconds), 46.9 seconds (25th and 75th percentiles, 40.3 and 51.1 seconds), and 52.2 seconds (25th and 75th percentiles, 46.3 and 58.6) (P<0.0001; normal 25th and 75th percentiles, 27 and 40 seconds). RB007 reversed the activated partial thromboplastin time to baseline levels within a median of 1 minute (25th and 75th percentiles, 1 and 2 minutes) with no rebound increase through 7 days. No major bleeding or other serious adverse events occurred.

Conclusions—This is the first experience of an RNA aptamer drug-antidote pair achieving inhibition and active restoration of factor IXa activity in combination with platelet-directed therapy in stable coronary artery disease. The preliminary clinical safety and predictable pharmacodynamic effects form the basis for ongoing studies in patients undergoing elective revascularization procedures. (Circulation. 2008;117:2865-2874.)

Key Words: anticoagulants ■ coronary artery disease ■ thrombosis ■ aspirin ■ coagulation ■ platelets ■ hemorrhage

Parenteral anticoagulation is used extensively to treat acute venous and arterial thrombosis during vascular instrumentation, during percutaneous coronary intervention, and in extracorporeal circuits. However, major hemorrhage occurs in ~4% of patients with acute coronary syndrome and 5% of patients undergoing percutaneous coronary intervention, whereas up to 5% of patients undergoing coronary artery bypass grafting require a second operation to control bleeding. A marked increase in mortality occurs with major hemorrhage, and even minor hemorrhage is associated with substantial resource use and hospitalization cost.

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Factor IXa is a nascent target for the development of direct and specific modulators of coagulation (Figure 1), and animal models of thrombosis have demonstrated favorable ratios of antithrombotic activity to bleeding risk with several pharmacological inhibitors of this coagulation protease. REG1...
consists of RB006 (drug), a synthetic RNA aptamer that binds factor IXa with high affinity and specificity, and RB007 (antidote), a rationally designed complementary oligonucleotide that neutralizes the effect of RB006 (Figure 1). Healthy volunteers treated with RB006 exhibited a clear dose-dependent increase in activated partial thromboplastin time (aPTT), whereas a single intravenous bolus of RB007 abolished >95% of its anticoagulant activity rapidly (<5 minutes) and durably (>24 hours).8

The pharmacodynamic effect and clinical safety of RB006 and RB007 in patients who require revascularization is unknown. This population is typically older, often requires several concomitant medications that affect hemostasis, exhibits reduced drug clearance, and possesses inherently greater propensity toward bleeding, making safe, rapid, and readily reversible anticoagulation an absolute prerequisite for optimal patient care. Therefore, in anticipation of phase 2 trials of revascularization therapy, we investigated the clinical safety and pharmacodynamic profiles of RB006 and RB007 in patients with stable coronary artery disease (CAD) on maintenance single or dual antiplatelet therapy.

**Methods**

We conducted a phase 1b randomized, placebo-controlled, double-blind, dose-escalation study of REG1 in patients with stable CAD on aspirin and/or clopidogrel.

**Study Objectives**

Our primary objective was to investigate the safety of a range of doses (dose escalation) of RB006 with and without RB007 in subjects with stable CAD on oral antiplatelet therapy. Our secondary objective was to characterize the dose-pharmacodynamic relationships of RB006 and RB007, with core laboratory aPTT at 10 minutes after RB006 administration selected as the principal pharmacodynamic outcome.

We further prespecified 2 subject populations for analysis. The safety population included all subjects receiving any amount of study drug or placebo, and the pharmacodynamic cohort included all subjects who completed per-protocol treatment.

**Study Organization**

Patients were recruited between April 2006 and March 2007 from 7 participating sites within the United States. The ethics committee at each institution approved the study, and all patients provided written informed consent. The Duke Clinical Research Institute (Durham, NC) organized the study and performed all data management and statistical analyses. The data were periodically reviewed by an independent Data and Safety Monitoring Committee, and primary outcomes (safety) events were adjudicated by site investigators blinded to study medication allocation.

**Study Population**

Patients were eligible if they were between 50 and 75 years of age; had documented stable CAD defined as prior myocardial infarction (>1 month), prior coronary revascularization (percutaneous coronary intervention >1 month or coronary artery bypass grafting >3 months), or documented angiographic coronary disease; and were receiving aspirin (>80 mg/d), clopidogrel (>75 mg/d), or both for >7 days before randomization. Major exclusion criteria were a recent episode of bleeding, significant renal or liver impairment, and abnormal baseline hemoglobin or platelet counts. All other exclusion criteria are listed in the online Data Supplement.

**Study Protocol**

All enrolled subjects were to receive an initial intravenous injection of active drug (RB006) or placebo drug over 1 minute, followed 3 hours later by active antidote (RB007) or placebo antidote. Subjects who qualified for the study had 2 intravenous access sites inserted, with 1 intravenous site used exclusively for study drug administration and the other for blood sampling. Heparin use, including heparin flushes of the intravenous cannula or tubing, was prohibited.
Sequential randomization was implemented by the study pharmacist or authorized designate by means of a Web-based central randomization service. The protocol randomly assigned subjects to 3 treatment groups: RB006 followed by placebo antidote, RB006 followed by RB007, or placebo drug followed by placebo antidote (double placebo) within each of 4 escalating dose levels of drug and antidote (Figure 2). All subjects assigned to receive RB007 would, by protocol, receive RB006 beforehand, with the dose ratio of RB006 to RB007 fixed at 1:2. In the 2 lowest dose levels, subjects were randomized in a 6:3:2 ratio to receive RB006 plus RB007, RB006 plus placebo antidote, or double placebo. At the 2 highest dose levels, subjects were randomized in an 8:4:2 ratio to receive RB006 plus RB007, RB006 plus placebo antidote, or double placebo. Sodium chloride (0.9%) was used for all placebo injections, and placebo injection. Information concerning clinical, safety, adverse events, and laboratory assessments was obtained during follow-up visits scheduled at days 2, 3, and 7 after randomization.

Laboratory Assessments
Samples were drawn for measurement of prothrombin time (PT) and aPTT at baseline; at 1, 10, and 30 minutes; and 1, 2, and 3 hours after the initial injection of RB006 or placebo drug. After the RB007 or placebo antidote injection 3 hours later, further PT and aPTT samples were drawn at 1, 10, and 30 minutes and at 1, 3, 5, 9, 21, 33, 45, and 165 hours after this second injection, corresponding to the time points of 3 hours 1 minute, 3 hours 10 minutes, 3 hours 30 minutes, and 6, 8, 12, 24, 36, 48, and 168 hours after the initial RB006 (or placebo drug) injection. Samples also were drawn for measurement of activated clotting time (ACT) at baseline; at 5, 10, and 30 minutes and 1, 2, and 3 hours after the injection of RB006 or placebo drug; at 1, 10, and 30 minutes after the injection of RB007 or placebo antidote; and at 4, 6, 8, 12, and 24 hours after the initial RB006 or placebo drug injection. Samples for PT, aPTT, and ACT were collected locally for immediate safety review by the site investigator. Additional laboratory samples were collected in 3.2% sodium citrate tubes for analysis at a core clinical coagulation laboratory (Icon Labs, Farmingdale, NY). Samples were centrifuged within 30 minutes of venipuncture at 1000g to 1300g for 15 minutes at room temperature. The plasma was placed in aliquots and stored at \(< -70°C\) until shipped on dry ice to the core laboratories for analysis. ACT assays (reference range, 117 to 178 s) were performed locally with a Hemochron Jr Signature (ITCMed, Edison, NJ) using the ACT Jr LR cartridge according to the manufacturer’s instructions. All core laboratory coagulation assays were performed on a Stago STA analyzer (Stago, Parsippany, NJ). PT (reference range, 12.6–15.4 seconds) and aPTT (reference range, 27 to 40 seconds) assays were performed with STANepolamine CI PLUS 10 and STA-PTT A5 (both Stago), respectively.

Safety evaluations included local measurements of chemistry, complete blood count, liver profile, and urinalysis with microscopic examination at baseline and at 24, 48, and 168 hours from the first injection; stool was assessed for occult blood at baseline and 48 and 168 hours; and complement Bb measurements were obtained at baseline, 10 minutes, 3 hours 10 minutes, 6 hours, and 24 hours. We were particularly interested in the potential for drug or antidote to cause complement activation, a class-specific toxicity noted with earlier-generation oligonucleotides.1 Complement Bb (reference range, 0.35 to 0.85 µg/dL) is a biomarker specific for complement-mediated oligonucleotide toxicity11 (see the online Data Supplement for a description), was measured with an ELISA (Quidel, San Diego, Calif) at the Diagnostic Complement Laboratory (National Jewish Medical and Research Center, Denver, CO).
Table. Baseline Characteristics

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Statistical Analysis

Unless otherwise stated, all hypotheses were tested at a 2-sided significance level of 5%. Statistical analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC). Baseline characteristics, adverse clinical events, local laboratory values, and core laboratory measures were summarized as numerator/denominator and percentages for categorical variables and as median (25th and 75th percentiles) for continuous measures. For the core laboratory-assessed complement Bb quantification, repeated-measures ANOVA was used to test for differences in complement levels between treatment groups. For all other safety outcomes, we compared the proportion of patients in each dose level who experienced ≥1 clinical adverse event or had ≥1 laboratory value falling outside the reference range with the placebo group. Because of the small sample size typical of a phase 1 dose-escalation design, this component of the safety analysis was descriptive without formal statistical hypothesis testing.

Pharmacodynamic Analysis

Pharmacodynamic analyses were performed with SAS version 9.1 (SAS Institute) and WinNonlin 5.2 (Pharsight Corporation, Cary, NC). Because of the sensitivity of the aPTT to preanalytic variables12 inherent in a multicenter study, we included—before database lock and study drug/antidote unblinding—a statistical quality control algorithm13 in the statistical analysis plan to identify biologically implausible PT results that reflected inappropriate handling conditions leading to spurious prolongation of coagulation times (see online Data Supplement). No measurements were excluded from the safety analysis.

The Kruskal-Wallis test was used to compare differences in coagulation measures at the prespecified 10-minute time point, and a repeated-measures ANOVA was used to assess differences in coagulation response over time. The relationship of weight-adjusted dose with the aPTT and ACT was assessed with Spearman’s rank-order correlation analysis. We then compared the dose-pharmacodynamic relationship in the present study with the 1a healthy volunteer study with the 1a healthy volunteer study with the 1a healthy volunteer study to identify biologically plausible PT results that reflected inappropriate handling conditions leading to spurious prolongation of coagulation times (see online Data Supplement). No measurements were excluded from the safety analysis.

Safety and Tolerability

Overall, both RB006 and RB007 were well tolerated, with no major bleeding or other serious adverse events occurring throughout the 7 days. No subjects experienced symptoms or signs of acute encephalopathy. Complement Bb levels remained within previously defined limits at all time points (Data Supplement).

There were 5 cases of minor bleeding in the form of intravenous-site hematomas, and transient cutaneous reactions (flushing and/or pruritus) were noted in 2 subjects at 8 and 15 minutes after injection of 30 mg RB006 and 100 mg RB007, respectively. Another subject had a positive stool occult blood test at 48 hours, although the 24-hour and 7-day
stool occult blood tests were negative. Microscopic hematuria was observed in 1 patient on day 7 (12 red blood cells per high-power field), but the concomitant presence of pyuria (5 white blood cells per high-power field) and bacteria on urine microscopy suggested an underlying urinary tract infection. A third subject experienced an episode of mild dizziness 5 minutes after receiving RB006. These symptoms were not accompanied by hypotension, tachycardia, or pyrexia. These 3 subjects belonged to the high-dose group and received 75 mg RB006 followed by 150 mg RB007.

To investigate the safety profile of RB006 alone beyond 3 hours, we analyzed the subgroup receiving RB006 followed by placebo antidote (n = 14). Access-site hematomas developed in 3 of these subjects, 2 from the high-intermediate dose group (n = 4) and 1 from the high-dose group (n = 4), at 24 to 48 hours after RB006. One subject receiving high-dose RB006 followed by placebo antidote experienced transient fluctuations in white blood cell and platelet counts. In retrospect, this subject’s counts were trending downward even before study drug administration. The serum lactate dehydrogenase was 284 U/L (reference range, 100 to 250 U/L) before enrollment in this same subject and remained mildly elevated throughout day 7, although the significance of this remains uncertain. The subject was otherwise asymptomatic and did not exhibit a fall in hemoglobin. For all other subjects in this study, hematologic measurements and serum chemistries, including electrolytes, creatinine, and blood urea nitrogen, remained stable throughout all 7 days.

We then compared adverse events in the subgroups receiving single versus dual antiplatelet therapy among all subjects receiving RB006 (n = 42). Among subjects receiving single antiplatelet therapy in combination with RB006 (n = 26), 2 experienced access-site hematomas, and 1 subject had microscopic hematuria. Among subjects receiving dual antiplatelet therapy in combination with RB006 (n = 16), 2 subjects experienced access-site hematomas, and 1 subject had a positive stool occult blood test. No other bleeding symptoms were reported among these patients in the screening questionnaire.
Pharmacodynamic Measurements

A single intravenous bolus of RB006 produced a dose-dependent increase in the aPTT (Figure 3a) and ACT (Figure 3b). The scatterplot of the aPTT and ACT versus weight-adjusted dose of RB006 showed a clear linear response ($r=0.70$, $P<0.001$) for the aPTT (Figure 4a) but a less linear relationship with the ACT ($r=0.67$, $P<0.001$; Figure 4b). The onset of effect was rapid, and the aPTT response was durable over the prespecified 0- to 3-hour duration (Figure 5).

We then monitored the pharmacodynamic response to antidote reversal in subjects given RB006 followed by RB007 ($n=27$), obtaining coagulation measurements after injection of RB007. Neutralization of anticoagulation with RB007 produced a rapid, predictable, and consistent reversal in the aPTT across the range of RB006 doses with a median time to normalization, defined as $<10\%$ above the subject’s baseline aPTT, of 1 minute (25th and 75th percentiles, 1 and 2 minutes; Figure 6).

We also studied the pharmacodynamic response beyond 3 hours in 14 subjects given RB006 alone without RB007 (Figure 7). These graphs showed wide fluctuations in the aPTT response over time, likely a result of the small within-group sample sizes. In the high-dose group, which expectedly experienced the greatest pharmacodynamic effect, the aPTT remained above the upper reference limit for 6 to 7 hours. At 12 hours, the median aPTT for the low- and low-intermediate–dose groups had returned to baseline values, but for the high-intermediate– and high-dose groups, a residual dose-dependent pharmacodynamic effect persisted.

Figure 5. aPTT response to RB006 from 0 to 3 hours. Horizontal dashed line denotes upper limit of normal. Placebo group includes all patients receiving placebo drug and placebo antidote ($n=8$). The 4 dose groups here included all patients receiving RB006 (active drug) ($n=42$). $P<0.001$ across all dose groups for repeated measurements from 0 to 3 hours.

Figure 6. APTT recovery after administration of antidote (RB007). Horizontal dashed line denotes upper limit of normal. Placebo group includes all patients receiving placebo drug and placebo antidote ($n=8$). The 4 dose groups here included only patients receiving active drug followed by active antidote ($n=27$). $P<0.001$ across all dose groups for repeated measurements from 0 to 3 hours.
Discussion

REG1, an RNA aptamer drug-antidote construct specific for factor IXa, safely achieved rapid dose-dependent anticoagulation, with prompt and durable restoration of normal coagulation in patients with stable CAD on oral antiplatelet therapy. The results of this phase 1b study closely mirror the pharmacodynamic and safety profiles of an earlier study performed in healthy volunteers (Figure 8), despite the presence of multiple comorbid conditions and concomitant medication use.

In the phase 1a study, we demonstrated that healthy volunteers treated with RB006 exhibited a clear dose-dependent aPTT increase after injection of 15, 30, 60, and 90 mg RB006. The present study was performed to investigate safety and pharmacodynamic reproducibility in a population at increased risk of cardiovascular events before transition to a percutaneous coronary intervention population with iatrogenic plaque disruption. In the healthy volunteer study, the protocol-specified stopping rule for aPTT level was triggered by a subject who had a peak aPTT of 92 seconds after administration of 90 mg RB006. As a precaution and to avoid premature study termination, the 2 higher doses of RB006 selected for this study were 50 and 75 mg instead of the 60- and 90-mg doses used in the healthy population study. Moreover, the staggering of dose levels across both studies allowed more precise characterization of the dose-response relationship within the therapeutic range (Figure 8). The inclusion criteria of chronic antiplatelet used in this phase 1 study allowed us to investigate early signals of increased bleeding risk with combination therapy, although larger studies are needed to accurately quantify this risk. The linear response of aPTT after normalization of RB006 dose to patient weight (Figure 4) indicates that a weight-adjusted dosing strategy, as opposed to a fixed dose, may be the optimal approach for future trials.

Safety in Stable CAD

Bleeding is a key concern with novel anticoagulants. Although major bleeding did not occur in this study, 10% of subjects experienced non-dose-dependent, mainly mucocutaneous bleeding. These minor bleeding rates are consistent with those seen in other phase 1 anticoagulant programs.

In an earlier study of healthy volunteers, 1 subject experienced transient encephalopathy after receiving 30 mg RB006 that was of unclear relation to the study drug. There were no cases of acute encephalopathy in this phase 1b cohort of older patients on multiple concomitant medications. We acknowledged a priori the largely exploratory nature of our safety and feasibility study and that smaller differences in bleeding, encephalopathy, and other clinical events would not be detectable between groups given the modest sample size. Nonetheless, the absence of acute encephalopathy in the present study provides reassurance during continued clinical development.

Many class-specific toxicities occurring during a rapid intravenous infusion of oligonucleotides such as pyrexia and hemodynamic instability are mediated largely by the alternative complement pathway. In our present study, there was no
Figure 8. aPTT response to RB006 in combined REG 1a and 1b data set. A, Fixed dose of RB006; B, weight-adjusted dose of RB006. Pink open circles indicate data points from the 1a healthy volunteer study; blue crosses, data points from the current 1b study.
significant increase in complement Bb levels, a marker specific for activation of the alternative pathway, implying an absence of detectable complement activation at the assigned doses.

Presently, antidotes to parenteral anticoagulants in clinical use are nonspecific, pharmacokinetically unpredictable, or associated with an unfavorable adverse effect profile. For example, protamine sulfate, frequently used to reverse the anticoagulant effect of unfractionated heparin during cardiopulmonary bypass and in acute bleeding events, has several well-known adverse effects.\(^{14}\) Mortality was shown to be as high as 36% in patients developing an acute allergic reaction to protamine while undergoing vascular surgery.\(^{15}\) The absence of hemodynamic disturbances or serious allergic reactions with RB007 in our study is therefore reassuring. Dissociation of the heparin-protamine complex (heparin rebound) leads to reemergence of heparin anticoagulant activity, limiting durability and the desired clinical effect of protamine.\(^{16}\) In contrast, full recovery of coagulation after administration of RB007 was found to be both rapid and durable (up to 7 days) in an earlier study of healthy volunteers\(^{17}\) and in the present study (Figure 6).

A critical concern with the reversal of anticoagulation in subjects at high risk of arterial thrombosis is the potential for triggering acute thrombosis. The administration of coagulation factor concentrates or recombinant coagulation factors (replacement therapy) has been reported to cause acute arterial and venous thrombosis in patients with preexisting CAD.\(^{18,19}\) Because RB007 restores hemostatic capacity by preventing association of RB006 with factor IXa, the maximal levels of factor IXa generated by RB007 are limited by preexisting biological levels of native factor IX/IXa.\(^{20}\) Accordingly, there is no theoretical risk of “overshooting” or exceeding intrinsic factor IXa activity.

**Passive Versus Active Reversal of Anticoagulation**

A direct thrombin inhibitor with a short circulating half-life has recently shown good clinical efficacy with less bleeding compared with either unfractionated heparin or low-molecular-weight heparin.\(^{21}\) Nonetheless, this reversibility is “passive,” requiring up to several hours for the restoration of normal coagulant potential, especially in patients with impaired renal clearance. We postulate that an “active” antidote that elicits an immediate response may be advantageous in situations in which rapid restoration of hemostasis is required, providing unparalleled therapeutic control (Figure 6). A short half-life further mandates a continuous infusion to maintain the required intensity of anticoagulation, which may be viewed by some as a significant, albeit small, practical limitation. In contrast, a single intravenous 75-mg injection of RB006 produced a durable anticoagulant effect over 3 hours (Figure 5), a sufficient period for most procedural anticoagulant needs.

**Pharmacodynamics in Stable CAD**

REG1 was designed to have minimal off-target effects on other hemostatic proteins or organ systems. In contrast, unfractionated heparin displays highly complex and unpredictable pharmacodynamics and is associated with heparin-induced thrombocytopenia, a potentially limb- and life-threatening, drug-related adverse effect.\(^{14}\) Compared with unfractionated heparin, low-molecular-weight heparin offers modest benefit in the treatment of patients with acute coronary syndromes but has failed to demonstrate a consistent reduction in clinical bleeding.\(^{22}\) Similarly, a synthetic pentasaccharide inhibiting factor Xa, while demonstrating an impressive safety profile in acute coronary syndrome development, may perform less optimally in the angioplasty suite and is nonreversible.\(^{23,24}\)

Gui et al\(^{25}\) showed a defect in shear-dependent platelet thrombosis in factor IX–deficient mice, suggesting a previously unrecognized interaction between functional factor IX and platelets. However, RB006 did not affect shear-dependent platelet hemostatic capacity in the platelet function analyzer 100 experiments performed in the REG 1a healthy volunteer study (online Data Supplement). The potential for an interactive effect between RB006 and antiplatelet therapy on overall hemostasis remains unknown. Therefore, we have begun incorporating thromboelastography experiments as measures of tissue factor–initiated thrombin generation in our phase 2 program.

**Conclusions**

The present study represents the first experience of a novel antidote-controlled factor IXa inhibitor in patients with stable CAD on oral antiplatelet therapy. Factor IXa inhibition by an RNA aptamer followed by active restoration of its coagulant activity with a rationally designed complementary antidote was safely achieved without major bleeding, ischemic events, or other serious adverse events. The pharmacodynamic profile of REG1 mirrored previous observations in both preclinical models and healthy volunteers, laying the foundation for ongoing studies in patients undergoing revascularization procedures.

**Acknowledgments**

We would like to thank Drs Jack Ansell (chair), Jeffrey Weitz, and Craig Kessler for their leadership and guidance as our Data and Safety Monitoring Board; Dr Lynda Szczech for her role as our medical monitor; Carl Schuler, Valerie Mouery, Roger DeRaad, Stella Palencia, Ida Guzman, Mary Wethington, Karen Arrowood, Mary Mata, Ioana Ghiu, Siobhan Gallagher, and Jessica Sutton for their role in site management; Patricia Giclas for her advice on immune toxicology; Bill Wargin for his assistance with pharmacodynamic modeling; Marie Davydan and Anastasios Tsiatis for statistical advice; Amanda McMillan for editorial assistance; and Kimberly Ferrer for graphics assistance. Steering committee members included Richard C. Becker (principal investigator and chair), Robert A. Harrington (co-chair), John H. Alexander, and Christopher P. Rusconi. Sites were located at the Division of Cardiology, University of North Carolina, Chapel Hill; Black Hills Cardiology, Rapid City, SD; Cardiovascular Research Center, University of Kentucky, Lexington; Hershey Medical Center, Pennsylvania State University, Hershey; the Methodist DeBakey Heart Center, Baylor College of Medicine, Houston, Tex; Mount Sinai Medical Center, New York University Hospital, New York; and Saint Joseph’s Translational Research Institute, Saint Joseph’s Hospital of Atlanta, Atlanta, Ga.

**Source of Funding**

This work was supported by Regado Biosciences, Durham, NC. Dr Chan receives salary support from the National Medical Research Council of Singapore.
Disclosures
Drs Chan, Cohen, Dyke, Lin, Walder, Steinhubl, Gilchrist, Kleinman, Vorchheimer, Chronos, Melloni, Alexander, Harrington, and Becker, S.K. Myles, and L.G. Aberle received research grants from Regado Biosciences. S.K. Myles and L.G. Aberle, Dr Harrington, and Dr Becker received consulting fees or honoraria from Regado Biosciences. Drs Tonkens and Rusconi are employees of Regado Biosciences.

References

CLINICAL PERSPECTIVE
Animal models of thrombosis have demonstrated favorable ratios of antithrombotic activity to bleeding risk with factor IXa inhibition. However, it is unknown whether factor IXa inhibition produces an appropriate anticoagulant effect when combined with platelet-directed therapy in patients with stable coronary artery disease. REG1 consists of RB006 (drug), an injectable RNA aptamer that specifically binds and inhibits factor IXa, and RB007 (antidote), the complementary oligonucleotide that neutralizes its anti-IXa activity. We evaluated the safety, tolerability, and pharmacodynamic profiles of REG1 in a randomized, double-blind, placebo-controlled study, assigning 50 subjects with coronary artery disease taking aspirin and/or clopidogrel to 4 dose levels of RB006 and RB007. RB006 achieved rapid dose-dependent anticoagulation, whereas RB007 restored normal coagulation promptly and durably, without any major bleeding or other serious adverse events. The pharmacodynamic profile of REG1 mirrored previous observations in both preclinical models and healthy volunteers, laying the foundation for ongoing studies in patients undergoing revascularization procedures. This RNA aptamer drug-antidote construct specific for factor IXa may have a future role in minimizing the bleeding risk associated with revascularization procedures.
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_Circulation_. 2008;117:2865-2874; originally published online May 27, 2008; doi: 10.1161/CIRCULATIONAHA.107.745687

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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