First-in-Human Experience of an Antidote-Controlled Anticoagulant Using RNA Aptamer Technology

Christopher K. Dyke, MD; Steven R. Steinhubl, MD; Neal S. Kleiman, MD; Richard O. Cannon, MD; Laura G. Aberle, MS; Min Lin, PhD; Shelley K. Myles, BS, RN; Chiara Melloni, MD; Robert A. Harrington, MD; John H. Alexander, MD; Richard C. Becker, MD; Christopher P. Rusconi, PhD

Background—Selectivity, titratability, rapidity of onset, and active reversibility are desirable pharmacological properties of anticoagulant therapy administered for acute indications and collectively represent an attractive platform to maximize patient safety. A novel anticoagulation system (REG1, Regado Biosciences), developed using a protein-binding oligonucleotide to factor IXa (drug, RB006) and its complementary oligonucleotide antidote (RB007), was evaluated in healthy volunteers. The primary objective was to determine the safety profile and to characterize the pharmacodynamic responses in this first-in-human study.

Methods and Results—Regado 1a was a subject-blinded, dose-escalation, placebo-controlled study that randomized 85 healthy volunteers to receive a bolus of drug or placebo followed 3 hours later by a bolus of antidote or placebo. Pharmacodynamic samples were collected serially. Subject characteristics were the following: median age, 32 years (interquartile range, 23 to 39 years); female gender, 35%; and median weight, 79 kg (interquartile range, 70 to 87 kg). No significant differences were found in median hemoglobin, platelet, creatinine, or liver function studies. There were no significant bleeding signals associated with RB006, and overall, both drug and antidote were well tolerated. One serious adverse event, an episode of transient encephalopathy, occurred in a subject receiving the low intermediate dose of RB006. The subject’s symptoms resolved rapidly, and no further sequelae occurred. A predictable dose-pharmacodynamic response, reflected in activated partial thromboplastin time measurements, was seen after administration of the bolus of drug, with a clear correlation between the peak posttreatment activated partial thromboplastin time and post hoc weight-adjusted dose of drug (correlation coefficient, 0.725; \( P < 0.001 \)). In subjects treated with drug, antidote administration reversed the pharmacological activity of the drug, with a rapid (mean time, 1 to 5 minutes across all dose levels) and sustained return of activated partial thromboplastin time to within the normal range. The activated clotting time followed a similar anticoagulant response and reversal pattern. As anticipated, prothrombin time remained unchanged compared with baseline.

Conclusions—These observations represent a first-in-human experience of an RNA aptamer and its complementary oligonucleotide antidote used as an anticoagulant system. The findings contribute to an emerging platform of selective, actively reversible anticoagulant drugs for use among patients with thrombotic disorders of the venous and arterial circulations. (Circulation. 2006;114:2490-2497.)

Key Words anticoagulants ■ coagulation ■ pharmacology ■ aptamers, nucleotide ■ thrombosis ■ trials

The short-term management of patients with coronary atherothrombosis and related disorders of the arterial circulatory system has traditionally focused on inhibiting 1 or more than 1 plasma proteases participating in coagulation. Because hemostasis is dependent on achieving a threshold level of thrombin generation through a complex and intricately orchestrated interface between cellular elements, proteases, and innate vascular responses, bleeding is not uncommon with present-day anticoagulant pharmacotherapy. The acceptance of bleeding as a “tradeoff” for effective anticoag-
ulation should not be endorsed in contemporary practice, however, given its profound impact on patient outcomes\(^1\)–\(^3\) and healthcare costs.\(^4\) Accordingly, there is a substantial clinical need to develop anticoagulants with more favorable safety profiles.

**Clinical Perspective p 2497**

A translatable platform for developing an optimal parenteral anticoagulant should consider several prerequisite properties: easy delivery, rapid onset of action, and predictable responses among the dose, pharmacokinetic profile, and pharmacodynamic effects to reduce the requirement for routine monitoring. Additionally, an optimal anticoagulant should be biologically selective and actively reversible.

Unfractionated heparin is currently the only antidote-reversible anticoagulant. Despite its wide-scale use, unfractionated heparin suffers from a number of well-described and clinically relevant limitations: Its complex pharmacokinetics make dose selection and titration difficult and imprecise;\(^5\) the effective use of its antidote, protamine sulfate, shares similar unwanted complexity, dose-response variability, and has the potential to elicit serious cardiovascular side effects;\(^6,7\) and perhaps of greatest concern, heparin compounds can cause a limb- and life-threatening drug-induced, immune-mediated syndrome known as heparin-induced thrombocytopenia.\(^8\)

The overall safety profile of anticoagulant therapy has been improved by the introduction of agents with short pharmacokinetic and biological half-lives.\(^9\) Antidote-controlled anticoagulants expand the safety paradigm further by providing active, more rapid control of biological activity, including a potential means to completely “shut off” the systemic effects of the drug. A novel platform for the rational design of drug-antidote pairs has emerged recently wherein the drug modality is an oligonucleotide-based aptamer.\(^10,11\) Aaptamer is the capacity to encode the information necessary to create complementary oligonucleotide antidotes that can alter its shape and actively reverse attendant pharmacological activity (Figure 1).\(^10,11\) The REG1 anticoagulant system is the first drug-antidote pair derived from this technology to be tested in humans. It is composed of RB006, a specific aptamer-based inhibitor of coagulation factor IXa, and RB007, an antidote rationally designed to neutralize the pharmacological activity of RB006.

RB006 is a direct factor IXa inhibitor that binds coagulation factor IXa with high affinity and specificity. RB006 is an RNA-based aptamer formulated to have a prolonged duration of effect by virtue of chemical stabilization to limit degradation by bodily nucleases and conjugation to a 40-kDa polyethylene glycol carrier. RB006 elicits an anticoagulant effect by selectively blocking the factor VIIIa/IXa–catalyzed conversion of factor X to factor Xa, a pivotal step in prothrombinase assembly and thrombin generation on the surface of tissue factor–bearing cells and activated platelets. Preclinical pharmacology studies have demonstrated that RB006 can provide durable systemic anticoagulation and antithrombotic activity after bolus intravenous administration.\(^10,11,13\) A thorough preclinical toxicology and safety development program led to the determination of suitable dosing for human subjects.

RB007 is an oligonucleotide complementary to a portion of RB006 that binds effectively to RB006, thereby neutralizing its anti-factor IXa activity. RB007 is a modified-RNA oligonucleotide formulated to provide sufficient in vivo stability for identifying and durably binding RB006 but otherwise retaining the ability to be cleared rapidly from the bloodstream. Preclinical pharmacological studies have shown that RB007 can rapidly and durably neutralize the anticoagulant activity of RB006 after intravenous bolus administration.\(^10,11,13\) The antidote has not exhibited anticoagulant or other pharmacological activity in either in vitro or in vivo experiments.

Regado 1a is a phase 1 clinical investigation that represents the first-in-human investigation of an aptamer-based inhibitor.
to factor IXa (RB006) and its complementary oligonucleotide antidote (RB007). We report in the present study the pharmacodynamic properties and safety of these 2 compounds in healthy volunteers.

Methods

Regado Ia was a randomized, subject-blinded, placebo-controlled, dose-escalation study that evaluated a bolus of drug (RB006) or placebo followed 3 hours later by a bolus of antidote (RB007) or placebo.

Study Subjects

Healthy volunteers >18 years of age were eligible for enrollment if they had no active or treated medical comorbidities and if they were not taking any prescription medication (including contraception). Exclusion criteria included the following: subject weight <50 kg or >120 kg; pregnancy or lactation; active menstruation; any medical condition other than a self-limited illness; any prescription medication; any use of nonsteroidal antiinflammatory drugs or aspirin in the prior 7 days; any contraindication to anticoagulation or increased bleeding risk; severe trauma, fracture, major surgery, or biopsy of a parenchymal organ <3 months; endoscopic peptic-ulcer disease <3 months; severe, persistent hypertension (>180/110 mm Hg); clinically significant laboratory abnormalities (hemoglobin <12.0 g/dL, prolonged prothrombin time [PT] or international normalized ratio, prolonged activated partial thromboplastin time [APTT], elevated liver enzymes, elevated serum creatinine or blood urea nitrogen, or platelet count <150,000/mm³ or >600,000/mm³); use of an investigational drug within the past month; illicit drug or alcohol use; any other factor that investigators thought would increase subject risk with participation; and inability to comply with the study protocol.

Subjects and Treatments

The study protocol was approved by the respective institutional review boards, and all study subjects gave written informed consent. Potential participants underwent screening and, if eligible, were then admitted to a general clinical research center or institution equivalent. Subjects were randomly assigned to treatment or placebo within the treatment arm of the study active at the time of their enrollment (Figure 2). Eight subjects were enrolled in each treatment arm, randomized 7:1 to active treatment versus placebo. Sodium chloride injection 0.9% United States Pharmacopeia (USP) was used for all placebo injections. The appearances of RB006, RB007, and placebo were identical after dilution. To minimize the risks to the subjects, a dose-escalation design consisting of 4 increasing doses of drug and antidote was chosen. At each dose level, subjects were sequentially enrolled in the 3 treatment arms (Figure 2). Doses of RB006 were fixed to enable an analysis of the relationship between pharmacodynamic response and weight-adjusted dose of RB006. The doses of drug RB006 were 15, 30, 60, and 90 mg (Table 1). These doses were selected to provide a high margin of safety to the study subjects (~130-fold based on the starting clinical dose) and to target pharmacodynamic effects that define the in vitro APTT dose-response curve to RB006: a low dose that elicits little to no response, 2 intermediate dose levels that bracket the midpoint of the response curve, and a high dose that approaches saturation of the response curve (nearly 100% inhibition of factor IXa activity). The fixed dose of antidote RB007 was twice the RB006 dose (30, 60, 120, and 180 mg; Table 1). This 2:1 antidote-to-drug ratio is 4-fold the minimal amount of RB007 required to neutralize the anticoagulant activity of RB006 in human plasma in vitro. Investigators and other study personnel were not blinded to treatment and therefore were prepared to appropriately treat any potential bleeding/clotting events. Per the study protocol, administration of antidote RB007 was the recommended first-line treatment for RB006-related bleeding events.

Clinical Assessments

Subjects were monitored for 24 hours (or until the APTT returned to baseline) after the study drug injection for bleeding events or other potential safety-related issues. Comprehensive examinations for external bruising and mucosal bleeding, as well as vital signs, were monitored at baseline and during predefined clinical assessments. A bleeding questionnaire was administered at baseline, immediately before antidote or placebo (3 hours after drug or placebo), and 24 hours after drug or placebo injection. Information concerning clinical, safety, adverse-event, 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 PT and APTT at baseline and at 1, 5, 15, and 30 minutes and 1 and 3 hours after the injection
of drug or placebo; at 1, 2, 4, 6, 8, 10, 15, and 30 minutes after the injection of antidote or placebo; and at 4, 6, 8, 12, 18, 24, 48, 72, and 168 hours after initial drug or placebo injection. Samples also were drawn for measurement of activated clotting time (ACT) at baseline; at 5, 15, and 30 minutes and 1, 2, and 3 hours after the injection of drug or placebo; at 1, 10, and 30 minutes after the injection of antidote or placebo; and at 4, 6, 8, 12, and 24 hours after initial drug or placebo injection. A sample to determine factor IX activity was collected at baseline.

Safety evaluations included local measurements of chemistry, complete blood count, liver profile, and urinalysis with microscopic examination at baseline and at hours 24, 48, 72, and 168; stool was assessed for occult blood at baseline and at hours 48, 72, and 168. Additional safety measurements included complement Bb, thrombin time, and fibrinogen levels.

**Assay Procedures and Analyses**

Samples for PT, APTT, and ACT were collected locally (for immediate safety review by the site investigator). Additional core 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 were performed locally with a Hemochron Jr Signature laboratory (ITCMed, Edison, NJ) with the ACT Jr LR cartridge according to the manufacturer’s instructions. All coagulation assays in the core laboratory were performed on a Stago STA analyzer (Stago, Parsippany, NJ). PT and APTT assays were performed using STA-Neoplastine CI PLUS 10 and STA-PTT A reagents, respectively. Thrombin time was measured by the clotting time method using STA-Neoplastine CI reagent; fibrinogen level was assessed by the clotting method of Clauss using STA fibrinogen reagent. Factor IX activity assays were performed using the STA Deficient IX Immuno-depleted Plasma for Factor IX assay kit. Complement Bb assays were performed using an enzyme-linked immunosorbent assay specific to this activation product at the Diagnostic Complement Laboratory at the National Jewish Medical and Research Center (Denver, Colo).

**Subject Safety and the Data Safety Monitoring Committee**

Adverse experiences, bleeding, local laboratory PT, APTT, serum creatinine, liver enzymes, platelet counts, and additional chemistry and hematology data were assessed throughout the study. Accumulated subject listings and summary tables were provided to an independent Data Safety Monitoring Board and reviewed at the conclusion of enrollment in treatment arms 2 and 3 at each of the 4 dose levels (Figure 2). Because of the first-in-human experience of this phase Ia trial, a pharmacodynamic stopping rule was adopted to maximize subject safety. Enrollment of additional subjects was to be discontinued if either of the following occurred: the mean of the highest APTT for each subject in any treatment arm at a dosing level (n=7 subjects) exceeds 2.5 times the upper limit of normal for the laboratory conducting the test or any single subject in the study achieves an APTT value that is ≥15% higher than 2.5 times the upper limit of normal for the laboratory conducting the test.

**Biostatistical Considerations**

This was a phase Ia investigation in healthy volunteers. The primary objective of the study was to evaluate the safety profile of the active drug (RB006) and antidote (RB007) components of the REG1 anticoagulation system, individually and together, and to estimate dose-response relationships. The sample size was typical of a phase Ia dose escalation design.

Baseline characteristics were summarized by treatment arm as percentages for categorical variables and as medians with interquartile ranges for continuous variables. Spearman correlation coefficients were generated to evaluate the relationship between baseline characteristics and pharmacodynamic response. Paired t tests were performed to investigate posttreatment change in laboratory measures. A Kruskal-Wallis test was conducted to assess the differences in time to neutralization after the antidote administration among dose levels.

The relationship between relative increases in APTT values engendered by RB006 administration versus the resulting reduction in percent factor IX activity was determined by interpolation of the mean increase in APTT at each dose level from the equation describing the relationship between the relative increase in APTT and percent factor IX activity for representative factor IX activity assay calibration curves. An area-under-the-curve analysis for the relative increase in APTT over time also was performed to evaluate the relationship between the pharmacodynamic effect and weight-adjusted dose of RB006. The area under the curve for the relative increase in APTT from administration to 3 hours after dosing (AUC0–3) was determined using the trapezoidal rule (Win-Nonlin, Pharsight Corp, Mountain View, Calif).

The authors had full access to the data and took full responsibility for their integrity. All authors have read and agree to the manuscript as written.

**Results**

In all, 85 healthy volunteers were randomized, and all but 1 received the assigned treatment without interruption or early discontinuation. Women represented 35% of the total study cohort. The median age was 32 years (interquartile range, 23 to 39 years); the median weight was 79 kg (interquartile range, 70 to 87 kg). Additional baseline characteristics are summarized in Table 2. Small differences between baseline characteristics of subjects enrolled at the 4 dose levels or assigned to placebo were observed. These differences are likely a result of the sequential randomization of treatment arms containing 8 subjects, and statistical analysis demonstrated no relationship between pharmacodynamic response and any baseline characteristic. Enrollment in the study was

<table>
<thead>
<tr>
<th>TABLE 1. Phase 1a Doses Planned for the 3 Treatment Arms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>Dose level 1: low dose</td>
</tr>
<tr>
<td>Dose level 2: low intermediate dose</td>
</tr>
<tr>
<td>Dose level 3: high intermediate dose</td>
</tr>
<tr>
<td>Dose level 4: high dose</td>
</tr>
</tbody>
</table>

Dyke et al Antidote-Controlled Factor IXa Inhibition 2493
TABLE 2. Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (n=11)</th>
<th>Low Dose (n=21)</th>
<th>Low Intermediate (n=21)*</th>
<th>High Intermediate (n=21)*</th>
<th>High Dose (n=11)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y†</td>
<td>28 (23, 31)</td>
<td>30 (22, 39)</td>
<td>34 (24, 39)</td>
<td>31 (23, 42)</td>
<td>27 (23, 35)</td>
</tr>
<tr>
<td>Female gender, %</td>
<td>45</td>
<td>14</td>
<td>33</td>
<td>38</td>
<td>55</td>
</tr>
<tr>
<td>White, %</td>
<td>45</td>
<td>86</td>
<td>42</td>
<td>67</td>
<td>75</td>
</tr>
<tr>
<td>Weight, kg†</td>
<td>76 (71, 89)</td>
<td>82 (71, 95)</td>
<td>81 (73, 87)</td>
<td>75 (67, 84)</td>
<td>73 (60, 84)</td>
</tr>
<tr>
<td>Height, cm†</td>
<td>173 (165, 176)</td>
<td>177 (173, 183)</td>
<td>173 (163, 180)</td>
<td>173 (164, 182)</td>
<td>173 (160, 177)</td>
</tr>
<tr>
<td>Body mass index, kg/m²†</td>
<td>26 (24, 48)</td>
<td>25 (23, 29)</td>
<td>27 (23, 30)</td>
<td>24 (23, 27)</td>
<td>23 (21, 27)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg†</td>
<td>126 (118, 130)</td>
<td>124 (116, 131)</td>
<td>117 (112, 127)</td>
<td>122 (113, 128)</td>
<td>124 (112, 135)</td>
</tr>
<tr>
<td>Pulse, bpm†</td>
<td>71 (62, 80)</td>
<td>73 (66, 76)</td>
<td>67 (64, 71)</td>
<td>64 (59, 69)</td>
<td>68 (52, 81)</td>
</tr>
<tr>
<td>Factor IX activity, %†</td>
<td>83 (77, 97)</td>
<td>91 (66, 104)</td>
<td>91 (81, 108)</td>
<td>96 (89, 110)</td>
<td>86 (71, 101)</td>
</tr>
</tbody>
</table>

*Unweighted sample size. Characteristic-specific sample sizes may be lower because of missing values.
†Values are median (first quartile, third quartile).

Pharmacodynamic Measurements

As expected for a specific inhibitor of factor IXa, only APTT and ACT values increased after administration of RB006. To characterize the initial response to RB006 administration, analysis of the change in APTT and ACT values over time was first conducted for all subjects who received RB006 (treatment arms 2 and 3) for the first 3 hours after drug administration (ie, before RB007 administration). In subjects treated with RB006, the APTT increased rapidly in a dose-dependent manner, and the effect was stable over this 3-hour window (Figure 3). Subjects treated with 15 mg RB006 exhibited a modest trend toward an increase in APTT (1.1-fold increase 15 minutes after administration), whereas subjects treated with 30, 60, or 90 mg RB006 demonstrated a clear response to the drug, with a mean relative increase in APTT of 1.3-, 2.1-, and 2.9-fold at 15 minutes after RB006 administration, respectively. To evaluate the underlying basis for APTT prolongation, we compared the relative increase in APTT for subjects treated with RB006 with the relative increase in APTT values versus percent factor IX activity derived from the factor IX assay calibration curve. This analysis indicates that a 1.1-fold increase in APTT in response to factor IXa inhibition is equivalent to a 35% to 40% loss of factor IX activity, a 1.3-fold increase represents a loss of 80% factor IX activity, a 2.1-fold increase represents a loss of 98% factor IX activity, and a 2.9-fold increase represents a loss of >99% factor IX activity. Thus, escalation of RB006 dose resulted in a stepwise inhibition of factor IX activity, with target inhibition reaching substantial levels at the 2 highest doses. ACT values followed a pattern similar to APTT values, with relative increases of 1.1-, 1.3-, 1.4-, and 1.5-fold, respectively, at the 4 dose levels 15 minutes after RB006 administration.

Doses of RB006 in this study were fixed to enable an analysis of the individual variability in response to RB006 based on weight-adjusted dosing. An area-under-the-curve analysis for the relative increase in APTT over the first 3 hours of RB006 treatment (AUC0–3) was performed to evaluate the relationship between the pharmacodynamic effect and weight-adjusted dose of RB006. By this analysis, a value of 3 represents no change in APTT over the 3-hour window, whereas a value of 6 reflects, on average, a doubling of the APTT value over the 3-hour window. As shown in Figure 4, there is a clear relationship between the pharmacodynamic effect of RB006 and the weight-adjusted dose of RB006. A clear correlation between the peak posttreatment APTT and weight-adjusted dose of drug also was observed (correlation coefficient, 0.725; P<0.001). In addition, the intersubject variability in response to RB006 appears modest, with a maximum range of effect of ≈2.5-fold for a given weight-adjusted dose in this study cohort. There were no other correlations between the observed individual response to RB006 and baseline subject demographics, including baseline factor IX activity, PT, thrombin time, or fibrinogen level.

The duration of the effect of RB006 was evaluated in subjects enrolled in arm 3 (drug alone). As shown in Figure 3, pharmacodynamic effects of RB006 at 0 to 3 hours after RB006 administration. The relative increase in APTT over baseline for each subject receiving RB006 before RB007 or placebo administration (all subjects assigned to arms 2 and 3) is shown vs subjects receiving placebo. Data represents the mean±SEM for all subjects receiving treatment at each dose level. Lines represent the best point-to-point fit of the data. ■ Indicates placebo; ○, 15 mg RB006; ●, 30 mg RB006; □, 60 mg RB006; and ▲, 90 mg RB006.
5, the elevation in APTT after RB006 administration persisted for a substantial period of time. The median duration of effect (defined in this study as the time required for APTT values to return to baseline after RB006 administration) was dose dependent, with a value of 3 hours in subjects receiving 15 mg RB006, 20 to 24 hours in subjects receiving 30 mg RB006, and 30 hours in subjects receiving 60 mg RB006.

Administration of RB007 after RB006 (arm 2) resulted in a rapid and durable return of the APTT value to baseline for all doses of RB006 (Figure 6), indicating that administration of RB007 restored factor IX activity levels to within the normal range. On average, neutralization of the pharmacodynamic effect of RB006 by RB007 occurred within 1 to 5 minutes after antidote administration, with no differences between dose levels. After neutralization of RB006 activity by RB007, no rebound anticoagulant effect was observed through the 7-day follow-up. ACT values exhibited a similar pattern.

Clinical Data
Overall, RB006 and RB007 were well tolerated (alone and in combination). Adverse events, specifically bleeding, were similar among placebo, RB006, and RB007 across all dose groups. Most of this minor bleeding consisted of ecchymoses at intravenous access sites.

Clinical laboratory measures did not change significantly within or between groups. Serum creatinine, liver function tests, and other chemistry values remained at baseline levels throughout the follow-up period. Median hemoglobin and platelet counts remained unchanged. There was also no change compared with baseline in complement Bb levels after drug and/or antidote delivery throughout the follow-up period.

One serious adverse event was reported, an episode of transient encephalopathy characterized by speech impairment, mood alteration, confusion, and eye droop that occurred 4 hours after administration of RB006. The event occurred in a subject randomized to the drug/placebo arm of the low intermediate dose level (30 mg drug). The onset of symptoms occurred after an emotional exchange with nursing staff and resolved rapidly. A clinical assessment by a staff neurologist and a brain computed tomogram failed to reveal any abnormalities; specifically, there was no evidence of intracranial bleeding. A causal relationship between the event and study drug could not be excluded. However, on the basis of recommendations from the Data Safety Monitoring Board, each subsequent subject screened and enrolled in Regado Ia underwent a comprehensive neurological examination. No subsequent similar episodes occurred.

Discussion
Refinements in the understanding of coronary atherothrombosis and related thrombotic disorders of the arterial circulatory system, coupled with the pharmacokinetic and pharmacodynamic shortcomings and narrow therapeutic indexes of
existing anticoagulants, provide a strong impetus to develop biology-based, titratable, and rapidly reversible therapeutics with improved safety and pharmacological profiles. Regado 1a heralds a new era in direct, selective, and actively reversible anticoagulants using target-specific oligonucleotide aptamers with their complementary antidotes. This novel construct represents, in essence, an anticoagulation system that provides a rapid and effective means to either attenuate or fully inhibit specific coagulation proteases while maintaining hemostatic control through tailored administration of an inherently safe neutralizing-specific antidote. In effect, this anticoagulation system offers a molecular “on-off” switch to pharmacological anticoagulation.

The primary objective of the present study in healthy volunteers was to determine the safety profile of RB006, a selective oligonucleotide aptamer-based inhibitor of factor IXa, its complementary oligonucleotide antidote (RB007), and their combination. Because this was the first administration of either agent (drug or antidote) in humans, the 2 compounds were examined individually and in combination in a dose-escalating manner. RB006 and RB007 were overall well tolerated, with both compounds exhibiting no adverse effects on renal function, hepatic function, platelet count, platelet function, or hemoglobin. There was no increase in bleeding-related adverse events in subjects treated with drug compared with those receiving placebo. An episode of transient encephalopathy, which resolved rapidly with no further sequelae, occurred in 1 subject receiving 30 mg RB006. Neurological examination and brain computed tomogram failed to reveal any abnormalities or cause for the event and specifically excluded intracranial bleeding. A causal link between the event and the study drug could not be excluded; however, there is no known mechanistic link. The subject’s psychological state and history of drug abuse (revealed during follow-up) further complicate interpretation of the event. Nonetheless, detailed neurological exams before and after treatment were incorporated into the protocol and are incorporated into ongoing studies of the REG1 system. Complement Bb levels, a sensitive measure of activation of the alternative complement pathway, remained at baseline levels throughout the study in subjects receiving drug and/or antidote, indicating that neither agent induced complement activation. This is reassuring from a safety perspective, given that complement activation was a class-specific toxicity observed with earlier generations of nucleic acid-based therapies.

A second objective of the Regado 1a study was to determine the pharmacodynamic profile of RB006 given alone and after the administration of RB007. As reflected in traditional coagulation measurement assays such as the APTT, a clear dose-response to RB006 and factor IXa inhibition was demonstrated, characterized by a rapid onset and sustained duration of effect. On RB007 administration, the observed pharmacodynamic effects of RB006 promptly (within 5 minutes) returned to baseline values. There was no evidence to support a “rebound” increase in RB006-mediated anticoagulant activity throughout the 7-day follow-up period. This is consistent with a lack of clinically discernible drug-antidote complex dissociation, confirming the strong binding affinity between RB006 and RB007, with eventual clearance of the compounds by plasma metabolism. A theoretical concern with active reversal of systemic anticoagulation is the potential for “rebound” thrombosis. Although this worthy concern could not be evaluated in our young and healthy study population, previous studies in animal models of cardiopulmonary bypass and vessel grafting have not revealed evidence of rebound thrombin generation or thrombotic events after antidote administration. RB007 appears to be pharmacologically inert, which is not surprising given its small molecular size and rapid metabolism when not complexed to drug.

The early termination of our study as a result of the “tripping” of a predefined stopping rule highlights the complexities of developing anticoagulant agents, wherein established relationships among well-known drugs, intensity of systemic anticoagulation as directly reflected in prolongation of standard laboratory coagulation assays, and hemorrhagic risk may not apply to new compounds. The subject in question reached an APTT value arbitrarily set during the design of the trial at a value 15% above 2.5 times the upper limit of normal. The rationale for incorporating a stopping rule in any subject-based investigation centers squarely on subject safety. Reaching and exceeding the designated APTT upper boundary provided valuable learning opportunities and underscores several key concepts. First, drug dosing in Regado 1a was fixed (not weight adjusted). The subject who triggered the stopping rule weighed 59.5 kg and received a weight-adjusted dose of RB006 of 1.5 mg/kg, the highest weight-adjusted dose administered in this study. This observation is consistent with the apparent relationship between the weight-adjusted dose of RB006 and pharmacodynamic activity, indicating that a weight-adjusted strategy for RB006 administration in future studies might further enhance dose selection and titratability. Second, administration of RB007 effectively reversed the subject’s elevated APTT, further proof of concept of the drug-antidote pair construct. Finally but importantly, the subject did not experience a hemorrhagic event or other safety issues.

The therapeutic range for RB006, whether determined in terms of APTT, factor IX activity, or other measures of coagulation, remains undefined and requires further investigation in patients with active arterial thrombosis. RB006 did, however, demonstrate the ability to inhibit a substantial portion of plasma factor IX activity in a stepwise, dose-dependent manner. Future studies establishing parameters for intensity of anticoagulation based on clinically dictated requirements will permit flexibility for dose titration and aid in the development of safe and effective dosing strategies.

Conclusions

The Regado phase 1a study represents a first-in-human experience with a nucleic acid aptamer (RB006) designed to target the pivotal coagulation protease factor IXa and its complementary oligonucleotide antidote (RB007). It epitomizes a novel platform for selective and actively reversible pharmacotherapeutics in patients with thrombotic disorders. Our ability to establish a close correlation among aptamer dose, factor IX activity, and APTT prolongation, as well as the safety and durable effectiveness of antidote administration, provided the requisite understanding to begin a phase 1b
study in patients with stable atherothrombotic coronary artery disease. Planned phase 2 trials will further define optimal dosing strategies, improve our understanding of safety, and provide initial assessments of efficacy for the REG1 anticoagulant system.

Acknowledgments
We gratefully acknowledge Drs Jack Ansell, Jeff Weitz, and Craig Kessler for their leadership and guidance as our Data Safety Monitoring Board; Dr Bill Warn GA, Pitoc GA, Quick G, Rusconi CP, Sullenger BA. RNA aptamers as reversible antagonists of coagulation factor IXa. Antithrombosis and Thrombolytic Therapy. 2003;126:287S–310S.

SOURCES OF FUNDING
Regado Biosciences, Inc (Durham, NC) provided research funding for the Regado Ia Clinical Trial.

DISCLOSURES
Regado Biosciences, Inc, is a spin-out of the Duke Department of Surgery, and Duke University has an ownership interest in the biopharmaceuticals company. The Duke Clinical Research Institute received research funding to coordinate the clinical trial, but neither the Duke Clinical Research Institute nor the Duke Clinical Research Institute-affiliated coauthors have an ownership interest in Regado Biosciences, Inc. Dr Dyke received research support from Regado Biosciences, Inc, and was an employee of Duke University. Drs Steinhhubl and Kleiman received research support as site investigators enrolling in Regado Ia. Laura Aberle, Shelly Myles, and Drs Lin, Melloni, Harrington, Alexander, and Becker receive research support from Regado Biosciences, Inc and are employees of Duke University. Dr Rusconi is an employee of and has ownership interest in Regado Biosciences, Inc. Dr Cannon has no disclosures.

REFERENCES

CLINICAL PERSPECTIVE
We report a first-in-human experience with an RNA aptamer and its complementary oligonucleotide antidote used as an anticoagulant system in a subject-blinded, randomized, placebo-controlled, dose-escalation trial in 85 healthy volunteers. RB006, an RNA-based aptamer, elicits an anticoagulant effect by binding directly to and inhibiting the activity of coagulation factor IXa. RB007 is an oligonucleotide complementary to a portion of RB006 that binds to RB006 and neutralizes its anti–factor IXa activity. In the present study, subjects received an intravenous bolus of RB006 or placebo followed 3 hours later by a bolus of RB007 or placebo. Escalation to the next of 4 fixed RB006 and RB007 doses occurred after Data Safety Monitoring Board review. Overall, RB006 and RB007 were well tolerated. Only minor bleeding occurred—mostly bruising at infusion sites—with a similar incidence across groups (including placebo). Chemistry, liver function, hemoglobin, and platelet values were unchanged. One subject experienced transient encephalopathy that resolved rapidly. Evaluation by a neurologist and cerebral imaging were unremarkable, and there was no evidence of intracranial bleeding. The trial was terminated early because the protocol-specified activated plasma thromboplastin time stopping rule was reached in 1 subject who received the highest dose (adjusted for weight) in the study. Activated plasma thromboplastin time strongly correlated with a dose-response relationship with a rapid and sustained return to baseline after antidote. These findings contribute to an emerging platform of selective, actively reversible anticoagulant drugs that may improve safety when used to treat patients with venous and arterial thrombotic disorders.
First-in-Human Experience of an Antidote-Controlled Anticoagulant Using RNA Aptamer Technology: A Phase 1a Pharmacodynamic Evaluation of a Drug-Antidote Pair for the Controlled Regulation of Factor IXa Activity


_Circulation._ 2006;114:2490-2497; originally published online November 13, 2006; doi: 10.1161/CIRCULATIONAHA.106.668434

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/114/23/2490