Pegnivacogin results in near complete FIX inhibition in acute coronary syndrome patients: RADAR pharmacokinetic and pharmacodynamic substudy

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Received 22 February 2011; revised 26 April 2011; accepted 13 May 2011; online publish-ahead-of-print 30 June 2011

Aims

Establishing factor IX inhibition in patients with acute coronary syndrome/non-ST-elevation myocardial infarction (ACS/NSTEMI), a setting characterized by increased factor IX activity, is critical to investigate the REG1 system in this target population. The REG1 system (Regado Biosciences, Basking Ridge, NJ) consists of pegnivacogin (RB006), an RNA aptamer that directly inhibits factor IXa, and anivamersen (RB007), its complementary control agent.

Methods and results

RADAR is a Phase 2b study investigating the use of pegnivacogin in patients (n = 800) with ACS undergoing planned early cardiac catheterization. To validate dose selection and stability of anticoagulation throughout the time of cardiac catheterization at an early stage of the clinical trial, 33 patients, 22 of whom had not received recent prior heparin, underwent thorough pharmacokinetic and pharmacodynamic assessment. Fold prolongation of activated partial thromboplastin time (aPTT) was used to impute factor IX inhibition. Pegnivacogin 1 mg/kg rapidly achieved a high pegnivacogin plasma concentration (26.1 ± 4.6 μg/mL), prolonged the aPTT (mean aPTT 93.0 ± 9.5 s), and approached near complete factor IX inhibition (mean fold increase from baseline 2.9 ± 0.3). These levels remained stable from the time of drug administration through completion of the catheterization.

Conclusion

Pegnivacogin administered at a weight-adjusted dose of 1 mg/kg consistently achieves a high level of factor IX activity inhibition among patients with ACS and provides stable anticoagulation during cardiac catheterization. These findings support the dose of pegnivacogin selected for the RADAR study.

Keywords

Pharmacodynamic • Pharmacokinetic • Factor IX inhibition • Acute coronary syndrome • Pegnivacogin • Anivamersen

Introduction

Antithrombotic therapy is the cornerstone of early management of patients with acute coronary syndrome (ACS), however, antithrombotic effectiveness is often limited by an inherent propensity for bleeding. As the relationship between bleeding and poor clinical outcomes has become increasingly apparent, an impetus to develop effective anticoagulants that also minimize bleeding risk has emerged.

Nucleic acid aptamers are oligonucleotides with unique three-dimensional structures that confer binding specificity. Using a serial selection process, aptamers specific for coagulation serine
proteases have been constructed. Aptamers are unique in their ability to encode for complementary controlling agents that bind to the active aptamer via Watson–Crick base pairing, alter its conformation, and facilitate either partial or complete reversal of activity.8,10

The REG1 anticoagulation system (Regado Biosciences, Basking Ridge, NJ) consists of pegnivacogin (RB006), an RNA aptamer that is a specific factor IXa inhibitor, and anivamersen (RB007), its 15-nucleotide-controlling agent.11 As in patients with haemophilia B, inhibition of factor IX causes prolongation of the activated partial thromboplastin time (aPTT) and anticoagulation, with the degree of aPTT prolongation correlating directly with the loss of factor IX activity.12 By establishing a relationship between the level of factor IX depletion in plasma samples and the degree of aPTT prolongation, we previously demonstrated in vitro that the degree of factor IX inhibition could be reliably determined using a laboratory aPTT assay.

REG1 has been investigated in three Phase 1 studies, including healthy volunteers and patients with stable coronary artery disease,11,13 and in a Phase 2a trial of patients undergoing elective percutaneous coronary intervention (PCI).14 Aggregate data from these studies suggested that a weight-based pegnivacogin dose of >0.7 mg/kg would achieve a high level of factor IX inhibition. Given the option of reversal with anivamersen and the need for high levels of anticoagulation during the conduct of PCI, particularly in patients with ACS in whom factor IX activity is increased,15,16 a dose of 1 mg/kg was selected for clinical development.14

The purpose of our pharmacokinetic (PK) and pharmacodynamics (PD) substudy was to determine whether pegnivacogin, administered intravenously at a dose of 1 mg/kg, could achieve a therapeutic plasma concentration, produce a consistent and predictable prolongation of aPTT values, and maintain a high degree of factor IX inhibition among patients with ACS participating in RADAR (A Randomized, Partially-Blinded, Multi-Center, Active-Controlled, Dose-ranging Study Assessing the Safety, Efficacy, and Pharmacodynamics of the REG1 Anticoagulation System Compared to Unfractionated Heparin or Low Molecular Heparin in Subjects with Acute Coronary Syndromes, www.clinicaltrials.gov, NCT 00932100).

Methods

This is a pre-specified PK and PD substudy of patients enrolled in the RADAR trial at seven early enrolling sites. The protocol was reviewed and approved by institutional review boards at each site, and all patients provided informed consent for the substudy prior to participation.

The design of the RADAR trial has been described previously and is shown in Figure 1.17 RADAR targets enrolment of patients with ACS/non-ST-segment elevation myocardial infarction (NSTEMI), and subjects are required to have angiina or anginal equivalent symptoms within 72 h of randomization and either (i) new or presumably new ST-depression on electrocardiogram (ECG), (ii) cardiac biomarkers consistent with NSTEMI, or (iii) a history of prior coronary artery disease as defined by flow-limiting stenosis on angiography or a prior history of coronary artery bypass grafting (CABG) or PCI. Patients were to undergo cardiac catheterization within 24 h of randomization, which represents the time of biological activity after a single dose of pegnivacogin.

Patients are randomised 3:1 to either pegnivacogin or heparin (unfractionated or low molecular) in an open-label fashion. Patients randomized to REG1 were eligible for the PK/PD substudy. The REG1 arm is further randomized 2:1:1:2 to blinded and varied degrees of anivamersen reversal. This PK/PD substudy focuses on the effects of pegnivacogin prior to anivamersen reversal.

Substudy specimen collection and analysis

Thirty-five patients, 24 of whom had not received recent prior heparin, were randomized to REG1 and underwent blood sample collection for central analysis at four time points. Blood (7 cc) was drawn (i) after randomization but prior to pegnivacogin administration; (ii) 20 min after initial pegnivacogin administration; (iii) immediately prior to cardiac catheterization; and (iv) after completion of the catheterization procedure, but prior to administration of blinded doses of anivamersen.

Blood was collected in EDTA- and citrate-containing tubes, centrifuged at 1500 × g for 7 min at 0–4°C, and the plasma was aspirated from the specimen using a transfer pipette. Plasma was dispensed in
1 mL vials, labelled, and placed into a −70°C freezer within 1 h of collection. Aliquots for PK and PD analysis were shipped in separate shipments to ACM Global Laboratories (Rochester, NY, USA) in ThermoSafe (Tegrant Corporation, Arlington Heights, IL, USA) shipping boxes with dry ice. Aliquots for PK analysis were subsequently shipped to PPD (Richmond, VA, USA) for analysis.

Sample analysis
Pegnivacogin concentration determination
Pegnivacogin plasma concentrations were measured using an ELISA method employing oligonucleotides complementary to pegnivacogin for capture and detection in human plasma, as follows: a biotinylated deoxyribonucleic acid capture probe complementary to the 3′-end of pegnivacogin (5′-[Biotin-TEG][spacer-18]GTGGAGGCAGCA TTA-3′) and a digoxigenin-labelled 2′-O-methyl detection probe complementary to the 5′-end of pegnivacogin (5′-CCCGGUAUAGUCCAC [spacer-18][Digoxigenin]-3′) were added to plasma samples containing pegnivacogin and incubated at 37°C for ~2 h in a 96-well microtiter plate to allow hybridization of the probes to pegnivacogin. The mixture was then transferred to a neutravidin-coated microtiter plate to immobilize the pegnivacogin-probe complex on a surface. Detection was accomplished using an anti-digoxigenin antibody conjugated to alkaline phosphatase coupled with the fluorescent substrate, AttoPhos (Promega Biosciences, Madison, WI, USA). Fluorescence intensity was measured using a Spectramax Gemini fluorescence plate reader (Molecular Devices, Silicon Valley, CA, USA), the signal of which was directly proportional to the amount of pegnivacogin present in the calibration standards, quality control samples, and study samples.

Pharmacodynamic analysis
Core laboratory coagulation assays were performed on a Stago STA analyser (Stago, Parsippany, NJ, USA). Activated partial thromboplastin time assays were performed with STA-PTT AS (Stago). Factor IX activity assays were performed using STA-Deficient IX immunodepleted plasma on the STA-R analyser calibrated with the STA-Unicalibrator (Diagnostica Stago, Asnieres, France). Fold increase in aPTT was determined by normalizing the aPTT value at each time point to the baseline value for that patient.

Imputation of factor IX activity
Calibration curves for the plasma factor IX assay generated during the analysis of samples in the study were generated to establish the relationship between relative aPTT prolongation and plasma percent of factor IX activity. The per cent of factor IX activity for individual plasma samples isolated from pegnivacogin-treated subjects was then imputed from the calibration curve based on the relative aPTT prolongation of each sample, and the per cent of inhibition of factor IX calculated (e.g. 100 per cent of factor IX activity = per cent of factor IX inhibition).

Statistical analysis
Descriptive statistics are expressed as mean values ± standard deviation and/or median values with 25th and 75th percentiles. All statistical analyses were considered significant with a P-value < 0.05. An ANOVA analysis was performed to assess differences at multiple time points and, where the overall ANOVA analysis reached statistical significance, pairwise comparisons for each pair of time points were performed using Tukey’s correction for multiple comparisons. Interaction between time and prior heparin dosing on pegnivacogin concentration was determined using a two-way ANOVA. The coefficient of variation was calculated from the standard deviation and means at each time point.

Results
RADAR began enrolment on 8 September 2009. Enrolment of the final patient in the pre-specified PK/PD cohort targeting enrolment of 30 patients was on 23 April 2010. Enrolment in RADAR was completed on 10 November 2010. Of the 35 patients enrolled, 24 had not received recent prior heparin and were eligible for PD assessments. Two patients were excluded from both the PK and PD analysis. One patient had non-viable PK samples upon receipt in the laboratory and, in addition, no PD samples were drawn. The second patient had mislabelled samples, precluding a correlation between pegnivacogin concentrations and PD assessment. An additional patient, included in the PK analysis, was excluded from the PD analysis as no samples for this purpose were provided.

Patient characteristics for the overall, PK, and PD analysis cohorts are shown in Table 1. The population was ~50% female and predominantly white with an average age of 62 years. There was a high prevalence of hypertension, hyperlipidaemia, and diabetes. A substantial proportion of patients were on medical therapy for atherosclerotic coronary artery disease and had a prior history of myocardial infarction (MI), PCI, or CABG. In addition, the majority of patients had either abnormal cardiac biomarkers or ongoing ECG changes.

All patients received 1 mg/kg pegnivacogin. Post-dosing samples were obtained at a mean (± standard deviation) and median (25th, 75th) of 40 ± 36 and 30 (21, 51) min following pegnivacogin dosing, respectively. Pre- and post-catheterization samples were obtained at 139 ± 218, 95 (42, 145) and 146 ± 195, 104 (54, 170) min following pegnivacogin dosing, respectively. The post-catheterization samples were drawn over a range of 11–1328 min or 19.9 h post-pegnivacogin administration.

Pharmacokinetic analysis
Thirty-three patients were included in the PK analysis. Twelve individual samples were not received: three after pegnivacogin dosing, eight pre-catheterization, and one post-catheterization. Three samples were excluded from the PK analysis due to spurious results. One sample obtained post-catheterization demonstrated very low pegnivacogin concentrations despite therapeutic pegnivacogin concentrations at the two other sampling points, as well as a simultaneous post-catheterization aPTT reflective of high levels of factor IX inhibition. Two samples (pre- and post-catheterization) from one patient were also excluded, displaying similar discordance between prior pegnivacogin concentrations and simultaneous aPTT elevation. Thus, the final PK analysis cohort reflects 117 samples obtained from 33 patients.

Pegnivacogin was not detectable at baseline (n = 33). After pegnivacogin administration, pre-catheterization, and immediately post-catheterization mean pegnivacogin concentrations were 26.1 ± 4.6, 25.8 ± 4.8, and 23.9 ± 4.4 μg/mL (Figure 2). Mean and median pegnivacogin concentrations were in the range targeted (18–30 μg/mL) at all time points following pegnivacogin dosing. One subject consistently had pegnivacogin concentrations
at the lower end of the targeted range (17.4, 15.7, and 13.6 μg/mL at time points 2, 3, and 4, respectively). There was no effect of recent prior heparin therapy on pegnivacogin plasma concentrations (Figure 3). As a measure of the degree of variation in pegnivacogin concentrations among the study cohort, a coefficient of variation was 18% at each time point. This degree of variation is consistent with that expected from the pegnivacogin concentration assay.

Pharmacodynamic analysis

The PD cohort comprised 21 patients who had not received any prior heparin therapy. Of these, six samples were not received, one post-pegnivacogin sample, and 5 pre-catheterization samples. Two samples were removed from the PD analysis: one with an aPTT value of 34.4 s pre-catheterization and values of 105.2 s post-pegnivacogin administration and 100.2 s post-catheterization, and one with an aPTT value of 240 s with aPTTs of 87.0 and 87.3 s just prior to and following this sample.

The mean aPTT values prior to and immediately after pegnivacogin administration were 30.8 ± 3.8 and 93.0 ± 9.5 s, respectively (Figure 4). During the sampling period, there was no change in observed PD effect of pegnivacogin, with mean aPTTs of 93.9 ± 26.8 s and 94.3 ± 16.0 s prior to and after completion of catheterization. The coefficient of variation ranged from 10.2% post-dosing to 28.6% pre-catheterization.

Pegnivacogin administration resulted in a three-fold mean increase of aPTT at time points post-administration (3.03 ± 0.38), pre-catheterization (2.95 ± 0.58), and post-catheterization (3.08 ± 0.52), with no difference over the time course of sampling (Figure 5). Other values of interest at these time points include: minimum fold aPTT increase (2.23, 2.26, and 2.10), median fold increase (3.03, 2.85, 2.98), and maximum fold increase (3.80, 4.77, 4.76).
Based on well-validated relationships established during the course of three Phase III,13,18 and a Phase 2a PCI study,14 relative aPTT prolongation was employed as a measure of factor IX inhibition. Using factor IX deficient mixed with factor IX replete plasma, a correlation curve was derived to determine the relationship between fold increase in aPTT and level of factor IX activity (Figure 6). This curve was used to impute the degree of factor IX inhibition from the fold increase in aPTT after pegnivacogin administration.

Near complete factor IX inhibition was observed following pegnivacogin administration at all 3 time points (Figure 7). The minimum fold increases in aPTT noted at each of the time points after pegnivacogin administration correspond to >99.8% factor IX inhibition.

The PK and PD relationship from the RADAR ACS cohort (Figure 8A) was compared with profiles from healthy volunteers (Figure 8B),11 and patients with stable coronary artery disease (Figure 8C),13 derived from the CLIN101 and CLIN102 studies, which utilized variable fixed doses of pegnivacogin to achieve a range of pegnivacogin concentrations. At the pegnivacogin concentrations achieved in RADAR, no dose–response was observed, consistent with achieving near complete factor IX inhibition in all patients at all time points and dosing at the upper plateau of the dosing curve.

Discussion

We found that 1 mg/kg pegnivacogin administered intravenously as a 1 min bolus consistently achieved a pegnivacogin plasma concentration between 18 and 30 μg/mL, prolonged the APTT by three-fold compared with baseline, and maintained near complete inhibition of factor IX activity in a cohort of patients with ACS undergoing cardiac catheterization. These data are in keeping with those derived from healthy volunteers11,18 and patients with stable coronary artery disease.13,14 However,
these data are the first to document that 1 mg/kg pegnivacogin, the dose selected for RADAR, reliably achieves near complete inhibition (99.8%) of factor IX activity in the setting of ACS, both immediately after initial dosing and during cardiac catheterization.

**Importance of pharmacodynamic monitoring**

The successful translation of novel therapies from preclinical development to use in clinical care is highly dependent on dose selection. This is especially true for new anti-thrombotics, which traditionally have a narrow therapeutic window, and the development of several anti-thrombotics has been delayed or harmed by improper dosing. Development of REG1 was predicated on the supposition that the availability of reversal would allow safe administration of high intensity anticoagulation for short periods of time. Therefore, we targeted near complete inhibition of the target, factor IX, thus maximizing in theory anti-ischaemic effect, while relying on active control to mitigate bleeding risk. While the dose of pegnivacogin achieving this level of FIX inhibition in stable patients had been theoretically derived from a combined analysis of a series of Phase 1 and 2a studies, the applicability of this dosing regimen to the ACS patient population was unknown when RADAR was initiated.

Accordingly, we performed a prospectively designed PK and PD substudy in the early stages of RADAR—a Phase 2b study. REG1, a novel anticoagulation system, is unique in its target (factor IXa), structure (oligonucleotide), and incorporation of both an active (pegnivacogin) and controlling agent (anivamersen), allowing complete or partial reversal should the clinical need arise. RADAR is the first clinical study investigating the utility of REG1 in the treatment of patients with ACS.

The pivotal role of factor IXa in each successive stage of coagulation, including initiation, priming, and propagation coupled with its requirement for platelet thrombus formation and thrombin generation in arterial injury, supports elevated factor IX activity as a risk factor for periprocedural MI. In the Study of Myocardial Infarctions Leiden (SMILE), which included 560 men <70 years of age with a first MI and 646 control subjects, mean factor IX activity was higher in patients than controls. Coronary heart disease-related events were assessed in relation to coagulation activation markers among 2997 men between 50 and 63 years of age (36 507 person-years of observation) participating in a Medical Research Council surveillance study. Factor IX activation peptide, reflecting either intrinsic pathway activation, tissue factor-mediated activation, thrombin-mediated bioamplification, or their combined effects was associated with MI. We have previously reported that pegnivacogin, by preventing association of factor X with the factor VIIa-factor IXa enzyme complex, blocks factor X
activation and subsequent thrombin generation. Our current findings show that the 1.0 mg/kg pegnivacogin dose inhibits factor IX activity in the highly thrombogenic environment of ACS.

We observed no relationship between pegnivacogin plasma concentration and fold increase in aPTT, confirming that the 1.0 mg/kg pegnivacogin dose is on the upper plateau of the dose-ranging curve. The consistency of our findings with those obtained in studies of healthy volunteers, patients with stable coronary artery disease, and in patients with coronary disease undergoing elective PCI further suggests that pegnivacogin PK and PD are stable across a wide range of clinical settings (Figure 7).

The verification of high factor IX activity inhibition was an early and pre-specified milestone for the continuation of the RADAR study as currently designed.

Reversal and RADAR results
Modelling from Phase 1 suggests that reversal results in a step-wise decrease in pegnivacogin concentrations, fold increase in the aPTT, and by inference degree of FIX inhibition. The focus of this sub-study was to assess in the open-label REG1 population the degree of factor IX inhibition achieved by 1 mg/kg dose of pegnivacogin. The PD surrounding variable levels of anivamersen reversal will only be known after completion of RADAR, which was closed to enrolment on 10 November 2010.

Limitations
During the conduct of this study, three PK samples were excluded because of low concentrations despite therapeutic concentrations at other time points, and two PD samples were excluded: one sample had an aPTT in the normal range despite elevated values at other post-dosing time points. The reasons for these anomalies are likely due to mishandling or mislabelling of samples, as repeated PD testing in Phase 1 studies demonstrated consistent aPTT prolongation after pegnivacogin dosing.

Conclusions
Dose selection in early phase drug development represents a cornerstone of the safe conduct of clinical investigation and positioning for active comparator Phase 3 clinical trials. Pegnivacogin, administered intravenously at a dose of 1 mg/kg, rapidly and consistently achieves high plasma concentrations, prolongs the aPTT by three-fold, and maintains near complete inhibition of factor IX activity in patients with ACS. These findings impact interpretation of RADAR and future clinical trials assessing the safety and efficacy of factor IXa inhibition and its partial or complete reversal on clinical outcomes.

Acknowledgements
The authors wish to thank Shelley Myles, Diane Joseph, Carolyn Darmiento, and Rebecca Cheren for their roles in the RADAR trial, and Elizabeth Cook for editorial assistance during the writing of this manuscript.

Funding
This work was supported by Regado Biosciences.


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