

Letter to the Editor

Pre-existing anti-PEG antibodies are associated with severe immediate allergic reactions to pegnivacogin, a PEGylated aptamer

To the Editor:

PEGylation is commonly used to extend half-life and limit volume of distribution of an increasing number of nucleic acid, peptide, and small molecule therapeutics. Pegnivacogin is a modified 31-nucleotide RNA aptamer that binds to and inhibits factor IXa conjugated to an inert 40-kD branched methoxypolyethylene glycol polymer. Although early clinical testing did not identify any safety concerns, the phase IIb 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 (RADAR) trial was stopped after 3 allergic reactions.¹ An extensive investigation demonstrated elevated levels of IgG anti-PEG antibodies in the 3 patients with allergic events, suggesting that the PEG moiety, and not the oligonucleotide, was the causative allergic agent.² On the basis of previous safety record of PEGylated products, investigators and regulatory authorities agreed that pegnivacogin should undergo additional definitive testing incorporating a risk mitigation and action plan in a phase III trial (Randomized, Open-label, Multi-Center, Active-Controlled, Parallel Group Study to Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention [REGULATE-PCI]) in which subjects undergoing percutaneous coronary intervention were randomized to pegnivacogin or bivalirudin.³ Methodology of the trial, planned biochemical analyses, statistical analyses, and allergy definitions are available in the first and second sections in this article's Online Repository at www.jacionline.org. REGULATE-PCI was ultimately terminated after enrollment of 3,232 of a planned 13,200 patients after an excess of allergic reactions in pegnivacogin-treated patients.³

The incidence and timing of allergic reactions are summarized in Table I. Descriptions of allergies meeting reporting criteria, as judged by the investigators, are provided in the third section in this article's Online Repository at www.jacionline.org. Assignment to pegnivacogin was associated with a statistically significant increase in allergic reactions. Of the clinical variables assessed, female sex, allergic reactions in the past year, current smoking, and previous percutaneous coronary

intervention were associated with severe allergic reactions (see Table E1 in this article's Online Repository at www.jacionline.org). There was no evidence of altered risk of allergic reactions in patients premedicated with H1 or H2 blockers, corticosteroids, beta blockers, or angiotensin-converting enzyme inhibitors (see Table E2 in this article's Online Repository at www.jacionline.org).

As stipulated in the risk mitigation and action plan, measurements of complement activation, tryptase release, and anti-PEG IgG antibodies were performed in all patients experiencing allergic reactions within 24 hours of pegnivacogin or bivalirudin (n = 34) dosing as well as a selected cohort of patients who did not manifest allergies (n = 144).

There were no significant differences in the baseline (prestudy drug) levels of C3a, C4a, C5a, CH50, Factor Bb, or tryptase between patients experiencing and not experiencing allergic responses, regardless of treatment group (see Fig E1 in this article's Online Repository at www.jacionline.org).

Fig 1, A, depicts the levels of baseline IgG anti-PEG antibodies normalized to the assay cutoff point. IgG anti-PEG antibodies were present in 15% of pegnivacogin-treated (13 of 87) and bivalirudin-treated (8 of 55) patients not experiencing reactions (consistent with published estimates^{4,5}), and in 71% (17 of 24) of patients with any allergic reaction and 83% (15 of 18) of patients with severe allergic reactions after pegnivacogin treatment. Of 16 patients with allergic reactions within 1 hour of pegnivacogin dosing, 15 (94%) had positive anti-PEG antibodies. None of the 10 patients with allergic reactions after bivalirudin dosing had preformed anti-PEG antibodies.

The levels of IgG anti-PEG antibodies were associated with the severity of clinical manifestation: 5.6 ± 4.6 for those with severe, 2.6 ± 3.2 for nonsevere, and 1.0 ± 1.4 for those with no reaction. Among patients with IgG anti-PEG antibodies more than $1 \times$ or $3 \times$ the assay cutoff point, the likelihood of having a severe allergic reaction was 5.1% (95% CI, 2.4-11.4) and 16.2% (95% CI, 7.4-25.0), respectively.

To obtain mechanistic insight into immediate allergic reactions to pegnivacogin, we analyzed measures of complement and tryptase activation before and 90 minutes after study drug (Fig 1, B and C). CH50 was significantly decreased, whereas C3a and Factor Bb were significantly increased. No significant differences were observed for changes in C4a or C5a, although 9 of 11 patients had increases in C4a. Tryptase levels were significantly increased; however, only some of the patients met proposed criteria for evidence of tryptase-mediated allergy

TABLE I. Incidence of allergic reactions within 24 hours by treatment arm

Type of allergic reaction	Bivalirudin (n = 1601)	Pegnivacogin (n = 1605)	Total (N = 3206)	OR (95% CI)
Any allergic reaction	10 (0.62)	24 (1.5)	34 (1.06)	2.4 (1.2-5.1)
Serious allergic reaction	1 (0.06)	10 (0.62)	11 (0.34)	10.0 (1.3-78.5)
Severe allergic reaction	4 (0.25)	18 (1.12)	22 (0.69)	4.5 (1.5-13.4)
Nonsevere allergic reaction	6 (0.37)	6 (0.37)	12 (0.37)	1.0 (0.3-3.1)
Anaphylaxis	1 (0.06)	10 (0.62)	11 (0.34)	10.0 (1.3-78.5)
Allergic reaction onset <1 h after study drug dosing	2 (0.12)	16 (1.0)	18 (0.56)	8.1 (1.9-35.1)
Severe allergic reaction onset <1 h after study drug dosing	1 (0.06)	12 (0.74)	13 (0.40)	12.1 (1.6-92.8)

Data presented as n (%). Bivalirudin was the reference group in the calculation of OR.
OR, Odds ratio.

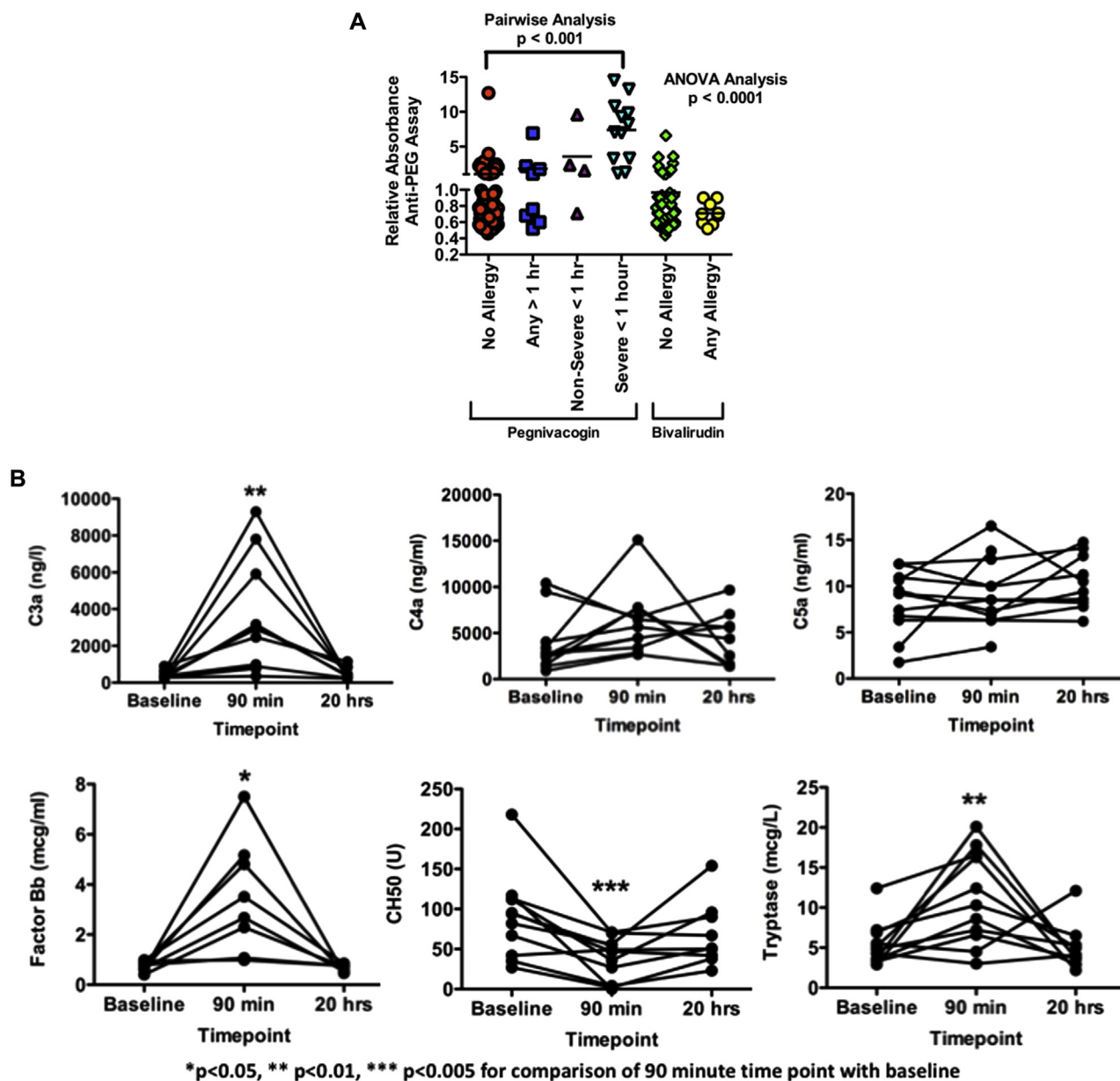


FIG 1. A, Relative levels of anti-PEG antibodies by allergy. B, Changes in individual complement levels in patients with severe immediate acute allergic reactions to pegnivacogin. C, Changes in individual complement and tryptase levels by treatment and occurrence and timing of allergy.

(doubling of tryptase levels, 6 of 11; follow-up tryptase >11.4 , 6 of 11; Valent criteria, 7 of 11; see Table E3 in this article's Online Repository at www.jacionline.org).

Although anti-PEG antibodies have been described with an incidence similar to our observed rate, their clinical importance is unknown. In contrast to reactions associated with induced antibodies, first-dose reactions have been rarely described.⁶ Some cases of acute allergy to PEG have been attributed to IgE anti-PEG antibodies, which have been documented by skin testing.⁷ First-dose reactions to pegloticase have been described, although the relationship to IgG anti-PEG antibody is unclear.⁵ Infusion reactions with PEGylated products have been reported, but a connection with the PEG component has not been established to date (see Table E4 in this article's Online Repository at www.jacionline.org).

Our work demonstrates that acute severe allergic reactions to pegnivacogin were observed exclusively in those with preformed anti-PEG antibodies, and were associated with complement activation and tryptase release. Increases in tryptase are consistent with IgE-dependent mast cell degranulation. We were unable to determine what role, if any, IgE antibodies played, because an assay for IgE anti-PEG antibodies was not available and skin testing was not practical in this planned 13,200 patient international cardiovascular trial; however, an IgE-dependent mechanism would not be expected to result in complement activation.

A possible alternative mechanism invokes direct IgG-PEG complex activation of the classical complement pathway, with C3a- and C5a-activated mast cells accounting for the elevated tryptase levels, a mechanism described in hyperimmune animal models of anaphylaxis.

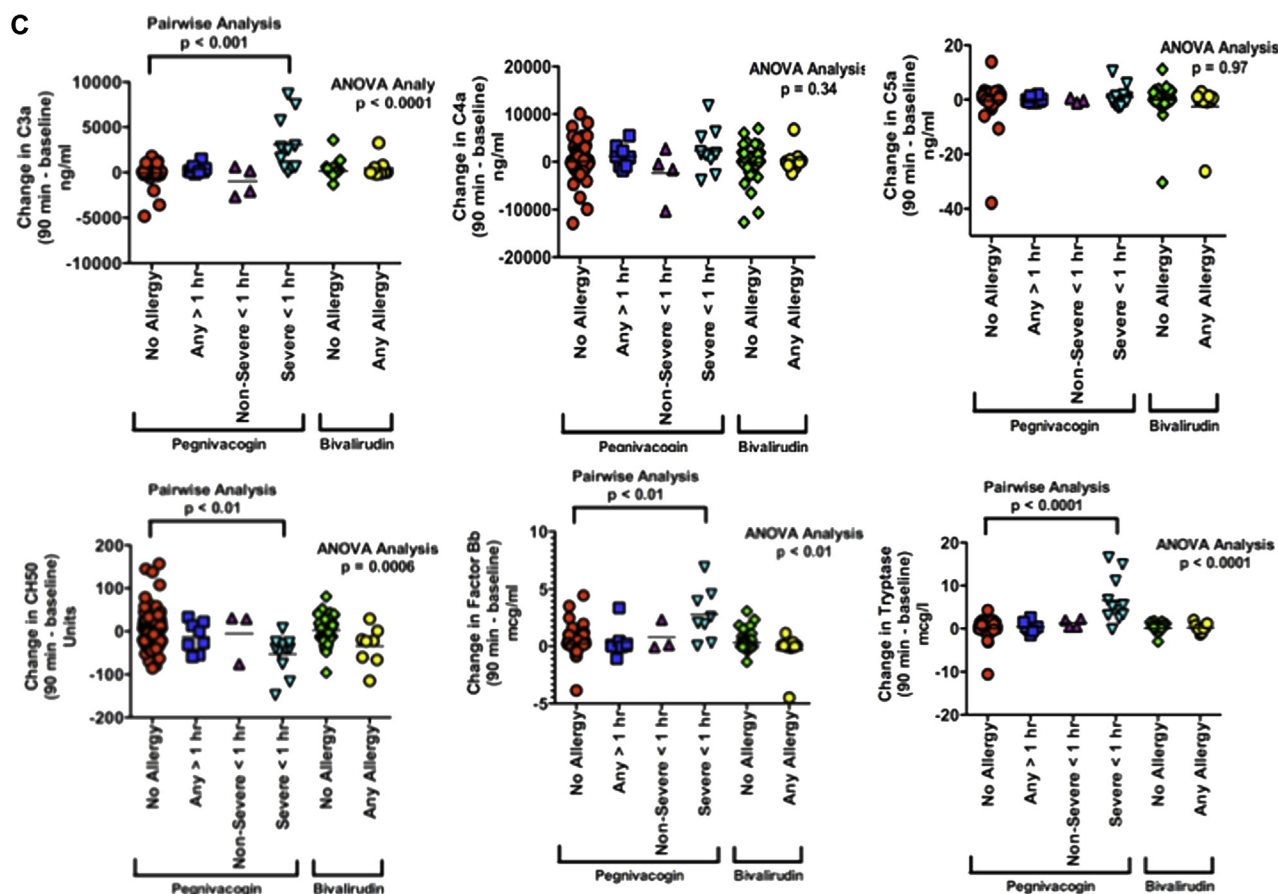


FIG 1. (Continued).

Murine models of anaphylaxis have suggested that IgG antibody/allergen complexes interacting with Fcγ receptors on basophils and neutrophils release platelet-activating factor and other vasoactive mediators.⁸ This mechanism has not been substantiated in humans; however, the robust baseline IgG anti-PEG titers and high plasma concentrations of PEG (~20 μg/mL) achieved after bolus pegnivacogin dosing are consistent with murine studies demonstrating high levels of both IgG and antigen as key factors in this type of reagenic IgG anaphylaxis.^{8,9}

We cannot definitively determine which of these or other mechanisms is applicable. The strong association of IgG anti-PEG antibodies with severe allergic reactions is consistent with the growing interest in IgG-mediated anaphylaxis, and the evidence of complement activation raises the possibility that complement may be involved in some cases of human anaphylaxis. Future studies aimed at identifying the subclasses of IgG anti-PEG antibodies involved and the coexistence of IgE anti-PEG antibodies would be important.

Elizabeth Cook of the Duke Clinical Research Institute provided editorial support.

Thomas J. Povsic, MD, PhD^a
Monica G. Lawrence, MD^b
A. Michael Lincoff, MD^c
Roxana Mehran, MD^d
Christopher P. Rusconi, PhD^e

Steven L. Zelenkofske, DO^e
Zhen Huang, MS^a
Jeffrey Sailstad, BS^f
Paul W. Armstrong, MD^g
P. Gabriel Steg, MD^{h,i,j}
Christoph Bode, MD^k
Richard C. Becker, MD^l
John H. Alexander, MD, MHS^a
N. Franklin Adkinson, MD^m
Arnold I. Levinson, MDⁿ

on behalf of the REGULATE-PCI Investigators

From ^aDuke Clinical Research Institute, Duke Medicine, Durham, NC; ^bthe University of Virginia, Charlottesville, Va; ^cCleveland Clinic Coordinating Center for Clinical Research (C5Research), Cleveland, Ohio; ^dMount Sinai School of Medicine, New York, NY; ^eRegado Biosciences, Basking Ridge, NJ; ^fSailstad and Associates, Inc, Durham, NC; ^gCanadian VIGOUR Centre, University of Alberta, Edmonton, Alberta, Canada; ^hUniversité Paris-Diderot, Sorbonne Paris Cité, Paris, France; ⁱAssistance Publique-Hôpitaux de Paris, Paris, France; ^jNHLI, Royal Brompton Hospital, Imperial College, London, United Kingdom; ^kthe University of Freiburg, Freiburg, Del; ^lthe University of Cincinnati College of Medicine, Cincinnati, Ohio; ^mJohns Hopkins Asthma and Allergy Center, Baltimore, Md; and ⁿPerelman School of Medicine, University of Pennsylvania, Philadelphia, Pa. E-mail: thomas.povsic@duke.edu.

The REGULATE-PCI trial and this analysis were funded by Regado Biosciences Inc. The sponsor of the trial, Regado Biosciences, participated in the design of the trial and the analysis plan for biospecimens collected in the trial, in collaboration with the investigators of the trial. The sponsor selected the core laboratories for the analysis, but did not perform the analyses or analyze the data. The corresponding author drafted this manuscript and made all revisions on the basis of input from the coauthors, including those employed by the sponsor. The sponsor had no role in the decision to submit the manuscript for publication.

Disclosure of potential conflict of interest: T. J. Povsic has received a grant from Regado Biosciences. M. G. Lawrence has received consulting and/or advisory board fees from Baxter Healthcare, has received fees for participation in review activities from Regado Biosciences/Faculty Connections, and has consultant arrangements with Merck/Faculty Connections. A. M. Lincoff has received a grant and travel support from Regado Biosciences; has received grants from Roche/Genentech, AstraZeneca, CSL, Eli Lilly, Pfizer, and Takeda; has received personal fees from CSL, Semonix, Amgen, Sarepta, and Abbott; and has received travel reimbursement from Eli Lilly, Pfizer, Takeda, Amgen, Sarepta, and Abbott. R. Mehran has received consulting and/or advisory board fees from Regado Biosciences, Abbott Vascular, AstraZeneca, Boston Scientific, Covidien, CSL-Behring, Janssen Pharmaceuticals, Maya Medical, Merck, and Sanofi-Aventis; grants from Eli Lilly/Daiichi Sankyo, AstraZeneca, The Medicines Company, Bristol-Myers Squibb/Sanofi-Aventis, and OrbusNeich; and has received consulting fees from Janssen Pharmaceuticals, Medscape, Osprey Medical Inc, and Watermark Research Partners. C. P. Rusconi has consultant arrangements with and is employed by Regado Biosciences, is current consultant for Tobira Therapeutics, is inventor on multiple pending and issued patents covering REG1 and its uses, has patents related to the composition of the subject of this article (no compensation received), and has stock options as an employee of Regado Biosciences. S. L. Zelenkofske is a former employee of Regado Biosciences and has a patent pending for anti-PEG antibodies. J. Sailstad has received consulting fees from Regado Biosciences. P. W. Armstrong has received research support from Boehringer-Ingelheim, AstraZeneca, GlaxoSmithKline, and Regado Biosciences and consulting and/or advisory board fees and/or honoraria from Boehringer-Ingelheim, Eli Lilly, Roche, Bayer, AstraZeneca, GlaxoSmithKline, and Regado Biosciences. P. G. Steg has received consulting fees and travel support from Regado Biosciences; has consultant arrangements with Amarin, AstraZeneca, Bayer, Boehringer-Ingelheim, Bristol-Myers-Squibb, CSL-Behring, Daiichi Sankyo, GlaxoSmithKline, Janssen, Lilly, MerckSharpeDohme, Novartis, Pfizer, Regeneron, Roche, Sanofi, Servier, Otsuka, Medtronic, Vivus, Janssen, Orexigen, and The Medicines Company; has received grants from Merck, Sanofi, and Servier; and has received payment for lectures from AstraZeneca, Amgen, and Merck. C. Bode has received a grant and consulting fees from Regado Biosciences; has received payment for lectures from Bayer, BMS, and Daiichi Sankyo; and has received consulting fees and/or honoraria from AstraZeneca, Bayer, Daiichi Sankyo, and Merck. R. C. Becker has received a grant from Duke University; has consultant arrangements with Janssen, AstraZeneca, and Portola; has received consulting fees and/or honoraria from Regado Biosciences; and has received research support from the National Heart, Lung, and Blood Institute. J. H. Alexander has consultant arrangements with Bristol-Myers-Squibb, CSL-Behring, Daiichi Sankyo, GlaxoSmithKline, Janssen, Pfizer, Sohalution, and Xoma; has received consulting fees from the American College of Cardiology, Portola, and the VA Cooperative Studies Program; has received a grant from Regado Biosciences, Boehringer-Ingelheim, Bristol-Myers-Squibb, CSL-Behring, Pfizer, Sanofi, Tenax, the National Institutes of Health, Tenex Therapeutics, and Vivus Pharmaceuticals; Duke University

owns a small amount of equity in Regado Biosciences. The amount and terms of this equity are unknown to anyone involved in this project. N. F. Adkinson has received fees for Data Safety Monitoring Board service from Duke University and for participation in review activities from Duke Clinical Trials. A. I. Levinson has received consulting fees from Regado Biosciences, Stallergenes, Endo Pharmaceuticals, and the US Department of Health and Human Services and has consultant arrangements with Janssen and Eisai Pharmaceuticals. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Povsic TJ, Vavalle JP, Aberle LH, Kasprzak JD, Cohen MG, Mehran R, et al. A phase 2, randomized, partially blinded, active-controlled study assessing the efficacy and safety of variable anticoagulation reversal using the REG1 system in patients with acute coronary syndromes: results of the RADAR trial. *Eur Heart J* 2013;34:2481-9.
2. Ganson NJ, Povsic TJ, Sullenger BA, Alexander JH, Zelenkofske SL, Sailstad JM, et al. Pre-existing anti-PEG antibody linked to first-exposure allergic reactions to pegnivacogin, a PEGylated RNA aptamer. *J Allergy Clin Immunol* 2016;137:1610-3.e7.
3. Lincoff AM, Mehran R, Povsic TJ, Zelenkofske SL, Huang Z, Armstrong PW, et al. Effect of the REG1 anticoagulation system versus bivalirudin on cardiovascular outcomes following PCI. The REGULATE-PCI randomized clinical trial. *Lancet* 2016;387:349-56.
4. Garay RP, El-Gewely R, Armstrong JK, Garratty G, Richette P. Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents. *Expert Opin Drug Deliv* 2012;9:1319-23.
5. Hershfield M, Ganson N, Kelly S, Scarlett E, Jaggars D, Sundry J. Induced and pre-existing anti-polyethylene glycol antibody in a trial of every 3-week dosing of pegloticase for refractory gout, including in organ transplant recipients. *Arthritis Res Ther* 2014;16:R63.
6. Lee SH, Cha JM, Lee JI, Joo KR, Shin HP, Baek IH, et al. Anaphylactic shock caused by ingestion of polyethylene glycol. *Intest Res* 2015;13:90-4.
7. Shah S, Prematta T, Adkinson NF, Ishmael FT. Hypersensitivity to polyethylene glycols. *J Clin Pharmacol* 2013;53:352-5.
8. Finkelman FD. Anaphylaxis: lessons from mouse models. *J Allergy Clin Immunol* 2007;120:506-15.
9. Jiao D, Liu Y, Lu X, Pan Q, Zheng J, Liu B, et al. Characteristics of anaphylaxis-inducing IgG immune complexes triggering murine passive systemic anaphylaxis. *Allergy* 2013;68:236-45.

<http://dx.doi.org/10.1016/j.jaci.2016.04.058>

Supplement

Section 1: REGULATE-PCI Methods

Patients undergoing percutaneous coronary intervention (PCI) were randomized 1:1 to receive pegnivacogin (1 mg/kg IV over 2 minutes) or bivalirudin (dosed per local label) as an anticoagulant. All patients underwent an allergy assessment prior to enrollment, including the number and severity of previous allergies, the specific allergen, type of reaction, treatment required, and date of the most recent allergic reaction. Use of concomitant medications was collected, including medications administered at time of catheterization, focused on use of antihistamines (H1 or H2 blockers), steroids, beta-blockers, and angiotensin converting enzyme (ACE) inhibitors. Study drug was administered in the catheterization laboratory immediately prior to PCI. All patients gave written informed consent.

A clinical events committee blinded to treatment and including an allergist independently adjudicated all suspected allergic reactions and classified them as fatal, severe, or non-severe. Events classified as severe were adjudicated as anaphylactic or not using criteria developed by Sampson et al.¹ Allergic reactions were also categorized as serious or non-serious using standard regulatory definitions.

Statistical Methodology

As pre-specified, the causal relationship between randomized treatment and allergic reactions was assessed and reported as odds ratios (OR) with 95% confidence intervals (CI). Baseline characteristics (including treatment received, age, race, region (NA, EE, WE), weight, BMI, history of allergy, number of allergies in the past year, randomization subgroup, reason for index PCI, ejection fraction, tobacco use, renal insufficiency, hyperlipidemia, previous stroke, previous MI, previous PCI, previous CABG, history of congestive heart failure, peripheral vascular disease) were compared among patients with and without allergic reactions within 24 hours of study drug dosing. Given the small number of events, a multivariate

logistic model included only factors with significant univariate association with allergic reactions ($p < 0.10$).

Baseline levels of complement components and tryptase levels between allergy subgroups were compared using t-tests. Two-way analysis of variance (ANOVA) was used to assess for interaction between allergy subgroup, time course, and levels of complement or tryptase.

To estimate the likelihood of developing a severe allergic reaction to pegnivacogin for subjects with baseline IgG anti-PEG antibodies, the crude rate from this subgroup was applied to the study population without allergies and randomized to pegnivacogin. A sensitivity analysis was carried out through inverse probability weighting.² First, the probability of being included in the subgroup was estimated through a logistic model. The inverse of this probability was then applied to the subgroup, and the estimate for the population was generated. Bootstrap method was used to obtain estimates of the standard error and the 95% confidence intervals.

Continuous variables are presented as means and standard deviation. Categorical variables are presented as frequencies and proportions. P-values are not adjusted for multiple comparisons. Analyses were performed at the Duke Clinical Research Institute using SAS version 9.3 (SAS Institute, Inc., Cary, NC) and Prism 5.0 (Microsoft Corporation, Redmond, WA).

Biochemical Testing

Serum and plasma samples (ethylenediaminetetraacetic acid [EDTA] tubes) obtained prior to, 90 minutes, and 20 hours post-study drug dosing were frozen, shipped on dry ice, and stored at -70°C (Pharmaceutical Product Development, LLC [PPD], Wilmington, NC) for analyses of complement activation (C3a, C4a, C5a, and Factor Bb [serum] and CH50 [plasma]), tryptase levels (plasma), and IgG anti-PEG antibodies (serum). Analysis was performed in samples from all patients with allergic reactions ≤ 24 hours after study

drug dosing and select non-reactive trial participants matched for age, region, weight, and sex and chosen based on sample availability and treatment with either bivalirudin or pegnivacogin. Levels of serum C3a, C4a (Advanced Diagnostic Laboratories, National Jewish Health, Denver, CO), C5a, Factor Bb (Central Laboratories, PPD), plasma tryptase (Mayo Clinic, Rochester, MN), and CH50 (Cleveland Clinic, Cleveland, OH) were analyzed in batch testing using standardized clinical assays.

Determination of IgG Anti-PEG Antibody

Anti-PEG Antibody Assay

An enzyme-linked immunosorbent assay (ELISA) for IgG anti-PEG antibodies was developed and validated (PBA11-248; WilResearch, Skokie, IL).

Overview

Serum samples were analyzed for the presence of antibodies reactive with PEG using a validated direct binding ELISA assay. The assay was validated for inter- and intra-assay precision, freeze-thaw stability, short term stability and drug tolerance per current industry recommendations.^{3,4} The assay screening cutpoint was statistically assigned per the methodology of Shankar by analysis of data obtained from analyzing 51 serum samples on 6 plates by 2 analysts over 3 separate days. A floating parametric cutpoint was determined to be 1.476 x negative control (NC) sample mean OD per plate. The confirmatory cutpoint was statically assigned by determining the upper limit of non-specific inhibition of antibody negative samples after the exclusion of outliers. The confirmatory cutpoint (99.9% confidence interval) was determined to be 21% inhibition of the mean sample OD in the presence as compared to absence of competitor, 20 kDa linear PEG (NOF). The estimated sensitivity at the assay cut point using a rabbit anti-PEG antibody was 1747 ng/mL. The assay was developed and validated at WIL Research (Skokie, IL). Analysis was conducted per WIL Research SOPs for ELISA assays.

Materials

Pegnivacogin was supplied as a lyophilized powder by Regado Biosciences and stored at -20°C. 20 kDa linear PEG (Sunbright ME-200CS, NOF) was used as the competitor (inhibitor) in confirmatory assays. Positive and negative control samples were identified by screening of individual normal human serum samples (Bioreclamation) during assay development. Positive control samples were classified as high (HC) and low (LC) positive based upon OD response obtained during assay development. The secondary antibody used for detection was alkaline phosphatase-conjugated goat anti-human IgG (Fab specific) from Sigma (Part A 8542). P-nitrophenyl phosphate (p-NPP) was also from Sigma. For the sensitivity assessment, the secondary antibody used for detection was alkaline phosphatase-AffiniPure Goat anti-Rabbit IgG F(ab)'₂ fragment from Jackson ImmunoResearch (Cat no. 111-055-005).

Procedure

A 96-well microtiter plate was coated with pegnivacogin. The positive controls (HC and LC), NC and unknown samples were diluted 20-fold (minimum required dilution), added to the wells of the microtiter plate and incubated with shaking at room temperature, followed by washing to remove any non-pegnivacogin reactive serum components. Detection of pegnivacogin-bound antibodies was achieved by incubation with anti-human IgG alkaline phosphatase conjugate, followed by washing and addition of pNPP substrate. Following incubation with pNPP, the reaction was stopped to terminate color development, and A₄₀₅ nm determined. The intensity of the color produced was proportional to the amount of anti-PEG antibodies in the sample. For confirmatory immunodepletion assays, the procedure was repeated (sample volume permitting), with samples analyzed in duplicate $\pm$ 20 kDa linear PEG and % inhibition calculated for each sample. Titers were determined using the end-point dilution method.

Sample Analysis

Serum samples were processed at investigative sites and shipped frozen at -70°C. The LC and HC were analyzed two times in duplicate on each plate. The NC was run three times in duplicate on each plate. The

mean OD of the three duplicate sets of the NC was used to calculate the plate-specific cutpoint. Each sample analyzed was compared to the plate-specific cutpoint.

All samples that were less than the plate-specific cutpoint were reported as negative. All samples that were equal to or greater than the plate-specific cutpoint were considered presumptive positive for anti-PEG antibodies and underwent confirmatory testing (sample volume permitting) to confirm positive samples and to exclude any false positives.

Any sample demonstrating a % Inhibition $\geq 21\%$, after the addition of 20 kDa PEG was reported as positive for anti-PEG antibodies. Any sample with % Inhibition $< 21\%$ was reported as negative for anti-PEG antibodies. Samples that were confirmed positive for the presence of anti-PEG antibodies in the confirmatory assay were titrated to estimate the anti-PEG antibody titer, sample volume permitting. A total of 540 samples were received to be analyzed for the presence of anti-PEG antibodies in human serum. One was QNS and was not able to be analyzed. The remaining 539 samples were screened for the presence of anti-PEG antibodies in human serum. One hundred and seventeen (117) samples yielded ODs above their corresponding plate-specific cutpoint and were presumed positive for anti-PEG antibodies. Of these, 3 were QNS for further testing and were not analyzed in the confirmatory assay. Of the 114 presumptive positive samples that were subjected to confirmatory immunodepletion analysis, 80 out of 539 samples (14.8%) were confirmed positive for the presence of anti-PEG antibodies and 34 samples were negative because the % Inhibition was less than 21%. These were considered false negatives and confirmed the desired false positive rate near 5% (actual 6.3%) Antibody titers were determined for the 80 confirmed positive samples.

References

1. Sampson HA, Muñoz-Furlong A, Bock SA, et al. Symposium on the definition and management of anaphylaxis: Summary report. *J Allergy Clin Immunol.* 2005;115:584–91.

2. Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. *Stat Methods Med Res.* 2013;22:278–95.
3. Mire-Sluis AR, Barrett YC, Devanarayan V, et al. Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. *J Immunol Methods* 2004;289:1–16.
4. Shankar G, Devanarayan V, Amaravadi L, et al. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. *J Pharm Biomed Anal* 2008;48:1267–81.

Section 2: Definitions of Allergic Events Used for Adjudication

Clinical presentation at baseline, including disease and procedural-related symptoms, will be considered when adjudicating all allergic events and determining specific organ system involvement.

Allergic Event Definitions

- 1) **Non-severe:** Acute onset of an illness (minutes to hours) with involvement of skin/mucosal tissue (e.g., hives, generalized itch/flush, swollen lips/tongue /uvula), airway compromise (e.g., dyspnea, wheeze/bronchospasm, stridor, reduced peak expiratory flow), reduced blood pressure or associated symptoms (e.g., hypotonia, syncope), other cardiovascular manifestations (e.g., chest pain, dysrhythmia), altered mental status, gastrointestinal (e.g., nausea, crampy abdominal pain, vomiting) or other symptoms (e.g., uterine contractions in women, aura of doom) not meeting criteria for severe allergic reaction but temporally related to administration of study drugs in a manner which suggests an allergic reaction.
- 2) **Severe:** Acute onset of an illness (minutes to hours) with involvement of skin/mucosal tissue (e.g., hives, generalized itch/flush, swollen lips/tongue/uvula), airway compromise (e.g., dyspnea, wheeze/bronchospasm, stridor, reduced peak expiratory flow), reduced blood pressure or associated symptoms (e.g., hypotonia, syncope), other cardiovascular manifestations (chest pain, dysrhythmia), altered mental status, gastrointestinal (e.g., nausea, crampy abdominal pain, vomiting) or other symptoms (e.g., uterine contractions in women, aura of doom) temporally related to administration of study drugs in a manner which suggests an allergic reaction **AND** which cause any of the below:
 - a. Requirement for hemodynamic support, including administration of more than 500 cc of intravenous fluid resuscitation, bolus or IV infusion of pressor agents, including but not limited to epinephrine, norepinephrine, dopamine, or vasopressin.
 - b. Requirement for respiratory intervention, including administration of B2 agonists to treat wheezing/airway obstruction, administration of positive pressure ventilation, or intubation.
 - c. Signs or symptoms which require treatment with systemic corticosteroids.
 - d. Events which result in prolongation of hospital stay.
- 3) **Fatal:** A fatal allergic reaction is an allergic reaction leading directly to death. Deaths after anaphylactic allergic reactions secondary to other complications (MI, stroke, CHF, arrhythmias) which are exacerbated or felt to be secondary to the allergic reaction will be considered deaths due to the allergic reaction. Deaths that occur during treatment of complications of allergic reactions (pressor support, intubation) or which occur prior to resolution of the signs/symptoms of the allergic reaction will be considered fatal allergic reactions. Deaths which occur after the patient has recovered from the allergic reaction (i.e. normal hemodynamics have been restored, pressor support [bolus or infusion] is no longer required, any respiratory compromise has resolved) who go on to develop other complications (e.g., MI, stroke, CHF, arrhythmias) which are not directly a result of the allergic reaction or its treatment will not be considered fatal allergic reactions.

- 4) **Anaphylactic:** Anaphylaxis is a severe allergic reaction that is rapid in onset and may cause death. An anaphylactic reaction will be defined as the acute onset of an illness (minutes to several hours) occurring in a temporally related fashion to administration of study product with involvement of skin or mucosal tissue or both (e.g., generalized hives, pruritus or flushing, swollen lips/tongue/uvula) **AND** at least one of the following:
- a. Respiratory compromise (e.g., dyspnea, wheeze/bronchospasm, stridor, reduced peak expiratory flow hypoxemia) requiring intervention (e.g., B2 agonist treatment, positive airway ventilation, intubation)
 - b. Hemodynamic compromise, including reduced blood pressure or end-organ compromise (e.g., hypotension, syncope, incontinence, tachycardia) requiring fluid resuscitation.
4. **Angioedema:** Angioedema is a result of extravasation of fluid into interstitial tissue leading to localized subcutaneous or submucosal swelling, and may occur with or without urticaria. Angioedema is characterized by rapid onset (minutes to hours), asymmetric distribution, tendency to not involve gravitationally dependent areas, involvement of the face, lips, larynx, or bowels, and association with some other signs and symptoms or allergic reactions. Angioedema will be diagnosed if:
- There are signs or symptoms (swelling, abdominal pain, respiratory compromise) of extravasation of fluid into subcutaneous or submucosal tissue **AND**
 - Edema changes rapidly over minutes to hours **AND**
 - The pattern of edema includes at least one of the following:
 - Asymmetric distribution
 - Not in a dependent position
 - Involves the face, lips, larynx or bowels
 - Is associated with other allergic manifestation

Section 3: Safety Narratives for All Serious Allergic Reactions

Allergic/anaphylactic reaction #1

Subject 12012048 was a 49-year-old female who participated in: *A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study To Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention*. Medical history included myocardial infarction (MI) (not in the last year), previous percutaneous coronary intervention (PCI), hyperlipidemia, current tobacco user, and no history of allergies. Medications taken within seven days before onset of the event included bisoprolol, ticagrelor, ECASA (enteric coated aspirin), trandolapril, Crestor (rosuvastatin calcium), and pantoprazole. Medications given on 07-Jan-2014 due to the PCI included Versed (midazolam), verapamil, lidocaine, Isovue (iopamidol injection) contrast dye, and nitroglycerin.

On 07-Jan-2014 at 08:16, the subject was randomized to study drug. On 07-Jan-2014 at 08:18, the subject received study drug. On 07-Jan-2014 at 08:20, the subject underwent PCI.

The subject's angiogram was completed. The subject was not pre-treated with steroids prior to her PCI since she did not have a history of allergies. On 07-Jan-2014 at 08:16, the registered nurse (RN) noticed that the subject's face was red, without hives. The subject was coughing; her coughing resolved. She had no complaints of shortness of breath (SOB) at that time. The subject had a severe anaphylactic reaction; classification was hypotension and respiratory. After study drug was given, the subject became agitated, short of breath, flushed, and wheezy; her blood pressure (BP) dropped to 80/53 mmHg, oxygen (O2) saturation dropped to 85%. She had nausea and vomiting. Treatment included epinephrine 0.5 mg times 2, oxygen, initiation of normal saline (NS) bolus, Solu-Cortef (hydrocortisone sodium succinate) 250 mg, Benadryl (diphenhydramine) 50 mg intravenously (IV), Zofran (ondansetron) 4 mg IV, intravenous fluids, Ventolin (albuterol sulfate), and epinephrine infusion. The PCI was completed. The subject's nausea and vomiting resolved in the cath lab. By the end of the procedure, the subject's color and shortness of breath had improved and her BP was 93/71 mmHg. The subject was admitted to the coronary care unit (CCU) for observation; her BP was low and an epinephrine infusion was started. The subject began to improve. On 07-Jan-2014 at 13:00, the subject's systolic BP was above 100 mmHg and the epinephrine infusion was discontinued at that time. There were no relevant laboratory/diagnostic tests.

On 07-Jan-2014 at 13:00, the outcome of the event was recovered/resolved.

The event was considered serious because it was an important medical event and required hospitalization or prolongation of an existing hospitalization.

The investigator has assessed the causal relationship between the study medication and the event as not related, stating the event may be related to contrast dye, as the subject started to have minor symptoms prior to the IP.

CEC Adjudication Results CEC re-reviewed: 17-Sep-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	07 Jan 2014 08:16
Type of allergic reaction	Severe
Severe subtype of reaction	Anaphylactic
If severe, did this qualify solely on the basis of the	No

administration of corticosteroids?	
Organ System Involvement	Mucocutaneous; Respiratory; Circulatory
Reviewer 1. How did the immunology sample impact the final adjudication decision?	Supported
Comments or Rationale (Specialty Reviewer)	Temporal relationship to study drug unclear (reaction may have begun 2 mins prior to administration).
Comments or Rationale (Phase II Committee):	Symptoms started before administration of study drug.

Allergic/anaphylactic reaction #2

Subject 12009002 was a 78-year-old male who participated in: *A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study To Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention*. Medical history included myocardial infarction (MI) within the past year, previous coronary artery bypass graft (CABG), previous percutaneous coronary intervention (PCI), hyperlipidemia; the subject had no history of allergies and never smoked tobacco. The subject received rabeprazole, enteric coated aspirin (EC ASA), ticagrelor, atorvastatin, bimatoprost eye drops, losartan, and tamsulosin CR (controlled-release) within seven days before onset of the event. Procedural-related medications included Gravol (dimenhydrinate), 2% Xylocaine (lidocaine), nitroglycerin, midazolam, Visipaque (iodixanol), and fentanyl.

On 12-Feb-2014 at 12:23, the subject was randomized to study drug. On 12-Feb-2014 at 13:07, the subject received study drug. On 12-Feb-2014 at 13:21, the subject underwent PCI.

The subject had no known prior allergies and was not pre-medicated for allergy prior to the catheterization. On 12-Feb-2014 at 13:14, three to five minutes after receiving study drug, the subject was noted to have profound hypotension, with systolic blood pressure (BP) 30-40 mmHg, upper body rash, flushing, and hives on his left shoulder; the subject's mucocutaneous and circulatory systems were involved. The subject's level of consciousness was assessed; he was still mentating. The subject's mouth was checked and there was no swelling. He was assessed for respiratory difficulties and was found to have no respiratory symptoms. The subject had no gastrointestinal (GI) symptoms. Treatment included normal saline 500 ml intravenous (IV) bolus times two, epinephrine 100 mcg IV times three, SoluCortef (hydrocortisone) 200 mg IV, Zantac 200 mg IV (to prevent GI symptoms), and Benadryl (diphenhydramine) 50 mg IV; study drug was stopped. The subject's BP stabilized and he had no increase in rash, hives, or further allergy symptoms. The subject's BP was borderline throughout but he was otherwise clinically stable and recovered very well at the end. The PCI procedure was completed uneventfully post reaction.

The subject's daughter indicated that, in January, the subject developed a rash after his hospitalization for myocardial infarction; it was thought that the subject was allergic to perindopril. The subject was switched to losartan. During the consent process, when the study coordinator inquired about allergies, the subject had forgotten about the rash and the perindopril.

On 12-Feb-2014 at 15:45, the outcome of the event was recovered/resolved.

Action taken with study drug was discontinued due to the event.

The event was considered serious because it was an important medical event.

The investigator has assessed the causal relationship between the study medication and the event as related, with rationale: acute onset within minutes of study drug administration. At the time of the event, the site investigator and sub-investigator assessed the event as not related to any of the usual procedural medications or activities that had taken place for the angiogram. Possible alternate causes of the SAE: none; within query response field it was confirmed, in the assessment of the site investigator, there were no other suspected causes. The site investigator and sub-investigators looked at the possibility of the allergic reaction being related to contrast dye. The subject had two angiograms in January and had developed the rash presumably related to perindopril afterward. It was concluded that allergy contrast was not likely because the subject was able to undergo the remaining procedure using more contrast after the allergic reaction symptoms had settled down.

CEC Adjudication Results Date of Review: 01-Jul-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	12Feb2014 13:14
Type of allergic reaction	Severe
Severe subtype of reaction	Anaphylactic
If severe, did this qualify solely on the basis of the administration of corticosteroids?	No
Organ System Involvement	Mucocutaneous; Circulatory
Reviewer 1. How did the immunology sample impact the final adjudication decision?	Supported
Comments or Rationale (Specialty Reviewer)	Hives, severe hypotension requiring epinephrine, NS, solumedrol, anti-H1
Comments or Rationale (Phase II Committee):	None

Allergic/anaphylactic reaction #3

Subject 11961013 was a 57 year-old male who participated in: *A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study To Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention*. Medical history included previous coronary artery bypass graft (CABG), previous percutaneous coronary intervention (PCI), diabetes mellitus, hyperlipidemia, tobacco use-former smoker, and history of allergies, none in the past year; allergic reaction to penicillin on 02-Apr-1994, type of reaction and treatment required: unknown. The subject received metformin, Omega 3, clopidogrel, aspirin, pravastatin, Versed (midazolam), Optiray 350 (Ioversol injection), cinnamon, niacin, morphine, esomeprazole, ibuprofen, propafenone, metoprolol, and digoxin within 7 days before onset of this event.

On 01-Apr-2014 at 15:54, the subject was randomized to study drug. On 01-Apr-2014 at 15:58, the subject received study drug. The PCI was not completed on this subject per physician (MD) discretion. The physician felt the subject was too unstable to continue the PCI procedure.

The subject was not prepped for allergy before the catheterization. On 01-Apr-2014 at 16:03, five minutes after study drug dosing, the subject experienced anaphylaxis reaction. Initial symptoms were flushing, itching, throat swelling, tachycardia, and hypertension followed by hypotension and fever. Other symptoms included swelling of mouth or lips, nausea, vomiting, and diarrhea. Systems involved included mucocutaneous, respiratory, circulatory, and gastrointestinal/genitourinary. The event was classified as anaphylactic due to severe and rapid onset of symptoms that involved skin and mucosal tissue, as well as

hemodynamic compromise of hypotension and tachycardia, which required fluid and drug resuscitation. Treatment included intravenous fluids, H2 antagonists, steroids, and vasopressors. Specific treatment medications were Benadryl (diphenhydramine), epinephrine, Decadron (dexamethasone), Neo-synephrine (phenylephrine), Zofran (ondansetron), dopamine, Lasix (furosemide), and oxygen (O2). On 01-Apr-2014 at 19:38, the subject's temperature was 101 degrees Fahrenheit (F) at which time blood cultures were drawn. All subsequent temperatures were afebrile. The source of fever and elevated white blood cell (WBC) count may have been from the anaphylaxis. The source of the positive blood culture was unknown but thought to have possibly been caused from possible contamination during collection. The subject was prophylactically treated with broad spectrum antibiotics. The subject's treatment response was manifested by return to baseline of blood pressure, body temperature, and O2 saturations, and stabilization of vital signs and urinary output. Until 02-Apr-2014, the subject's femoral sheath was left in place for monitoring arterial line pressures. That same day, the subject's pressure stabilized and he was subsequently weaned from vasopressors. On 04-Apr-2014, the subject was discharged to home in stable condition and with no sequelae resulting from this event.

On 04-Apr-2014 at 13:32, the outcome of the event was recovered/resolved.

The event was considered serious because it was an important medical event, was life-threatening, and resulted in hospitalization and prolonged hospitalization. The subject's hospitalization was prolonged due to administration of prophylactic antibiotics. The subject was unstable based on vital signs and presentation. Per the investigator, the subject would have died without intervention; thus, the event was considered to be life-threatening

The investigator has assessed the causal relationship between the study medication and the event as related. Other medications proximal to the event included Versed and morphine. The investigator's rationale for the causal relationship was the close timing between the time of study drug administration and the timing of the reaction. According to the report, there were no other alternative exposures for potential causes for the reaction.

CEC Adjudication Results Date of Review: 01-Jul-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	01Apr2014 16:03
Type of allergic reaction	Severe
Severe subtype of reaction	Anaphylactic
If severe, did this qualify solely on the basis of the administration of corticosteroids?	No
Organ System Involvement	Mucocutaneous; Respiratory; Circulatory; Gastrointestinal or Genitourinary
Reviewer 1. How did the immunology sample impact the final adjudication decision?	Supported
Comments or Rationale (Specialty Reviewer)	Anaphylaxis with markedly elevated inflammatory mediators in blood
Comments or Rationale (Phase II Committee):	Anaphylaxis with markedly elevated inflammatory mediators in blood

Allergic/anaphylactic reaction #4

Subject 19015002 was a 59-year-old female who participated in: *A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study To Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention*. Medical history included previous percutaneous coronary intervention (PCI), hyperlipidemia, former tobacco smoker, normal left ventricular function (>55%) on 29-Jan-2014, and no known allergies. Concomitant medications given within seven days before onset of the event included acetylsalicylic acid, Brilique (ticagrelor), bisoprolol, simvastatin, atacand, imeron, and aspirin.

On 24-Apr-2014 at 12:33, the subject was randomized to study drug. On 24-Apr-2014 at 13:12, the subject received study drug. On 24-Apr-2014 at 13:15, the subject underwent PCI.

The subject was not pre-medicated for allergy before the catheterization. On 24-Apr-2014 at 13:15, approximately two to five minutes after study drug dosing, the subject suddenly reported heat sensation followed by face swelling, including mouth or lips, rash, and shortness of breath. In addition, the subject experienced flushing, edema, and chest pain. The subject was monitored by electrocardiogram (ECG) and oximetry. Systems involved included mucocutaneous and respiratory. Treatment included Fenistil (dimetindene), Ranitic (ranitidine) and corticosteroids (prednisolone 250 mg) with subsequent relief. Symptoms' duration was forty five minutes. The subject responded well to treatment and her symptoms resolved rapidly. The PCI was completed. Nothing related to the PCI contributed. Imeron 100 ml and aspirin 500 mg were given. Clinical manifestation was obvious to be identified as allergic reaction and was temporally associated with study drug administration. The subject was discharged after twenty four hours in good condition.

The event was considered serious because it was reportedly life-threatening.

The investigator has assessed the causal relationship between the study medication and the event as relate with rationale: reaction immediately after administration. The contrast agent was previously well tolerated. Possible alternate causes of the event included Imeron (lomeprolol) 100 ml and aspirin 500 mg.

CEC Adjudication Results Date of Review: 17-Sep-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	24Apr2014; 1:15 PM
Type of allergic reaction	Severe
Severe subtype of reaction	Anaphylactic
If severe, did this qualify solely on the basis of the administration of corticosteroids?	No
Organ System Involvement	Mucocutaneous; Respiratory; Circulatory
Reviewer 1. How did the immunology sample impact the final adjudication decision?	Supported and Contributed
Comments or Rationale (Specialty Reviewer)	Significant elevation in tryptase and other inflammatory markers consistent with anaphylaxis
Comments or Rationale (Phase II Committee):	This allergic reaction had a possible temporal relationship to study drug administration (minutes after)

Allergic/anaphylactic reaction #5

Subject 12004028 was a 46 year-old female who participated in: *A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study To Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention*. Medical history included myocardial infarction (MI), most recent MI was within the past year, previous percutaneous coronary intervention (PCI), and current tobacco smoker. The subject received Aspirin (acetylsalicylic acid), Brilinta (ticagrelor), nitroglycerin, amlodipine, atorvastatin, metoprolol, perindopril, fentanyl, Versed (midazolam), verapamil, nitroglycerin, and atropine within seven days before onset of the event.

On 03-May-2014, the subject was admitted to the hospital for ST segment elevation myocardial infarction (STEMI). The subject underwent PCI to left anterior descending (LAD) artery. Ticagrelor 180 mg loading dose then 90 mg twice daily (BID) were administered.

On 06-May-2014 at 13:34, the subject was randomized to study drug but she was bumped and rescheduled. The subject reported no allergies pre catheterization (cath) therefore no allergy prep was provided before the cath. On 07-May-2014 at 14:19, the subject received study drug. On 07-May-2014 at 14:25, atropine and normal saline bolus 500 ml were given for heart rate (HR) 42 beats per minute (bpm) and systolic blood pressure (BP) 80 mmHg. At 14:27, BP was 114/80 mmHg and HR was 102 bpm. The investigator did not think the transient bradycardia and hypotension to be adverse events related to the study drug. On 07-May-2014 at 14:32, the subject underwent PCI. On the morning of 08-May-2014, the subject was discharged home.

On 08-May-2014 at 19:00, greater than 24 hours post study drug dosing, the subject noticed a rash appearing on both arms. As time went on, the rash covered her entire body and was very itchy. On 09-May-2014 at 09:15, the subject presented to her local hospital and was admitted. The rash covered her entire body and was very itchy and painful. The emergency room (ER) report indicated, "Given Brilinta 90 mg po nurse admit;" although there were no corresponding signature, date, time, drug, or dose signed for. The allergic reaction was mainly integumentary. The subject experienced hives and diarrhea but had no swelling of lips or tongue. The subject had no airway or cardiac involvement but nurses' notes indicated subject said throat was "tight." The subject was treated with steroids and antihistamines (H1 and H2) and intravenous (IV) fluids. All medications subject was receiving, including Lipitor, coversyl, amlodipine, and metoprolol, were discontinued then slowly reintroduced. The ER physician consulted with a cardiologist who thought the allergic reaction was due to Brilinta (ticagrelor); the last dose of Brilinta was questionably given on 09-May-2014 at 09:00. On 09-May-2014 at 22:00, as suggested by the cardiologist, a loading dose of Plavix (clopidogrel) 300 mg was given to subject. On 10-May-2014, the rash remained and was still itchy. At 22:00, maintenance dose of Plavix 75 mg/day was started. Other cardiac medications were slowly reintroduced and tolerated by subject. Once Brilinta was stopped and with the use of steroids/antihistamines, the rash and itchiness slowly resolved. Diarrhea resolved. Steroids were slowly weaned. On 11-May-2014, the rash improved slightly. On 12-May-2014, the rash was improving, subject was less itchy. On 13-May-2014, the rash continued to improve, with less itchiness, and the subject was more mobile. On 14-May-2014, the rash still remained but continued to fade and the subject was discharged home. All lab work was reportedly normal. On 16-May-2014, the rash was completely gone.

On 18-Jun-2014, the study coordinator learned of the event from the subject. Subject confirmed rash never returned and she was tolerating Plavix well. Brilinta remained discontinued.

On 14-May-2014 at an unknown time, the outcome of the event was recovered/resolved.

The event was considered serious because it required inpatient hospitalization or prolongation of existing hospitalization.

The investigator has assessed the causal relationship between the study medication and the event as not related. Possible alternate causes of the event: Concomitant medication - Brilinta (ticagrelor), discontinued, rash slowly disappeared with steroid/antihistamine course. Reloaded Plavix. No rash.

CEC Adjudication Results Date of Review: 17-Sep-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	08 May 2014 @ UNK
Type of allergic reaction	Severe
Severe subtype of reaction	Anaphylactic
If severe, did this qualify solely on the basis of the administration of corticosteroids?	Yes
Organ System Involvement	Mucocutaneous; Gastrointestinal
Reviewer 1. How did the immunology sample impact the final adjudication decision?	Supported
Comments or Rationale (Specialty Reviewer)	No significant elevation in tryptase documented, may be due to Brilinta
Comments or Rationale (Phase II Committee):	No significant increase in tryptase. May be due to ticagrelor.

Allergic/anaphylactic reaction #6

Subject 11952016 was a 60-year-old male who participated in: A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study to Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention. Medical history included myocardial infarction (MI) (not within the past year), previous coronary artery bypass graft (CABG), hyperlipidemia, and current tobacco smoker. Medical records confirmed no known allergies and, during screening, subject denied having allergies. The subject received nabumetone, Pravachol (pravastatin), Proair HF (albuterol sulfate), Cialis (tadalafil), aspirin, lisinopril, Protonix (pantoprazole), gabapentin, and Flomax (tamsulosin) within seven days before onset of the event. On 14-May-2014, prior to his catheterization, the subject received Omnipaque (iohexol), Versed (midazolam), Plavix (clopidogrel), and fentanyl.

On 14-May-2014 at 08:30, prior to his catheterization and per the hospital's standard pre-operative prep for all heart cath patients, the subject was given Benadryl (diphenhydramine) 50 mg orally. On 14-May-2014 at 10:00, femoral arterial access was obtained. On 14-May-2014 at 10:09, the subject was randomized to study drug. On 14-May-2014 at 10:25, the subject received study drug. On 14-May-2014 at 10:27, the subject underwent PCI.

On 14-May-2014 at 10:09, the subject was given a loading dose of Plavix 600 mg oral. At 10:23, the subject complained of heart burn. He was given sips of water and his heart burn resolved. At 10:26, one minute after study drug dosing, the subject stated he was having trouble breathing. At the time of the reaction, the subject was diffusely erythematous. The subject was not assessed or examined for wheezing, stridor, or other evidence of airway obstruction. As per the sub-investigator performing the cath procedure, "Pt complained of choking and shortness of breath; did not examine pt. for wheezing, etc. Pt. diffusely erythematous." The subject experienced a reaction involving his respiratory and circulatory systems. Signs and symptoms included shortness of breath and hypotension. During the event, heart rhythm was reportedly sinus bradycardic to sinus tachycardic; the subject did not experience cardiac

arrhythmia. At 10:34, atropine 1 mg intravenous (IV) push and epinephrine (Epi) 1 mg IV push were administered. At 10:35, chest compressions and bag ventilation were initiated. Blood pressure (BP) and heart rate (HR) increased following atropine/Epi administration. At 10:37, Solu-medrol (methylprednisolone) 125 mg IV push was administered and chest compressions were stopped. At 10:52, the physician performed repeat diagnostic image of the left anterior descending (LAD) artery; the LAD lesion was 100% compared to 80 % lesion prior to study drug administration. At 10:54, a bare metal stent was implanted in the LAD lesion with good results. TIMI (thrombolysis in myocardial infarction) flow was 3. All PCI equipment was out. Arterial blood gases were obtained; the subject was acidotic. At 11:14, the subject was intubated. At 11:20, the subject was transferred to intensive care unit (ICU) on the ventilator. On 17-May-2014, the subject was successfully weaned off IV vasopressors. On the morning of 18-May-2014, the subject was extubated. On 20-May-2014, the subject was on oxygen 2 liters via nasal cannula; tolerating his diet; without IVs at that time; and awaiting transfer from step down unit to a regular room. The subject was doing well, sitting up in bed, talking to staff and talking to family on his cell phone. He was alert and oriented times three, moving all extremities, and fully intact neurologically. Cardiology okayed the subject for discharge from a cardiac standpoint however pulmonary wanted the subject to stay 'another day or two.' At the time of report, the plan was for the subject to be evaluated for sleep apnea on an outpatient basis after discharge. On 21-May-2014, the subject was discharged home in stable condition and to undergo routine follow-up at the outpatient clinic in one week.

On 18-May-2014, the outcome of the event was recovered/resolved.

The event was considered serious because it was life-threatening and required prolongation of existing hospitalization.

The investigator has assessed the causal relationship between the study medication and the event as related, with the rationale: severe shortness of breath after study drug was given. There were no possible alternate causes of the SAE. As per the sub-investigator performing the cath procedure, this was not an acute occlusion of vessel, "...the angio prior to hemodynamic collapse showed artery patent with TIMI flow 3." As per the principal investigator and the two sub-investigators, this was an allergic reaction to the study drug.

CEC Adjudication Results Date of Review: 17-Sep-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	14May2014; 10:26 AM
Type of allergic reaction	Severe
Severe subtype of reaction	Anaphylactic
If severe, did this qualify solely on the basis of the administration of corticosteroids?	No
Organ System Involvement	Respiratory; Circulatory
Reviewer 1. How did the immunology sample impact the final adjudication decision?	Supported
Comments or Rationale (Specialty Reviewer)	Elevated tryptase 16.3 at 08:14 is unusual, but returns to baseline at 10:10 then sig increased at 11:42
Comments or Rationale (Phase II Committee):	This event had temporal relationship with the study drug (possible relationship)

Allergic/anaphylactic reaction #7

Subject 11567010 was a 67-year-old male who participated in: *A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study To Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention*. Medical history included previous percutaneous coronary intervention (PCI), hyperlipidemia, former tobacco smoker, and 1-5 allergic reactions in the past year. On 25-May-2014, the subject experienced hay fever, caused by an allergic reaction to pollen, and was treated with antihistamines. The subject received aspirin, clopidogrel, hydrochlorothiazide (HCTZ), lisinopril, metoprolol tartrate, nitroglycerin, sertraline, and simvastatin within seven days before onset of the event. On 28-May-2014, prior to the PCI, the subject received aspirin, clopidogrel, Versed (midazolam), fentanyl, lidocaine, verapamil, heparin, nitroglycerin, and Omnipaque (iohexol).

On 09-Apr-2014, the subject underwent a PCI procedure without problems; he was not prepped for allergy before the index PCI. On 28-May-2014 at 09:41, the subject was randomized to study drug. On 28-May-2014 at 09:53, the subject received study drug. The PCI procedure was not performed due to this event.

On 28-May-2014 at 09:59, approximately five minutes after study drug dosing, the subject complained of headache. Left anterior descending (LAD) appeared to have intense spasm. The subject complained of chest pain and was flushed/swollen in the face and had possible tongue swelling. He became hypotensive and bradycardic, showed changes on his electrocardiogram (ECG) then went into ventricular tachycardia. The subject experienced an anaphylactic reaction; his mucocutaneous and circulatory systems were affected. The subject did not require cardioversion or defibrillation. At 10:04, blood pressure (BP) was 51/29 mmHg and Neo-Syneprine (phenylephrine) was given. At 10:06, epinephrine was administered. After the subject converted back to sinus rhythm, he remained hypotensive and at 10:06, 10:07, and 10:25 required multiple boluses of Neo-Syneprine. The investigator confirmed there was no respiratory involvement and no skin rash after the procedure but the subject was given high dose of steroids during and immediately after the procedure; the subject did experience flushing and, at 10:17, complained of throat pain. The machine was unable to read the subject's BP until 10:22 when BP was 95/63 mmHg. Treatment also included Solu-Medrol (methylprednisolone), Benadryl (diphenhydramine), Pepcid (famotidine), and epinephrine. There was concern about left main dissection versus spasm. The investigator indicated the event could have been coronary spasm but there was nothing done new in the procedure to initiate a spasm event in his opinion. Once the subject was stabilized, he underwent intravascular ultrasound (IVUS) of the left main and LAD; there were no signs of dissection. Upon completion of the aborted PCI procedure, the subject was hemodynamically stable, awake, alert, and oriented.

On 28-May-2014 at 10:53, the outcome of the event was recovered/resolved.

The event was considered serious because it was life-threatening, an important medical event, and prolonged hospitalization.

The investigator has assessed the causal relationship between the study medication and the event as possibly related, with comment "event began within five minutes of study drug. The subject developed flushing and swollen face/possibly tongue. Event was brief and resolved with epinephrine injection and fluid resuscitation. Assessment is allergic reaction; cannot say definitively until lab results are back." Possible alternate causes of the SAE: possible coronary spasm.

CEC Adjudication Results Date of Review: 17-Sep-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	28May2014; 08:43 AM
Type of allergic reaction	Severe
Severe subtype of reaction	Anaphylactic
If severe, did this qualify solely on the basis of the administration of corticosteroids?	No
Organ System Involvement	Mucocutaneous; Respiratory; Gastrointestinal
Reviewer 1. How did the immunology sample impact the final adjudication decision?	
Comments or Rationale (Specialty Reviewer)	No relevant labs provided
Comments or Rationale (Phase II Committee):	This event had temporal relationship with the study drug (possible relationship)

Allergic/anaphylactic reaction #8

Subject 15006003 was a 56-year-old female who participated in: *A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study To Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention*. Medical history included myocardial infarction (MI), the most recent MI was within the past year; previous percutaneous coronary intervention (PCI), 15-May-2014 left ventricular function (ejection fraction): normal (> 55%), peripheral vascular disease (PVD), diabetes mellitus, hyperlipidemia, and current tobacco smoker. The subject received moxonidin mylan, furosemide, trandolapril, irbesartan, Ketosteril (ketoanalogues and amino acids), atorvastatin, spironolactone, Lantus (insulin glargine), and Humalog (insulin lispro) within seven days before onset of the event.

On 23-Jun-2014 at 09:17, the subject was randomized to study drug. Subject was a subgroup B patient. On 23-Jun-2014 at 09:28, the subject received study drug. The subject did not undergo PCI due to this SAE.

The subject was prepped for allergy before the catheterization with two tablets of bisulepine 2 mg. On 23-Jun-2014 at 09:29, within one minute after study drug administration, the subject reported itching of her face that spread to her entire body, followed promptly by choking, due to bronchospasm, and severe hypotension, i.e. complex of symptoms consistent with anaphylactic reaction. The subject also experienced shortness of breath, wheezing, and cardiac arrhythmia. There were mucocutaneous, respiratory, and circulatory manifestations. Extended life support was immediately provided with early tracheal intubation, artificial ventilation, and administration of hydrocortisone 200 mg intravenous (IV), bisulepine IV, and other drugs including high dose epinephrine IV (nine vials of 1 mg), amiodarone, atropine, mesocain IV, H2 antagonist IV, and norepinephrine IV bolus followed by continuous infusion. During resuscitation, the subject was given normal saline for volume expansion. There was no response to treatment and the status of the subject continued to worsen. Despite treatment, there was a severe electrical instability beginning with bradycardia followed by alternating asystole, ventricular fibrillation (VF), and ventricular tachycardia (VT). Eight direct current (DC) shocks were applied between 10:00 and 11:00 am. There was intermittent improvement of circulatory status. Finally, efficient circulation was restored and normalization of blood pressure but the subject was on a high dose of continuous norepinephrine and already had fixed dilation of her pupils. The subject died at 17:00 (5:00 pm) in the intensive care unit (ICU).

Autopsy report: all findings were consistent with clinical history and presentation, with no additional findings. Autopsy report is available with source documents.

The investigator has assessed the causal relationship between the study medication and the event as related, stating anaphylactic reaction immediately after study drug administration. Possible alternate causes of the event: none.

CEC Adjudication Results Date of Review: 17-Sep-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	23Jun2014; 09:29 AM
Type of allergic reaction	Fatal
Severe subtype of reaction	Fatal
If severe, did this qualify solely on the basis of the administration of corticosteroids?	No
Organ System Involvement	Mucocutaneous; Respiratory; Circulatory
Reviewer 1. How did the immunology sample impact the final adjudication decision?	Supported
Comments or Rationale (Specialty Reviewer)	Significant elevation in tryptase and other inflammatory
Comments or Rationale (Phase II Committee):	Significant elevation in tryptase and other inflammatory

Allergic/anaphylactic reaction #9

Subject 11888013 was a 70-year-old female who participated in: *A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study To Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention*. Medical history included hyperlipidemia, current tobacco use and an allergic reaction, (0 reactions in past year). Concomitant medications within seven days before onset of the event included Toprol XL (metoprolol succinate), amlodipine/benazepril, atorvastatin, vitamin D-3, Plavix (clopidogrel), Synthroid (levothyroxine), omeprazole, chlordiazepoxide HCL, and Zoloft (sertraline). For allergy prophylaxis for the procedure, the subject received oral Benadryl (diphenhydramine) 50 mg and Valium (diazepam) 10 mg.

On 05-Jun-2014 at 08:22, the subject was randomized to study drug. On 05-Jun-2014 at 08:36, the subject received study drug. At 08:43, approximately five minutes post study drug administration, the subject experienced shortness of breath (SOB), wheezing, flushing, and nausea, and the subject's lips were noted to be cyanotic. Treatment included epinephrine 0.1 mg intravenously (IV) followed by an infusion, Solumedrol (methylprednisolone) 100 mg IV, Benadryl 25 mg IV, and Pepcid (famotidine) 20 mg IV, and 100% re-breather oxygen support. The subject reported resolution of SOB and nausea. On exam, no flushing or wheezing was noted. Post-procedure, the subject remained stable without chest pain. Her electrocardiogram (EKG) was normal; cardiac enzymes were negative; and echocardiogram revealed normal left ventricular (LV) function. On 06-Jun-2014, the subject was discharged to home in stable condition without sequelae.

On 05-Jun-2014 at 08:47, the outcome of the event was recovered/resolved

On 05-Jun-2014 at 08:51, the subject underwent PCI.

The event was considered serious because it was an important medical event.

The investigator has assessed the causal relationship between the study medication and the event as related stating SOB, wheezing, flushing experienced five minutes post drug. No possible alternate causes were provided. In the previous two and one-half months, this was the fourth diagnostic study/intervention the subject had in which she received contrast dye and premedication with Benadryl and Valium. This was the first allergic response noted and it occurred within five minutes post study drug administration. Therefore, the investigator believed the allergic reaction was most likely related to the study medication.

CEC Adjudication Results Date of Review: 17-Sep-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	5Jun2014; 08:43 AM
Type of allergic reaction	Severe
Severe subtype of reaction	Anaphylactic
If severe, did this qualify solely on the basis of the administration of corticosteroids?	No
Organ System Involvement	Mucocutaneous; Respiratory; Gastrointestinal or Genitourinary
Reviewer 1. How did the immunology sample impact the final adjudication decision?	Supported
Comments or Rationale (Specialty Reviewer)	Significant elevation in tryptase documented confirming anaphylaxis diagnosed clinically
Comments or Rationale (Phase II Committee):	Clinical presentation met the definitions for severe allergic reaction, and the elevated tryptase supported it.

Allergic/anaphylactic reaction #10

Subject 12006018 was a 63-year-old male who participated in: *A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study To Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention*. Medical history included myocardial infarction, most recent MI within the past year, previous percutaneous coronary intervention (PCI), hyperlipidemia, no tobacco smoking, and no allergies. The subject received the following concomitant medications within seven days before onset of the event; aspirin, clopidogrel, bisoprolol, Crestor (rosuvastatin), and Coversyl (perindopril).

On 26-Jun-2014 at 15:42, the subject was randomized to study drug. The subject received intravenous Valium (diazepam) 5 mg, nitro 100 mcg intra-arterially (IA), verapamil 1 mg IA, and heparin 5000 IU IA infusion. Radial access was achieved. No prophylactic allergy medications were given. On 26-Jun-2014 at 16:04, the subject received study drug.

On 26-Jun-2014 at 16:05, one minute after study drug dosing, the subject experienced hypotension with a systolic blood pressure (BP) of 53 mmHg, diaphoresis, chest heaviness, trouble breathing, cardiac arrhythmia (atrial fibrillation), and a rash described as redness from head to toe. At 16:10, epinephrine 1 mg IV was administered followed by an infusion started at 16:24. At 16:11 intravenous Benadryl (diphenhydramine) and intravenous Solu-cortef were given. At 16:19 dopamine IV was started and

administered until 16:27. The subject's oxygen saturation was 90% with some expiratory wheezes. The subject was admitted to the coronary care unit (CCU): Levophed (norepinephrine) was initiated; subject was administered ten liters of oxygen with non-rebreather mask and bronchodilator for expiratory wheezing. Vital signs included BP 88/50 mmHg, heart rate (HR) 110 (atrial fibrillation) beats per minute (bpm), oxygen saturation 98%, respiratory rate 20 per minute, and afebrile. The subject was hemodynamically stable overnight on epinephrine and norepinephrine infusions. Fluid resuscitation with three liters total of normal saline and Ringer's Lactate was administered. On 27-Jun-2014, Levophed infusion was off at 02:25 and epinephrine infusion was stopped at 06:15. The subject was treated with IV pressor support and fluids; he had no other symptoms, delayed reaction, or sequelae during his hospitalization and was discharged home from the CCU. On 18-Jul-2014 at 13:56, the subject underwent repeat catheterization and PCI.

On 28-Jun-2014 at 10:25, the outcome of the event was recovered/resolved.

The event was considered life-threatening and resulted in a prolonged hospitalization.

The investigator has assessed the causal relationship between the study medication and the event as related, with rationale: temporal relationship between study drug and event onset. Prior PCI done on 08-May-2014 with no problems or reactions noted. Possible alternate causes of the event: none.

CEC Adjudication Results Date of Review: 17-Sep-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	26Jun2014; 04:05 PM
Type of allergic reaction	Severe
Severe subtype of reaction	Anaphylactic
If severe, did this qualify solely on the basis of the administration of corticosteroids?	No
Organ System Involvement	Mucocutaneous; Respiratory; Circulatory
Reviewer 1. How did the immunology sample impact the final adjudication decision?	Supported
Comments or Rationale (Specialty Reviewer)	Tryptase sig elevated consistent with anaphylaxis
Comments or Rationale (Phase II Committee):	This event had a possible temporal relationship with the study drug because the allergic event (symptom and hypotension) occurred after 2 minutes of drug administration

Bivalirudin Patient

Subject 11884001 was a 63-year-old male who participated in: A Randomized, Open-Label, Multi-Center, Active-Controlled, Parallel Group Study To Determine the Efficacy and Safety of the REG1 Anticoagulation System Compared to Bivalirudin in Patients Undergoing Percutaneous Coronary Intervention. Medical history included myocardial infarction, previous coronary artery bypass graft (CABG), previous percutaneous coronary intervention (PCI), renal insufficiency, hyperlipidemia, former tobacco use; allergy history included: 23-Jan-2014, mucocutaneous and circulatory allergic reaction to intravenous (IV) contrast, treated with IV fluids, antihistamines, and steroids; Mar-2012, mucocutaneous allergic reactions to metoprolol and furosemide, treated with antihistamines.

The subject received Androgel (testosterone gel), aspirin, benazepril, clopidogrel, hydrocodone, acetaminophen, isosorbide mononitrate, meloxicam, Nitrostat (nitroglycerin), pravastatin, Ranexa (ranolazine), verapamil, Benadryl (diphenhydramine), Solu-Cortef (hydrocortisone sodium succinate), Versed (midazolam) , fentanyl, and Isovue, within seven days before event onset.

On 25-Jun-2014 at 11:58, the subject was randomized to study drug. On 25-Jun-2014 at 12:05, the subject received study drug. On 25-Jun-2014 at 12:07, the subject underwent PCI.

The subject was prepped for allergy with Benadryl and Solu-Cortef, prior to catheterization. On 25-Jun-2014 at approximately 15:00, three hours after study drug dosing and post PCI, the subject experienced chest pain. Intravenous (IV) fluids, H1/H2 blockers and vasopressors were initiated. The subject was transferred to the intensive care unit (ICU) and started on Cardizem (diltiazem hydrochloride) and heparin drip. While in the ICU, the subject started to experience shortness of breath, skin redness and cold chills. Hypotension started at about midnight post index procedure. The subject's mucocutaneous, respiratory, and circulatory systems were affected. While in the ICU, the subject was started on Neo-Synephrine drip, Benadryl, and Pepcid. The allergic reaction, including hypotension, gradually resolved and the subject was stabilized. The heparin drip was discontinued. After a two day stay for observation, the subject was transferred out of ICU back to the regular floor. He was subsequently discharged home, prior to midnight, on Day 3.

As of 06-Jul-2014, the outcome of the event was recovered/resolved.

There was no action taken with the study drug due to the event.

The event was considered serious because it required inpatient hospitalization.

The investigator has assessed the causal relationship between the study medication and the event as not related, stating, the subject experienced an allergic reaction to IV contrast in Jan-2014 after PCI, as well. The investigator believed that the allergic reaction was due to IV contrast and not related to study drug.

CEC Adjudication Results Date of Review: 17-Sep-2014	
Did the potential allergic reaction occur within 24 hours of study drug administration?	Yes
Date and time of allergic reaction onset	25 Jun 2014 @ 3:00 PM
Type of allergic reaction	Severe
Severe subtype of reaction	Anaphylactic
If severe, did this qualify solely on the basis of the administration of corticosteroids?	No
Organ System Involvement	Mucocutaneous; Respiratory; Circulatory
Reviewer 1. How did the immunology sample impact the final adjudication decision?	Contributed
Comments or Rationale (Specialty Reviewer)	HoTN seems related to cardiac dz rather than allergy; no sig change in tryptase argues against anaphylaxis
Comments or Rationale (Phase II Committee):	All the lab information was drawn before the symptoms onset.

Supplementary Table 1A. Univariate association of clinical factors and allergic reactions, controlled for treatment assignment

	Severe Allergy (n=22)	Any Allergy (n=34)	No Allergy (n=3172)	Severe vs. None OR (95% CI)	Any vs. None OR (95% CI)
Treatment received					
Pegnivacogin	18/22 (82%)	24/34 (71%)	1581/3172 (50%)	4.5 (1.5–13.3)	2.4 (1.1–5.1)
Bivalirudin	4/22 (18%)	10/34 (29%)	1591/3172 (50%)	Reference	Reference
Age, mean (SD), 10 yrs	60.7 (9.5)	62.3 (9.9)	65.3 (10.7)	0.7 (0.5–1.0)	0.8 (0.6–1.0)
Female	11/22 (50%)	15/34 (44%)	809/3172 (26%)	3.0 (1.3–7.0)	2.4 (1.2–4.7)
White race	22/22 (100%)	33/34 (97%)	3021/3150 (96%)	NA	1.3 (0.2–9.6)
Region					
North America	14/22 (64%)	23/34 (68%)	2213/3172 (70%)	Reference	Reference
Eastern Europe	5/22 (23%)	6/34 (18%)	558/3172 (18%)	1.5 (0.5–4.1)	1.2 (0.4–2.6)
Western Europe	3/22 (14%)	5/34 (15%)	401/3172 (13%)	1.1 (0.3–4.0)	1.2 (0.4–3.1)
Weight, mean (SD), 10 kg	90.1 (20.0)	90.0 (19.6)	88.7 (19.4)	1.0 (0.8–1.3)	1.0 (0.9–1.2)
BMI, mean (SD), kg/m ²	32.0 (6.4)	31.0 (5.9)	29.9 (6.0)	1.0 (1.0–1.1)	1.0 (1.0–1.1)
History of any allergy	6/22 (27%)	13/34 (38%)	1037/3170 (32.7%)	0.8 (0.3–2.0)	1.3 (0.6–2.6)
Allergies in last year					
No allergies	3/6 (50%)	9/13 (69%)	908/1037 (88%)	Reference	Reference
1–5 allergies	3/6 (50%)	4/13 (31%)	116/1037 (11%)	3.8 (1.1–13.1)	3.3 (1.2–9.6)
>5 allergies	0/5	0/13	13/1037 (1%)	NA	NA
Current tobacco use	10/22 (45%)	15/34 (44%)	651/3171 (21%)	3.1 (1.3–7.3)	3.0 (1.5–5.9)
Previous PCI	17/22 (77%)	26/34 (76%)	1127/3171 (36%)	3.3 (1.2–9.1)	3.1 (1.4–7.0)
Previous CABG	4/22 (18%)	6/34 (18%)	536/3171 (17%)	1.1 (0.4–3.2)	1.0 (0.4–2.5)
Previous MI	13/22 (59%)	20/34 (59%)	1127/3171 (36%)	2.6 (1.1–6.2)	2.6 (1.3–5.2)
Congestive HF	1/22 (5%)	4/34 (12%)	391/3171 (12%)	0.4 (0.1–2.6)	1.0 (0.3–2.8)
PVD	1/22 (5%)	3/34 (9%)	435/3171 (14%)	0.3 (0.04–2.2)	0.6 (0.2–2.0)

Previous stroke	0/22	0/34	134/3171 (4%)	NA	NA
Hyperlipidemia	18/22 (82%)	29/34 (85%)	2336/3171 (74%)	1.5 (0.5–4.6)	2.0 (0.8–5.2)
CrCl <60, mL/min	1/22 (5%)	2/23 (6%)	388/3171 (12%)	0.4 (0.1–2.6)	0.5 (0.1–1.9)
Diabetes	7/22 (32%)	8/34 (24%)	1110/3171 (35%)	0.8 (0.4–2.1)	0.6 (0.2–1.2)

Data presented as n/N (%), unless otherwise indicated. BMI indicates body mass index; CABG, coronary artery bypass grafting; CI, confidence interval; CrCl, creatinine clearance; HF, heart failure; MI, myocardial infarction; NA, not applicable; OR, odds ratio; PVD, peripheral vascular disease; SD, standard deviation.

Supplementary Table 1B. Multivariate association of clinical factors and allergic reactions, controlled for treatment assignment

	<u>Severe vs. None</u>		<u>Any vs. None</u>	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Pegnivacogin	4.5 (1.5–13.4)	0.007	2.4 (1.1–5.1)	0.02
Female	2.9 (1.2- 6.9)	0.01	2.3 (1.2–4.7)	0.02
1–5 allergies in last year	3.2 (0.9–11.3)	0.07	2.9 (1.0–8.6)	0.06
Current tobacco use	3.6 (1.5–8.4)	0.004	3.4 (1.7–6.8)	0.001
Previous PCI	3.7 (1.4–10.3)	0.01	3.5 (1.6–7.8)	0.002

CI, confidence interval; OR, odds ratio; PCI, percutaneous coronary intervention.

Supplementary Table 2. Select pre-study drug medication use among subjects who had allergic reactions within 24 hours of initial dose of randomized treatment*

	Adjudicated Severe Allergic Reactions (N=22)	Any Adjudicated Allergic Reaction (N=34)	No Adjudicated Allergic Reactions (N=3172)
Medication within 48 hrs prior to randomization			
H1 blocker	4/22 (18.18%)	7/34 (20.59%)	733/3172 (23.11%)
H2 blocker	1/22 (4.55%)	1/34 (2.94%)	178/3172 (5.61%)
Steroids	1/22 (4.55%)	1/34 (2.94%)	112/3172 (3.53%)
Beta blocker	16/22 (72.73%)	26/34 (76.47%)	2274/3171 (71.71%)
ACE inhibitor	12/22 (54.55%)	16/34 (47.06%)	1538/3171 (48.50%)
GP IIb/IIIa	1/22 (4.55%)	1/34 (2.94%)	39/3172 (1.23%)

*There were no factors with significant associations with development of any or severe allergic reactions ($p < 0.05$), controlling for treatment.

Supplementary Table 3. Individual tryptase levels in patients with allergic reactions within 1 hour of pegnivacogin dosing (all values in ng/mL)¹

	Baseline Tryptase	90-minute Tryptase	20-hour Tryptase
1	3.5	8.6	2.9
2	5.5	4.5	12.1
3	4.3	3.0	4.1
4	3.6	20.1	3.5
5	3.3	6.6	3.7
6	5.2	16.3	2.2
7	6.9	12.4	5.0
8	7.2	10.3	6.5
9	12.4	16.5	
10	4.8	7.2	5.3
11	2.9	17.8	3.8

¹ Valent P, Akin C, Arock M, et al. Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. *Int Arch Allergy Immunol* 2012;157:215-25.

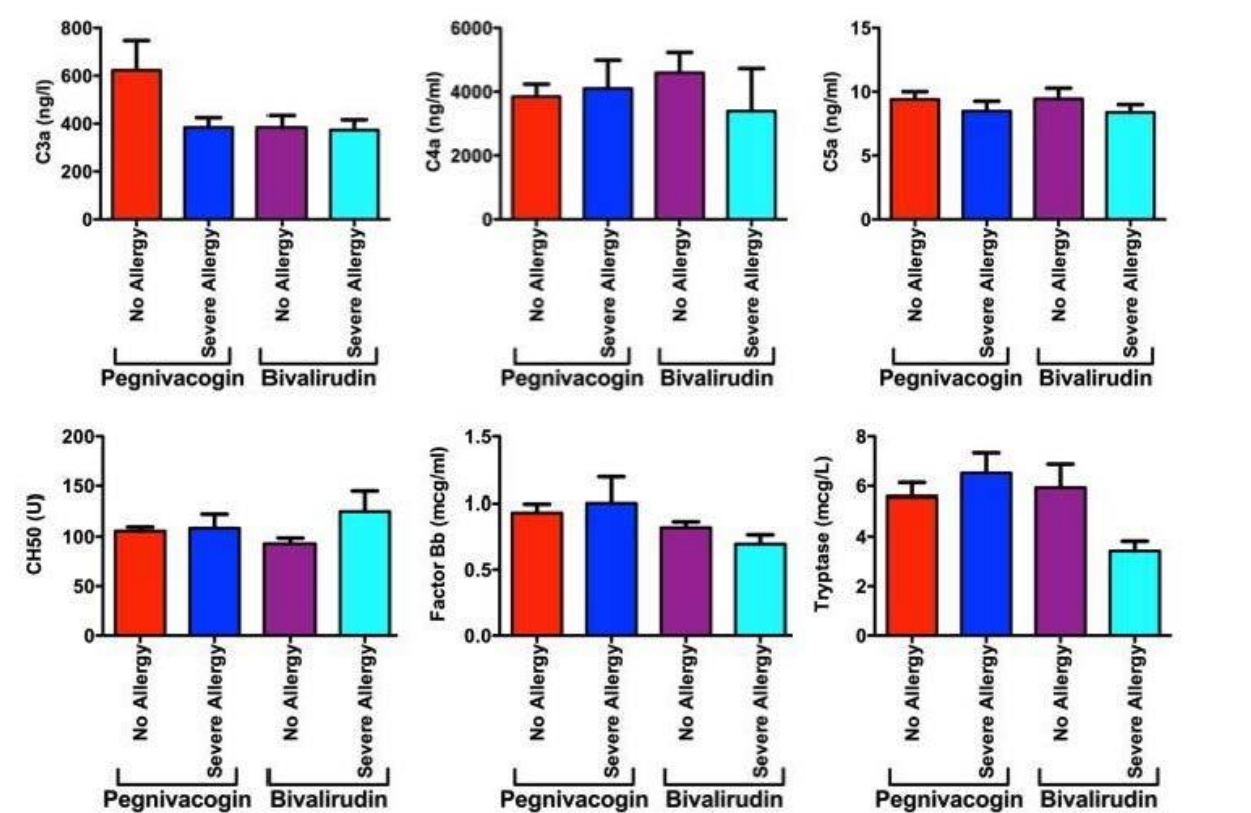
Supplementary Table 4. PEGylated products approved for use in the United States

Compound	Therapeutic	Approved	Route	No. of Patients Studied*	Size of PEG	PEG Content	Estimated PEG Dose	Notes
Adagen	Bovine adenosine deaminase	1993	IM	6	5 kD	63%	NA	Dose based on activity
Oncaspar	Asparaginase	1994	IM	109	5 kD	73%	NA	Dose based on activity
Doxil	Doxorubicin	1995	Slow IV	563	MPEG-DSPE	1/6:1 w/w	137 mg	
Pegintron	Interferon alpha 2b	2001	SC	1124	12 kD	28%	~0.022 mg	
Definity	ECHO contrasting agent	2001	IV			0.15 mg/mL	0.12 mg	
Pegasys	Interferon alpha 2a	2002	SC	1000	40 kD	67%	0.12 mg	
Neulasta	Granulocyte stimulating factor	2002	SC	467	20 kD	51%	3 mg	
Macugen	Anti-VEGF aptamer	2004	IO	892	40 kD	81%	1.3 mg	
Mircera	Epoetin-B	2007	IV or SC	716	30 kD	50%	0.0003 mg	Pulled due to safety concerns
Cimzia	Anti-TNF antibody fragment	2008	SC	2367	40 kD	44%	176 mg	
Pegloticase	Uricase	2010	Slow IV	169	10 kD	75%	6 mg	3% first dose

								reactions, 42%
								develop neutralizing
								antibodies to PEG
Omontys	Erythropoietin mimic	2012	IV or SC	1066	40 kD	89%	2.85 mg	Pulled from market
								Jan 2013 due to
								severe/fatal allergic
								events
Movantik	Naloxone	2014	PO	901	308 D	41.5%	10.3 mg	
Pegnivacogin	Anti-factor IXa	--	IV	2095	20 kD	80%	64 mg	

*Represents number of patients treated with study drug in safety analysis in package insert. TNF indicates tumor necrosis factor.

Supplementary Figure 1. Complement and tryptase levels at baseline by treatment and occurrence of allergy



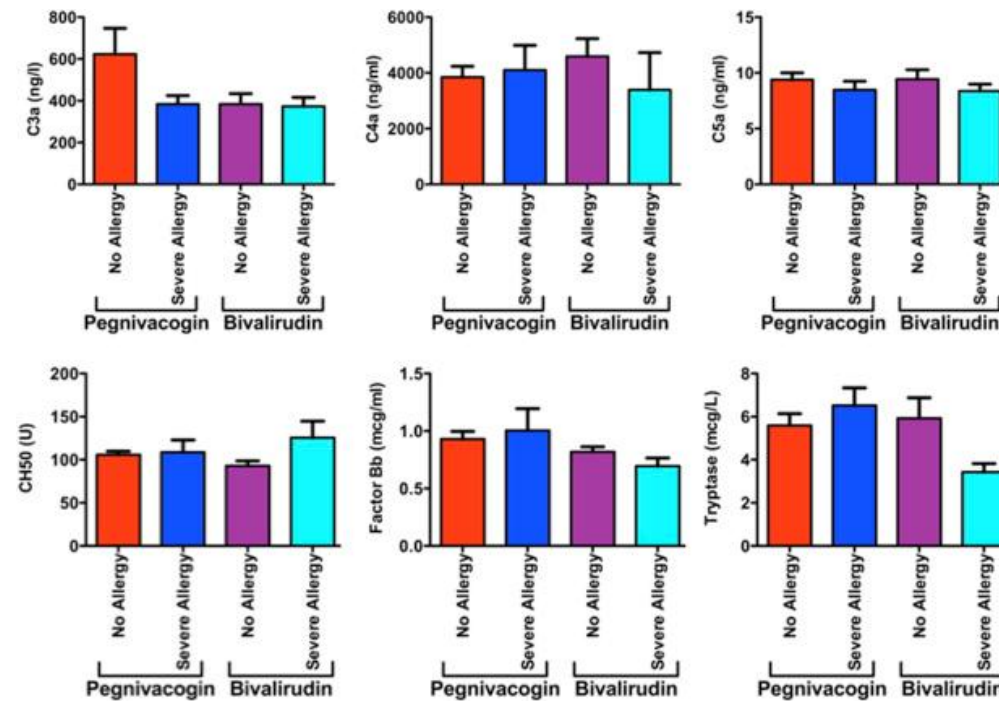


Fig E1.

Thomas J. Povsic, Monica G. Lawrence, A. Michael Lincoff, Roxana Mehran, Christopher P. Rusconi, Steven L. Zelenkofske, Zhen Huang, Jeffrey Sailstad, Paul W. Armstrong, P. Gabriel Steg, Christoph Bode, Richard C. Becker, John H. Alexander, N. Franklin Adkinson, Arnold I. Levinson
Pre-existing anti-PEG antibodies are associated with severe immediate allergic reactions to pegnivacogin, a PEGylated aptamer

Journal of Allergy and Clinical Immunology, 2016, Available online 14 July 2016

<http://dx.doi.org/10.1016/j.jaci.2016.04.058>