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The REG1 anticoagulation system: a novel actively controlled factor IX inhibitor using RNA aptamer technology for treatment of acute coronary syndrome

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Abstract

Antithrombotic agents including anticoagulants and antiplatelets are the cornerstone of treatment of acute coronary syndromes. Currently available anticoagulants have several important limitations including unpredictable pharmacodynamics, immunogenicity, and difficulty in reversibility. A potent anticoagulant that has predictable efficacy, is easily reversible should the clinical need arise, and reduces ischemic events without an increase in bleeding risk would overcome many of the current limitations. Inhibition of factor IX in the coagulation cascade has shown promise as a target for development of a novel anticoagulant with a favorable bleeding risk. Aptamers are small oligonucleotides that can be developed to inhibit specific protein targets with high affinity and used as active drugs. Because aptamers are made of oligonucleotide sequences, they provide the code for their own complement (reversal agent) that can be developed and used to inhibit their function. The REG1 anticoagulation system is a novel, aptamer-based, factor IXa inhibitor that is being developed for use in patients undergoing percutaneous coronary intervention and the treatment of acute coronary syndrome.

Keywords

anivamersen; antidote-controlled anticoagulation; aptamer; factor IX; pegnivacogin; REG1 anticoagulation system

Acute coronary syndrome

Patients with acute coronary syndrome (ACS) undergoing coronary revascularization procedures, either with percutaneous coronary interventions (PCI) or through coronary artery bypass grafting (CABG), require periprocedural anticoagulation to minimize ischemic complications [1]. Treatment of ACS centers around the mainstay of antiplatelet and anticoagulation therapy. Currently used anticoagulants include unfractionated heparin (UFH), low molecular weight heparins (LMWH), an indirect factor Xa inhibitor (fondaparinux), and the direct thrombin inhibitor (DTI) bivalirudin. The characteristics of

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each of these anticoagulants are summarized in Table 1. The American College of Cardiology/American Heart Association guidelines for the treatment of ACS and PCI include recommendations for use of all of the above [2–4]. However, there are several important limitations to each of these anticoagulants.

Shortcomings of current anticoagulation therapy

As with any anticoagulant therapy, there is a trade-off between the antithrombotic effect to reduce ischemic events and the bleeding risk. It is estimated that major hemorrhage occurs in approximately 4% of ACS patients and 5% of patients undergoing PCI or CABG [5–7]. It is now widely recognized that any bleeding associated with ACS management or coronary revascularization procedures negatively affects long-term outcomes and is associated with increased morbidity and mortality [8,9]. In fact, there is a stepwise relationship between bleeding severity and adverse clinical outcomes; and blood transfusions appear to further worsen that risk [10]. Therefore, there is a current need for anticoagulants that minimize bleeding risk while maximizing anti-ischemic effects. In particular, an anticoagulant that is easily and safely reversible or titratable in real time, and with a nonrenal clearance mechanism would be highly desirable.

Bivalirudin has been shown to reduce the risk of bleeding as compared with UFH and LMWH in the setting of elective or urgent PCI, which is attributable to its short half-life of 25 min in patients with normal renal function [11–13]. In the HORIZONS trial, bivalirudin was associated with an unexpected reduction in 30-day cardiac mortality (1.8 vs 2.9%; $p = 0.03$) that was attributed to decreased bleeding in patients undergoing primary PCI for treatment of ST-elevation myocardial infarction [12]. On the other hand, this study also showed that the rates of stent thrombosis within the first 24 h after PCI was greater in the bivalirudin group (1.3 vs 0.3%, $p < 0.001$), although without any impact on mortality and without a significant difference at 30 days.

Fondaparinux demonstrated marked reductions in bleeding risk and lower mortality when compared with enoxaparin. However, fondaparinux should not be used as a standalone anticoagulant to support PCI due to an increased risk of catheter thrombosis [14,15].

The heparins are commonly used for both the medical ACS management and to support percutaneous or surgical revascularization. UFH has an unpredictable pharmacodynamic response, can activate platelets, requires careful monitoring and has recently had quality issues leading to serious side effects and death due to adulteration during the manufacturing process. UFH is also associated with heparin-induced thrombocytopenia (HIT); a potentially life-threatening complication. While UFH is reversible through the use of an antidote, protamine sulfate, there are also potentially serious side effects from protamine use and the effective dose is difficult to predict [16].

Enoxaparin has been extensively studied for use in ACS and PCI [17,18]. Its advantages are its ease of use, more predictable pharmacokinetics and pharmacodynamics, and the long half-life. However, unlike UFH, the anticoagulant effect is difficult to monitor and protamine is not an effective antidote for enoxaparin. Furthermore, in case of bleeding complications, its long half-life and inability to be actively reversed are disadvantageous.

Factor IXa as a novel target for anticoagulant development

Factor IXa represents an attractive target for the development of novel anticoagulants for several reasons. Factor IXa is involved in both the initiation and propagation phases of coagulation, and serves as an important link between these two phases (Figure 1) [19]. It is initially activated by the tissue factor (TF)–VIIa complex, leading factor IXa to form a

complex with VIIIa that binds to platelets. The factor IXa–VIIIa complex then serves as a very potent activator of factor X, and represents the rate-limiting enzyme in thrombin generation [20].

Several antifactor IXa agents have been developed with promising results in preclinical studies [19]. In these studies, chemical or immunologic inhibition of factor IXa resulted in a favorable risk ratio of bleeding to anticoagulant effect [21–24]. When used at their minimal effective doses, they have demonstrated effective anticoagulation with reduced bleeding risks in animal models as compared with heparin [25–27]. In a canine model of cardiopulmonary bypass, inhibition of factor IXa demonstrated adequate anticoagulation with limited formation of fibrin in the extracorporeal circuit, as well as diminished blood loss when compared with UFH [28]. Similar results have been seen in other studies comparing factor IX inhibition to UFH [22,25,27]. As a result of these data, inhibition of factor IX appears to be a very promising target for the development of novel anticoagulants.

Aptamers as active drugs

Aptamers (*L. aptus*, to fit) are short, single-stranded oligonucleotides (<50 bases) whose 3D conformations allow them to bind and inhibit specific protein targets with high affinity and specificity. Aptamers encode for their own control agents, which are complementary oligonucleotides that bind to the aptamers through Watson–Crick base pairing and inactivate their function (Figure 2). Introduction of an oligonucleotide control agent complementary to the active aptamer results in a 3D structural change of the aptamer drug that prevents target protein binding and neutralizes the effect of the aptamer drug. Like other small, structured RNAs (e.g., tRNAs, rRNAs and siRNAs) aptamers are not protein coding, and as they are not naturally occurring, aptamers do not have any biologic function beyond the selected target protein-binding activity.

Aptamers are identified through an iterative purification process that was originally described in 1990 [29]. From a large combinatorial library of single-stranded nucleic acids, an *in vitro* screening process called SELEX (Systemic Evolution of Ligands by EXponential enrichment) is used to isolate a specific aptamer (Figure 3) [29]. In this process, an RNA nucleic acid combinatorial library of oligonucleotides is first incubated with the target protein of interest. The oligonucleotide molecules that bind to the target protein are then separated from the others. The identified oligonucleotides that have bound to the target protein are then amplified by reverse transcription and PCR. Lastly, this selected library is then transcribed for further use in additional rounds of SELEX. This process is then repeated approximately eight to 12-times with increasing selective pressure for high-affinity ligands until the affinity of the oligonucleotide library for the target protein is high and can no longer be increased. The selected oligonucleotides are then sequenced and studied for their ability to bind to and inhibit the target protein of interest.

Once an aptamer has been identified through the above process, several steps are required prior to *in vivo* evaluation. This typically includes minimizing aptamer length, increasing serum stability through ribonucleotide substitution of ribonucleotides with stabilizing modifications, and decreasing renal clearance typically by conjugation with a polyethylene glycol (PEG) carrier [30,31]. Through these postselection modifications, the pharmacologic half-life of an aptamer can be designed to be anywhere from several minutes to over 100 h following intravenous administration [32].

Aptamers demonstrate high affinity for their target proteins, similar to that seen for monoclonal antibodies against antigens. This affinity, in conjunction with the specific 3D binding between aptamer and protein, allows for highly discriminatory binding and inhibition of the target protein. Currently, there is only one aptamer approved for clinical use

by the US FDA. Pegaptanib (Macugen®, Eyetech, Inc., FL, USA) is an anti-VEGF aptamer used for treatment of wet age-related macular degeneration [33].

The REG1 anticoagulation system

The REG1 anticoagulation system is an aptamer-based, direct factor IXa inhibitor and its active control agent being developed by Regado Biosciences, Inc. (NJ, USA) for use in the setting of ACS, PCI and open heart surgery. It is composed of the active aptamer drug, pegnivacogin, and its active control agent, anivamersen (Figure 4) [34–37]. Pegnivacogin is a modified RNA made of 31 nucleotides and is stabilized from endonuclease reduction by 2'-fluoro and 2'-O-methyl sugar containing residues, and from exonuclease degradation by a 3' inverted to a deoxythymidine cap [38]. The nucleic acid portion is conjugated to a 40 kDa PEG carrier to limit its volume of distribution to the plasma compartment and prolongs its half-life by limiting renal clearance and by affording additional protection from exonuclease degradation. The active control agent for pegnivacogin, anivamersen, is a 2'-O-methyl RNA oligonucleotide that is 15 nucleotides in length. It binds to pegnivacogin via traditional Watson–Crick base pairing to inhibit its function and reverse its anticoagulant effect. This mechanism allows for full or partial reversal of anticoagulation that is titratable to clinical needs based upon the dose of anivamersen administered.

Pharmacokinetics

The binding of pegnivacogin to factor IXa blocks the activation of factor X by the factor IXa–VIIIa complex, thereby inducing its anticoagulant effect [32,39]. Pegnivacogin binds to factor IXa with a K_d of 3 nM, and an equilibrium between free and bound pegnivacogin with factor IXa is established within 5 min [40]. Pegnivacogin reaches maximum plasma concentration (C_{max}) following bolus intravenous administration within 2–5 min. At doses greater than 0.7 mg/kg, the duration of action is longer than 24 h [34–36]. There is a linear relationship between weight-adjusted pegnivacogin and C_{max} , which can be predicted using the following formula: C_{max} (in $\mu\text{g/ml}$) = 27.98 (pegnivacogin dose mg/kg) + 0.217 (Figure 5) [40].

Anivamersen neutralizes the effect of pegnivacogin within 5 min after intravenous bolus injection. The pegnivacogin/anivamersen complex is stable and biologically inert. Pegnivacogin, anivamersen and the complex formed by the two are cleared via nuclease-mediated degradation. Following administration of anivamersen, there is no evidence of rebound anticoagulant effect [36].

Pharmacodynamics

There is a consistent relationship between pegnivacogin dose, factor IX activity, and activated partial thromboplastin time (aPTT). The relationship between pegnivacogin dose and percentage inhibition of factor IX activity, as well as pegnivacogin concentration and percent inhibition of factor IX, are depicted in (Figure 6). It was determined that a pegnivacogin dose greater than 0.7 mg/kg is associated with >99% inhibition of factor IX activity and an approximate 2.5-fold elevation in plasma aPTT values. Data from Phase I clinical studies confirmed the relationship between pegnivacogin concentration and the relative rise in aPTT (Figure 7). After >99% inhibition of factor IXa, there was an associated maximum of approximately 2.5-fold prolongation of the aPTT.

There is a graded response to reversal with anivamersen based on the dose ratios of anivamersen to pegnivacogin [36]. Anivamersen to pegnivacogin ratios of at least 1:1 fully reversed the pharmacodynamic effect of pegnivacogin and returned aPTT levels to baseline within 1–5 min. Complete pegnivacogin reversal remained stable over 14 days clearly shows

the titratable nature of the reversibility of pegnivacogin and its dependence on the ratio of anivamersen to pegnivacogin administered (Figure 8) [36,40].

Based on combined data from three Phase I studies, a pegnivacogin dose of 1 mg/kg was selected for further clinical study during elective PCI, as it was felt this would provide reproducible inhibition of at least 99% of factor IX activity and posed no increased safety risk as compared with a 0.7 mg/kg dose based upon preclinical and Phase I clinical studies [41]. It was also felt that at least a 1:1 ratio of anivamersen to pegnivacogin was needed to be administered to fully reverse the anticoagulant effect of pegnivacogin. However, to allow for a safety margin, a 2:1 dose ratio of anivamersen:pegnivacogin was selected to provide complete pegnivacogin reversal prior to sheath removal in a Phase IIa pilot study [37].

Clinical evaluation program

To date, three Phase I studies and two Phase II studies have been completed (Table 2) [34–37,42]. These studies have characterized the pharmacokinetic and pharmacodynamic effects of the REG1 anticoagulation system well and have demonstrated promise for its use as an anticoagulant for ACS and percutaneous and surgical coronary revascularization. Here we provide a brief description and review of these studies.

Phase Ia

This was a single-blinded, placebo-controlled, dose-escalation study of 85 healthy volunteers who received a bolus of pegnivacogin or placebo, followed 3 h later by a bolus of the control agent anivamersen or placebo [34]. Among patients who received pegnivacogin, the aPTT and activated clotting time levels increased quickly in a dose-dependent fashion. This effect was observed to be stable over several hours. There was a stepwise increase in relative aPTT values with increasing pegnivacogin doses (Figure 9). Approximately 99% inhibition of factor IXa correlated with a 2.9-fold increase in aPTT. Administration of anivamersen caused rapid reversal of the anticoagulant effect within 5 min and the aPTT levels remained normal in the ensuing 7 days. Overall both pegnivacogin and anivamersen were well tolerated in this study. This was the first human experience of an anticoagulant RNA aptamer and its complementary control agent.

Phase Ib

In this randomized, double-blind, placebo-controlled study, a single escalating intravenous dose of pegnivacogin was studied in patients with stable coronary artery disease on either aspirin, clopidogrel, or both [35]. Patients were randomized to four different doses of pegnivacogin or placebo. Patients receiving active drug were further randomized to receive placebo or anivamersen. A total of 50 subjects were enrolled with a median age of 61 years old. Consistent with the Phase Ia study, a dose-dependent rise in the aPTT was observed after pegnivacogin administration, followed by reversal of aPTT to baseline values within a median of 1 min after anivamersen administration with no rebound seen after 7 days. This study represented the first experience of an aptamer drug/control-agent pair achieving inhibition and active restoration of Factor IXa activity in patients with stable coronary artery disease on antiplatelet therapy.

Phase Ic

This study tested the safety and pharmacodynamic reproducibility of repeat doses of pegnivacogin and the relationship between anivamersen dose and pegnivacogin reversal in 39 healthy volunteers. Subjects were randomized either to placebo or REG1, and treated with the assigned therapy every other day for 5 days [36]. The dose of pegnivacogin was 0.75 mg/kg. Anivamersen was dosed 1 h following pegnivacogin treatment in dose ratios

ranging from 0.125:1 to 2:1. The study showed that repeat doses of pegnivacogin achieved highly reproducible aPTT levels and that repeat doses of anivamersen showed reproducible and dose-dependent decreases in aPTT (Figure 8). This study also demonstrated that there was a lack of residual effect of anivamersen when subjects were re-dosed with pegnivacogin. Both drugs were tolerated well in this study and there were no major bleeding or other serious side effects.

Phase IIa

This study evaluated the use of the REG1 anticoagulation system during elective PCI in a total of 26 patients enrolled in five hospitals [37]. Subjects were randomized to REG1 or UFH in a 5:1 fashion. In the first part of the study patients allocated to REG1 received full anticoagulation with pegnivacogin, followed by partial 50% reversal with anivamersen after the procedure and 100% reversal 4 h later, prior to femoral sheath removal. In the second part of the study, pegnivacogin was 100% reversed with anivamersen immediately after the PCI and followed by femoral sheath removal. This study demonstrated the applicability of a novel anticoagulation system with titratable reversibility in the setting of PCI. Both reversal strategies enabled scheduled femoral sheath removal. There were two ischemic events in the REG1 group consisting of post-procedural cardiac enzyme elevation and a repeat nontarget vessel revascularization procedure. More importantly, there were no serious alarming events, episodes of stent thrombosis, or clot formation in catheters or interventional equipment. There was one ischemic event consisting of cardiac enzyme elevation and one major bleeding event occurred in the UFH group. The study demonstrated the feasibility of supporting PCI with the REG1 system and formed the basis for further investigation in a Phase IIb study.

Phase IIb

The RADAR clinical trial was a Phase IIb study of REG1 in ACS patients undergoing cardiac catheterization. The study had three main objectives: to verify that a 1 mg/kg dose resulted in >99% inhibition of factor IXa in patients with ACS, to determine the dose-response of anivamersen necessary for early and safe sheath removal, and to assess the safety and efficacy of REG1 as an anticoagulant for invasively managed ACS patients [43].

RADAR was designed to enroll a total of 800 subjects randomized 3:1 to pegnivacogin or UFH. Anticoagulation and sheath removal in patients randomized to UFH were managed per local standard of care. The use of glycoprotein 2b3a inhibitors was encouraged in the UFH arm. Patients randomized to pegnivacogin were then randomized in a 2:1:1:2 ratio to target levels of 25, 50, 75 or 100% reversal with anivamersen. It was constructed as an adaptive study design, whereby if excess bleeding or ischemic complications were seen in the extreme arms of reversal, those arms could be closed during the conduct of the study and subsequent patients would be enrolled in the remaining arms. Open label 100% anivamersen could be given to any subject in the pegnivacogin arm who experienced persistent bleeding or needed to undergo CABG. The primary end point was 30-day major or minor bleeding according to a definition used in the ACUTY trial [11]. The secondary end point was a 30-day composite of death, myocardial infarction, urgent target vessel revascularization, or recurrent ischemia.

Enrollment was halted after 640 subjects (479 to pegnivacogin, 161 to heparin) were randomized because of the occurrence of three serious allergic reactions in the REG1 arm. It was determined that the 640 patients enrolled in the study provided enough statistical power to analyze the primary trial end point. During the conduct of the trial, the 25% reversal arm was terminated due to excessive bleeding events. In the REG1 arm, almost 60% of patients

underwent PCI and over 9% had CABG. In the heparin arm, almost 70% underwent PCI, with just over 6% undergoing CABG.

Except for the 25% reversal arm, which was associated with an excessive 65% bleeding rate, total bleeding event rates were similar across all arms (range: 30.4–34.5%). Of note, major bleeding was significantly reduced in the REG1 – 100% reversal arm (7.3%) as compared with the heparin arm (10.1%). In addition, a numerically lower number of ischemic events were noted in the REG1 arm as compared with the UFH arm (3.0 vs 5.7%). However, it is important to note that this study was not powered to show a difference in ischemic events and should be interpreted with caution, but does provide promise for REG1 as an appealing anticoagulant agent in ACS patients undergoing cardiac catheterization [42].

RADAR confirmed that a dose 1 mg/kg of pegnivacogin can effectively achieve >99% inhibition of factor IXa [41]. It also demonstrated that at least 50% reversal with anivamersen is required for safe femoral artery sheath removal and suggested that with higher doses of reversal (75 or 100%), there may be less bleeding than with UFH [42].

Safety

As previously noted, three subjects developed severe allergic-like adverse events shortly after receiving pegnivacogin. All three subjects were enrolled late during the conduct of the trial after several hundred subjects had already been exposed and without safety signals. These reactions occurred in three women and ranged from dermatologic manifestations to hemodynamic compromise requiring support. However, when evaluating other allergic-like nonserious adverse events reported in RADAR, there was no difference in events such as hives, hypotension, rash or dyspnea between the REG1 arm and the UFH arm [42]. A clear etiology of these allergic reactions has not been identified, but after an extensive investigation, the reactions appear not to be specific to pegnivacogin. Determination of the rate of occurrence of these events and the underlying mechanism should be better defined during upcoming Phase III studies in PCI and Phase II studies in open heart surgery as the REG1 system continues to the next phase of clinical development.

Conclusion

The REG1 anticoagulation system is a first-in-class, titratable anticoagulant that inhibits factor IXa using RNA aptamer technology. Early clinical studies have demonstrated that pegnivacogin effectively inhibits >99% of factor IXa with high specificity and reproducible pharmacokinetics. The clinical program suggests that REG1 is a safe anticoagulation strategy in the setting of ACS and PCI. REG1 was well-tolerated in most clinical studies, with no suggestions of severe allergic reactions in the early Phase I and Phase IIa studies. Given the conclusion that the severe allergic reactions seen in Phase IIb were not specific to REG1, further investigation in Phase III studies in PCI is planned. It should be noted that preclinical and clinical studies have consistently demonstrated that aptamers, as biologically active drugs, have low immunogenicity and no risk of allergic reactions have been identified in repeat dose nonclinical studies of REG1.

REG1 is a novel, actively controllable anticoagulation system that possesses many of the qualities lacking in currently available anticoagulants. Its predictable pharmacokinetics and reversibility hold potential advantages over currently available agents. Furthermore, its target (factor IXa) is an advantageous characteristic of this drug as early studies show promising results of reduced ischemic complications without increases in bleeding complications. This constellation of characteristics makes the REG1 anticoagulation system potentially one of the more revolutionary developments in anticoagulation therapy in recent history.

Future perspective

The prospect of having an anticoagulant that is easily titratable and reversible in real time for use in the critical pathway of care of ACS patients from the emergency room to the catheterization laboratory is very appealing. It is hypothesized that this feature could substantially reduce bleeding risk and mitigate the long-term adverse effects of bleeding events. Factor IXa as a target for anticoagulation is an additional appealing feature of REG1. There is now substantial data to suggest that factor IXa inhibition may provide reductions in ischemia without an increase in bleeding complications. In general, aptamers are felt to have low immunogenicity; however, the observation of severe allergic reaction to REG1 in Phase IIb warrants further exploration in future studies. Phase III studies of REG1 in PCI and Phase II studies in open heart surgery are in the planning stages, and will be initiated in the near future. Ultimately, these studies will determine the safety profile and clinical efficacy of REG1 for use in revascularization procedures for the treatment of patients with coronary artery disease.

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References

Papers of special note have been highlighted as:

■ of interest

■ ■ of considerable interest

1. Popma JJ, Berger P, Ohman EM, Harrington RA, Grines C, Weitz JI. Antithrombotic therapy during percutaneous coronary intervention: the seventh ACCP Conference on antithrombotic and thrombolytic therapy. *Chest*. 2004; 126(3 Suppl):576S–599S. [PubMed: 15383485]
2. Antman EM, Anbe DT, Armstrong PW, et al. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the Management of Patients with Acute Myocardial Infarction). *Circulation*. 2004; 110(9):e82–e292. [PubMed: 15339869]
3. Smith SC Jr, Feldman TE, Hirshfeld JW Jr, et al. ACC/AHA/SCAI 2005 Guideline Update for Percutaneous Coronary Intervention – summary article: a report of the American College of Cardiology/ American Heart Association Task Force on Practice Guidelines (ACC/AHA/SCAI Writing Committee to Update the 2001 Guidelines for Percutaneous Coronary Intervention). *Circulation*. 2006; 113(1):156–175. [PubMed: 16391169]
4. Anderson JL, Adams CD, Antman EM, et al. ACC/AHA 2007 guidelines for the management of patients with unstable angina/non-ST-Elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 2002 Guidelines for the Management of Patients With Unstable Angina/ Non-ST-Elevation Myocardial Infarction) developed in collaboration with the American College of Emergency Physicians, the Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation and the Society for Academic Emergency Medicine. *J. Am. Coll. Cardiol.* 2007; 50(7):e1–e157. [PubMed: 17692738]
5. Moscucci M, Fox KA, Cannon CP, et al. Predictors of major bleeding in acute coronary syndromes: the Global Registry of Acute Coronary Events (GRACE). *Eur. Heart J.* 2003; 24(20):1815–1823. [PubMed: 14563340]

6. Kinnaird TD, Stabile E, Mintz GS, et al. Incidence, predictors, and prognostic implications of bleeding and blood transfusion following percutaneous coronary interventions. *Am. J. Cardiol.* 2003; 92(8):930–935. [PubMed: 14556868]
7. Herwaldt LA, Swartzendruber SK, Zimmerman MB, Scholz DA, Franklin JA, Caldarone CA. Hemorrhage after coronary artery bypass graft procedures. *Infect. Control Hosp. Epidemiol.* 2003; 24(1):44–50. [PubMed: 12558235]
8. Rao SV, O'Grady K, Pieper KS, et al. Impact of bleeding severity on clinical outcomes among patients with acute coronary syndromes. *Am. J. Cardiol.* 2005; 96:1200–1206. [PubMed: 16253582]
9. Mehran R, Pocock S, Nikolsky E, et al. Impact of bleeding on mortality after percutaneous coronary intervention results from a patient-level pooled analysis of the REPLACE-2 (randomized evaluation of PCI linking angiogram to reduced clinical events), ACUTY (acute catheterization and urgent intervention triage strategy), and HORIZONS-AMI (harmonizing outcomes with revascularization and stents in acute myocardial infarction) trials. *JACC Cardiovasc. Interv.* 2011; 4(6):654–664. [PubMed: 21700252]
10. Rao SV, Jollis JG, Harrington RA, et al. The relationship of blood transfusion and clinical outcomes in patients with acute coronary syndromes. *JAMA.* 2004; 292:1555–1562. [PubMed: 15467057]
11. Stone GW, McLaurin BT, Cox DA, et al. Bivalirudin for patients with acute coronary syndromes. *N. Engl. J. Med.* 2006; 355(21):2203–2216. [PubMed: 17124018]
12. Stone GW, Witzenbichler B, Guagliumi G, et al. Bivalirudin during primary PCI in acute myocardial infarction. *N. Engl. J. Med.* 2008; 358(21):2218–2230. [PubMed: 18499566]
13. Lincoff AM, Bittl JA, Harrington RA, et al. Bivalirudin and provisional glycoprotein IIb/ IIIa blockade compared with heparin and planned glycoprotein IIb/IIIa blockade during percutaneous coronary intervention: REPLACE-2 randomized trial. *JAMA.* 2003; 289(7):853–863. [PubMed: 12588269]
14. Yusuf S, Mehta SR, Chrolavicius S, et al. Comparison of fondaparinux and enoxaparin in acute coronary syndromes. *N. Engl. J. Med.* 2006; 354(14):1464–1476. [PubMed: 16537663]
15. Yusuf S, Mehta SR, Chrolavicius S, et al. Effects of fondaparinux on mortality and reinfarction in patients with acute ST-segment elevation myocardial infarction: the OASIS-6 randomized trial. *JAMA.* 2006; 295(13):1519–1530. [PubMed: 16537725]
16. Hirsh J, Anand SS, Halperin JL, Fuster V. Guide to anticoagulant therapy: heparin : a statement for healthcare professionals from the American Heart Association. *Circulation.* 2001; 103(24):2994–3018. [PubMed: 11413093]
17. Mahaffey KW, Cohen M, Garg J, et al. High-risk patients with acute coronary syndromes treated with low-molecular-weight or unfractionated heparin: outcomes at 6 months and 1 year in the SYNERGY trial. *JAMA.* 2005; 294(20):2594–2600. [PubMed: 16304073]
18. Montalescot G, White HD, Gallo R, et al. Enoxaparin versus unfractionated heparin in elective percutaneous coronary intervention. *N. Engl. J. Med.* 2006; 355(10):1006–1017. [PubMed: 16957147]
19. Howard EL, Becker KC, Rusconi CP, Becker RC. Factor IXa inhibitors as novel anticoagulants. *Arterioscler. Thromb. Vasc. Biol.* 2007; 27(4):722–727. [PubMed: 17272750]
20. Lawson JH, Mann KG. Cooperative activation of human factor IX by the human extrinsic pathway of blood coagulation. *J. Biol. Chem.* 1991; 266(17):11317–11327. [PubMed: 2040636]
21. Wang X, Cheng Q, Xu L, et al. Effects of factor IX or factor XI deficiency on ferric chloride-induced carotid artery occlusion in mice. *J. Thromb. Haemost.* 2005; 3(4):695–702. [PubMed: 15733058]
22. Benedict CR, Ryan J, Wolitzky B, et al. Active site-blocked factor IXa prevents intravascular thrombus formation in the coronary vasculature without inhibiting extravascular coagulation in a canine thrombosis model. *J. Clin. Invest.* 1991; 88(5):1760–1765. [PubMed: 1939660]
23. Spanier TB, Oz MC, Madigan JD, et al. Selective anticoagulation with active site blocked factor IXa in synthetic patch vascular repair results in decreased blood loss and operative time. *ASAIO J.* 1997; 43(5):M526–M530. [PubMed: 9360098]

24. Refino CJ, Jeet S, DeGuzman L, Bunting S, Kirchhofer D. A human antibody that inhibits factor IX/IXa function potently inhibits arterial thrombosis without increasing bleeding. *Arterioscler. Thromb. Vasc. Biol.* 2002; 22(3):517–522. [PubMed: 11884300]
25. Spanier TB, Chen JM, Oz MC, et al. Selective anticoagulation with active site-blocked factor IXA suggests separate roles for intrinsic and extrinsic coagulation pathways in cardiopulmonary bypass. *J. Thorac. Cardiovasc. Surg.* 1998; 116(5):860–869. [PubMed: 9806393]
26. Feuerstein GZ, Toomey JR, Valocik R, Koster P, Patel A, Blackburn MN. An inhibitory anti-factor IX antibody effectively reduces thrombus formation in a rat model of venous thrombosis. *Thromb. Haemost.* 1999; 82(5):1443–1445. [PubMed: 10595635]
27. Choudhri TF, Hoh BL, Prestigiacomo CJ, et al. Targeted inhibition of intrinsic coagulation limits cerebral injury in stroke without increasing intracerebral hemorrhage. *J Exp Med.* 1999; 190(1): 91–99. [PubMed: 10429673]
28. Spanier TB, Oz MC, Minanov OP, et al. Heparinless cardiopulmonary bypass with active-site blocked factor IXa: a preliminary study on the dog. *J. Thorac. Cardiovasc. Surg.* 1998; 115(5): 1179–1188. [PubMed: 9605089]
29. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science.* 1990; 249(4968):505–510. [PubMed: 2200121]
30. Pieken WA, Olsen DB, Benseler F, Aurup H, Eckstein F. Kinetic characterization of ribonuclease-resistant 2'-modified hammerhead ribozymes. *Science.* 1991; 253(5017):314–317. [PubMed: 1857967]
31. Beigelman L, McSwiggen JA, Draper KG, et al. Chemical modification of hammerhead ribozymes. Catalytic activity and nuclease resistance. *J. Biol. Chem.* 1995; 270(43):25702–25708. [PubMed: 7592749]
32. Becker RC, Rusconi C, Sullenger B. Nucleic acid aptamers in therapeutic anticoagulation. Technology, development and clinical application. *Thromb. Haemost.* 2005; 93(6):1014–1020. [PubMed: 15968382]
33. Campa C, Harding SP. Anti-VEGF compounds in the treatment of neovascular age related macular degeneration. *Curr. Drug Targets.* 2011; 12(2):173–181. [PubMed: 20887245]
34. Dyke CK, Steinhubl SR, Kleiman NS, et al. 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(23):2490–2497. [PubMed: 17101847] ■ **Phase Ia study of REG1.**
35. Chan MY, Cohen MG, Dyke CK, et al. Phase 1b randomized study of antidote-controlled modulation of factor IXa activity in patients with stable coronary artery disease. *Circulation.* 2008; 117(22):2865–2874. [PubMed: 18506005] ■ **Phase Ib study of REG1.**
36. Chan MY, Rusconi CP, Alexander JH, Tonkens RM, Harrington RA, Becker RC. A randomized, repeat-dose, pharmacodynamic and safety study of an antidote-controlled factor IXa inhibitor. *J. Thromb. Haemost.* 2008; 6(5):789–796. [PubMed: 18284597] ■ **Phase Ic study of REG1.**
37. Cohen MG, Purdy DA, Rossi JS, et al. First clinical application of an actively reversible direct factor IXa inhibitor as an anticoagulation strategy in patients undergoing percutaneous coronary intervention. *Circulation.* 2010; 122(6):614–622. [PubMed: 20660806] ■ **Phase IIa study of REG1.**
38. Becker RC, Oney S, Becker KC, Rusconi CP, Sullenger B. Nucleic acid aptamers and their complimentary antidotes. Entering an era of antithrombotic pharmacobiologic therapy. *Hamostaseologie.* 2007; 27(5):378–382. [PubMed: 18060250]
39. Rusconi CP, Scardino E, Layzer J, et al. RNA aptamers as reversible antagonists of coagulation factor IXa. *Nature.* 2002; 419(6902):90–94. [PubMed: 12214238] ■■ First report of an RNA aptamer as a reversible factor IX inhibitor.
40. Povsic TJ, Cohen MG, Chan MY, et al. Dose selection for a direct and selective factor IXa inhibitor and its complementary reversal agent: translating pharmacokinetic and pharmacodynamic properties of the REG1 system to clinical trial design. *J. Thromb. Thrombolysis.* 2011; 32(1):21–31. [PubMed: 21503856]

41. Povsic TJ, Wargin WA, Alexander JH, et al. Pegnivacogin results in near complete FIX inhibition in acute coronary syndrome patients: RADAR pharmacokinetic and pharmacodynamic substudy. *Eur. Heart J.* 2011; 32(19):2412–2419. [PubMed: 21724623]
42. Povsic TJ, Vavalle JP, Kasprzak JD, et al. A randomized, partially-blinded, multi-center, active-controlled, dose-ranging study assessing the safety, efficacy, and pharmacodynamics of the REG1 anticoagulation system compared with unfractionated heparin or low molecular weight heparin in subjects with acute coronary syndromes: primary results of the RADAR randomized clinical trial. Presented at: The 60th Annual Scientific Session of the American College of Cardiology New Orleans, LA, USA 2–5 April 2011 ■ **Phase IIb clinical trial of REG1.**
43. Povsic TJ, Cohen MG, Mehran R, et al. A randomized, partially blinded, multicenter, active-controlled, dose-ranging study assessing the safety, efficacy, and pharmacodynamics of the REG1 anticoagulation system in patients with acute coronary syndromes: design and rationale of the RADAR Phase IIb trial. *Am. Heart J.* 2011; 161(2):261–268. [PubMed: 21315207]

Executive summary

Acute coronary syndrome

- Acute coronary syndromes affect approximately 1 million people in the USA each year.
- Given the central pathophysiologic role of thrombosis in acute ischemic heart disease, anticoagulation together with antiplatelet therapy are the cornerstones of therapy.
- Patients undergoing percutaneous or surgical coronary revascularization also require periprocedural anticoagulation to minimize ischemic complications.

Shortcomings of current anticoagulants

- Currently used anticoagulants include unfractionated heparin, the low molecular weight heparins, an indirect factor Xa inhibitor (fondaparinux), and the direct of thrombin inhibitor bivalirudin.
- These anticoagulants have a number of limitations that include unpredictable pharmacokinetics, lack of reversibility (bivalirudin and low molecular weight heparin), immunogenicity (heparins), and catheter thrombus formation (fondaparinux).

Aptamers as drugs

- Aptamers are single-stranded oligonucleotides (<50 bases) whose 3D conformations allow them to bind and inhibit specific protein targets with high affinity and specificity.
- Since aptamers are made of nucleic acids, they encode for their own antidotes, which are the Watson–Crick complements that bind to and inactivate their function.
- Aptamers are identified through an iterative purification process called SELEX (Systemic Evolution of Ligands by EXponential enrichment).

The REG1 anticoagulation system

- REG1 is a novel, titratable, anticoagulant that directly inhibits factor IXa and is comprised of pegnivacogin, the aptamer drug, and anivamersen as its Watson–Crick pair control agent.
- REG1 has reproducible pharmacokinetics and pharmacodynamics.
- REG1 inhibits >99% of factor IXa, which correlates to an approximate 2.5-times relative increase in aPTT values.
- The anticoagulant effect is reversible within minutes of administering the control agent, anivamersen.

Clinical studies

- Early clinical studies of REG1 have demonstrated that a dose of at least 0.75 mg/kg of pegnivacogin is required to inhibit >99% of factor IXa.
- There is a dose-dependent response to both pegnivacogin, and its control agent anivamersen.
- A ratio of at least 1:1 anivamersen to pegnivacogin has been demonstrated to fully reverse pegnivacogin

- Phase II studies demonstrate that REG1 uniquely has the potential to reduce ischemia and bleeding risk simultaneously in acute coronary syndrome and elective percutaneous coronary intervention.
- There is a safety signal for a possible allergic reaction to this drug that warrants further investigation.

Figure 1. The role of factor IXa in both the initiation and propagation phases of a cell-based model of coagulation

TF: Tissue factor; vWF: Von Willebrand factor.

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Figure 2.
Conceptual function of factor IXa inhibition by pegnivacogin, and reversal with anivamersen

Figure 3.
SELEX process for identification and purification of aptamers for use as drugs.

Figure 4. The REG1 anticoagulation system is made of the active drug aptamer, pegnivacogin and oligonucleotide antidote, anivamersen, which binds to pegnivacogin via Watson–Crick base pairing and inhibits its affect
 Reproduced with permission from [34].

Figure 5. Data from Phase Ia and Ib studies show a linear dose-dependent relationship between pegnivacogin dose and the maximal concentration of pegnivacogin ($C_{\max}$ in mg/ml) Reproduced with permission from [40].

Figure 6. Percent factor IXa inhibition versus weight-adjusted pegnivacogin (A) or plasma concentration of pegnivacogin (B)

Open diamonds are from Phase Ia, and closed squares are from Phase Ib studies, respectively.

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Figure 7. Relationship between pegnivacogin concentration and relative activated partial thromboplastin time

Diamonds are from Phase Ia, and squares are from Phase Ib studies, respectively. aPTT: Activated partial thromboplastin time.

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Figure 8. Relationship between dose of anivamersen and reversal of prolonged activated partial thromboplastin time from pegnivacogin

Ani: Anivamersen; aPTT: Activated partial thromboplastin time; Peg: Pegnivacogin.
Reproduced with permission from [40].

Figure 9. Pharmacodynamic effect of pegnivacogin administration over 3 h

Data points are means $\pm$ SEM. Lines represent the best point-to-point fit of the data. Closed box is placebo, open box is 15 mg, closed circle is 30 mg, open circle is 60 mg, closed triangle is 90 mg of pegnivacogin, respectively. aPTT: Activated partial thromboplastin time.

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Table 1

Principal characteristics of currently available anticoagulants.

	UFH	LMWH	Fondaparinux	Bivalirudin
Anticoagulation target	IIa, Xa, IXa, XIa, and XIIa	IIa and Xa	Xa	IIa
PCI-approved indication	Yes	Yes	No	Yes
Plasma half-life (h)	30–60 min (iv.) Longer at ⁺⁺ doses	3–6 h (sq.)	17–21 h (sq.)	25 min (iv.)
ACC/AHA guideline recommendation for NSTEMI/ACS invasive management	I (LOE: A)	I (LOE: A)	I (LOE: B)	I (LOE: B)
Need for iv. infusion	Yes	No	No	Yes
Elimination	Cellular mechanisms and renal	Renal	Renal	Renal (20%)
Need for monitoring	Yes	No	No	No
Recommended monitoring test	ACT (during PCI)	Anti-Xa level	Anti-Xa level	ACT (not ideal)
Widely available point-of-care test	Yes	No	No	No
Reversibility	Yes: protamine (1 mg/100 IU of UFH)	Partial: protamine (1 mg/1 mg of enoxaparin)	No	No

ACC: American College of Cardiology; ACT: Activated clotting time; AHA: American Heart Association; IU: International unit; iv.: Intravenous; LMWH: Low molecular weight heparin; LOE: Level of evidence; NSTEMI: Non-ST elevation acute coronary syndrome; PCI: Percutaneous coronary intervention; sq.: Subcutaneous;

UFH: Unfractionated heparin.

Table 2

Summary table of clinical studies of REG1.

Study phase	n	Setting	Main findings	Ref.
Phase Ia	85	Healthy volunteers	First human experience with REG1 Stepwise increase in relative aPTT with increasing pegnivacogin doses Rapid reversal observed with anivamersen	[34]
Phase Ib	50	Stable coronary artery disease on antiplatelet therapy	Dose-dependent rise in aPTT, with a rapid reversal with anivamersen	[35]
Phase Ic	39	Healthy volunteers	Repeat doses of pegnivacogin achieved highly reproducible aPTT levels Repeat doses of anivamersen showed reproducible and dose-dependent decreases in aPTT	[36]
Phase IIa	26	Elective PCI	Demonstrated feasibility of REG1 in the setting of PCI	[37]
Phase IIb (RADAR)	640	NSTE-ACS	Established 50% reversal as minimal amount needed for safe arterial sheath removal Confirmed that a dose of 1 mg/kg of pegnivacogin effectively achieves >99% inhibition of factor IXa Fewer ischemic events noted with REG1 as compared with heparin Decreasing bleeding with higher reversal strategies Concern over allergic reaction raised	[42]

aPTT: Activated partial thromboplastin time; NSTE-ACS: Non-ST elevation acute coronary syndrome; PCI: Percutaneous coronary intervention.