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× or 3× 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.

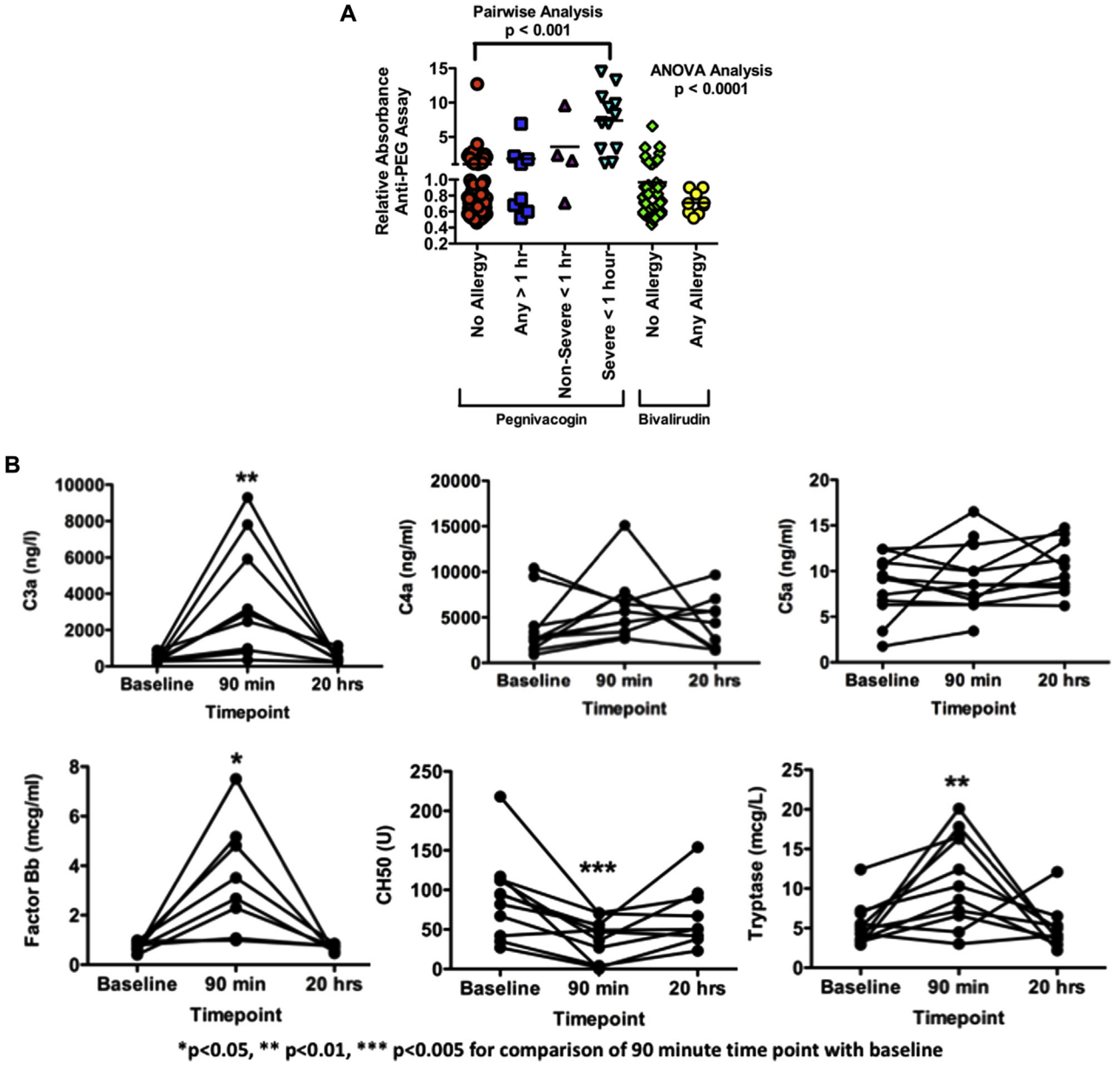

FIG 1. A, Relative levels of anti-PEG antibodies by allergy. **B,** Changes in individual complement levels in patients with severe immediate acute allergic reactions to pegnivacogin. **C,** Changes in individual complement and tryptase levels by treatment and occurrence and timing of allergy.

(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.

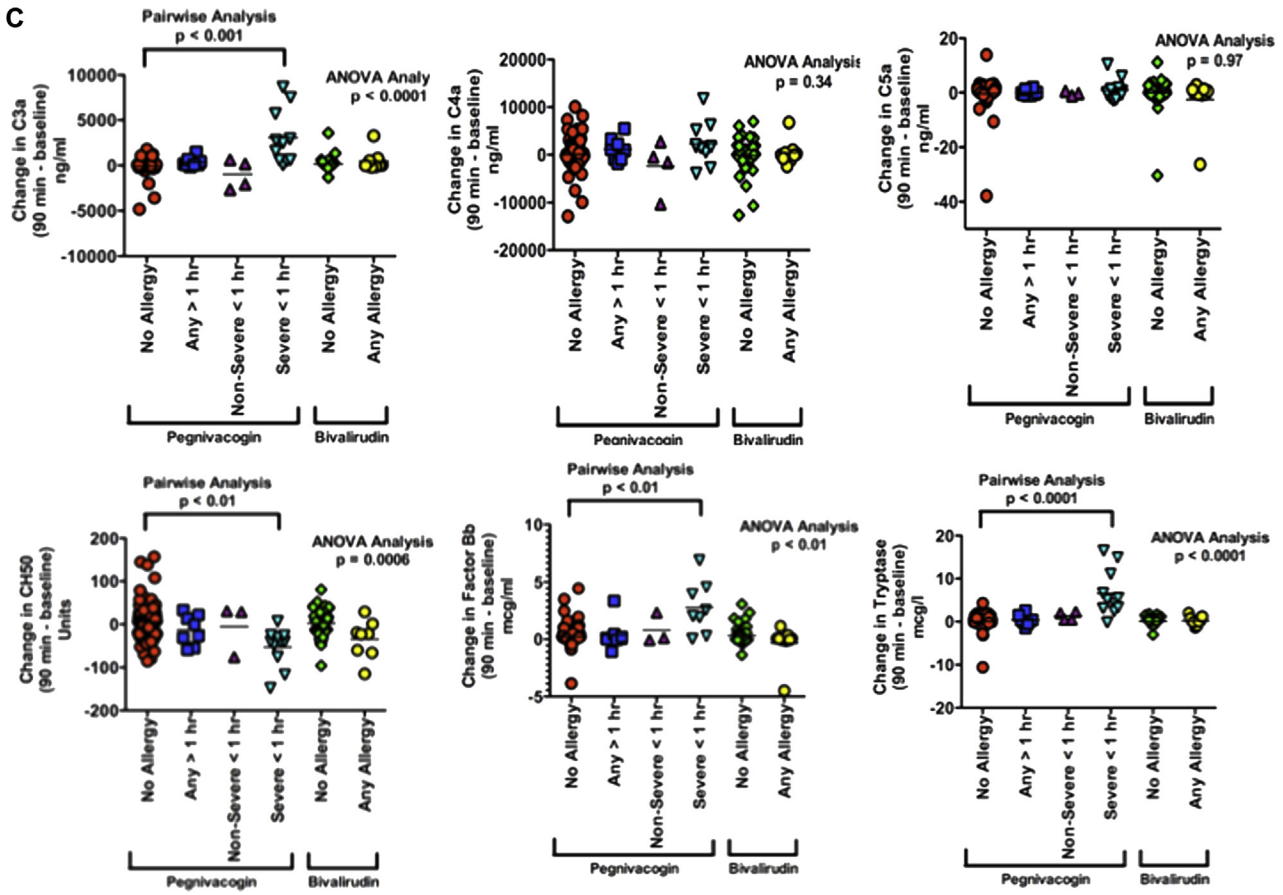

FIG 1. *(Continued).*

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 reaginic 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.

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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, Sohmalution, 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.

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