M1072

The Correlation of an Anti-PEG Antibody Assay Results with Allergic Reactions

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1 ABSTRACT

Purpose

Pegnivacogin (RB006) is a single-stranded, nucleic acid aptamer which binds to blood coagulation Factor IXa, inhibiting its activity and preventing blood clot formation. An anti-PEG (polyethylene glycol) antibody assay was developed, validated, and used to assess antibodies from a Phase 3 randomized, open-label clinical trial on the safety and efficacy of pegnivacogin.

Methods

A method to detect anti-PEG antibodies was developed, in which the PEGylated drug (RB006) was coated onto plates and antibodies from controls and unknowns were captured by the drug. An anti-human IgG alkaline phosphatase conjugate was used for detection antibody, and a pNPP substrate solution generated a colorimetric response. In order to show specificity to PEG, any samples that were presumptive positive underwent confirmatory testing by inhibition with 20K PEG. A positive control pool was prepared from individual human serum samples that were positive for anti-PEG antibodies. Human serum samples from the clinical trial were assessed via a tiered approach of screening, confirmatory assay (through a measurement in the presence of PEG), and titering.

Results

The ADA assay in human serum was validated for intra- and inter-assay precision of the screening positive controls, precision of the confirmatory positive controls, sensitivity, drug tolerance, and stability. A screening and confirmatory cut point were determined statistically for use in analysis of clinical samples. For each patient, immunogenicity samples were taken pre-dose, 90 minutes post-dose, and 20 hours post-dose. In the case of a serious allergic reaction, an additional immunogenicity sample was taken as close to the serious allergic reaction as possible considering the safety of the patient. All samples for patients with allergic reactions were tested in the anti-PEG assay; in addition, a sampling from patients with no adverse response were tested. The results of this analysis are given in Figure 1. The lower anti-PEG antibody response in post-dose samples was likely due to drug interference with the assay.

Conclusion

The validated anti-PEG antibody assay demonstrated that a preexisting anti-PEG response in patient pre-dose samples correlated with first-exposure serious allergic reactions to the drug.

2 INTRODUCTION

Pegnivacogin is coupled to a 40-kDa branched PEG to increase its half-life in plasma. During the Phase 2b clinical trial in patients with acute coronary syndrome, allergic reactions occurred within minutes of a first dose in 3 patients (Ganson, Nancy J. et al. *Journal of Allergy and Clinical Immunology*, IN PRESS).

Free PEGs are used as additives in many consumer products and medications, and anti-PEG antibodies have been detected by various methods in several study populations at a prevalence of approximately 3% to more than 40%.

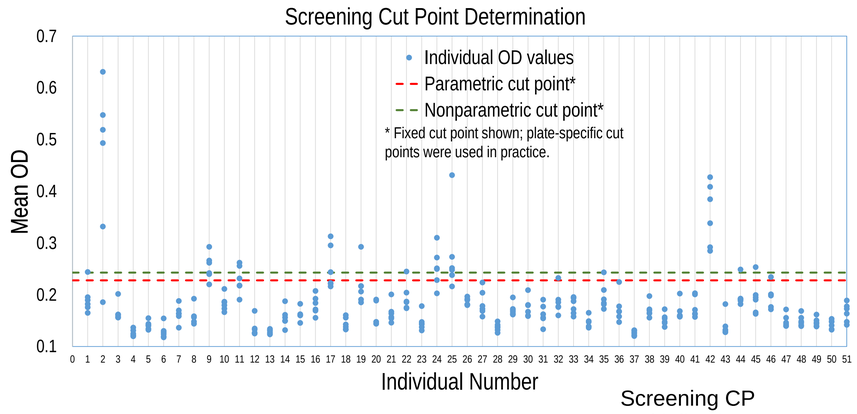
Based on the Phase 2b results, an anti-PEG assay was validated and used to assess samples from a Phase 3 study.

3 METHODS

- Plate coated with RB006
- Positive control prepared from positive human serum
- Rabbit anti-PEG antibody (Epitomics) used to determine sensitivity
- Minimum required dilution (MRD) = 20
- Detected with anti-human IgG-alkaline phosphatase and pNPP
- Plate read at 405 nm (absorbance)
- Confirmed with inhibition by 10 µg/mL of 20k PEG (NOF)

4 RESULTS - VALIDATION

The assay was validated for intra- and inter-assay precision, sensitivity, drug tolerance, confirmatory precision, titer precision, and stability. A plate-specific screening assay cut point correction factor and a confirmatory cut point were determined by assessing 51 individuals by 2 analysts over multiple days.



Screening CP multiplication factor used: **1.476**
Confirmatory CP: **21% Inhibition**

5 RESULTS – SAMPLE ANALYSIS

Immunogenicity samples taken:

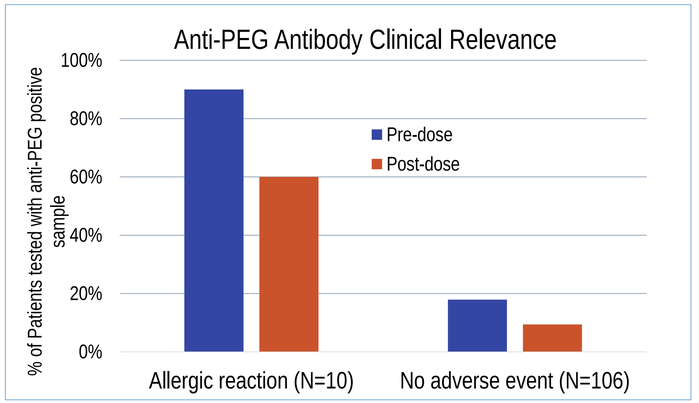
- Baseline
- 90 minutes post-dose
- 20 hours post-dose
- In case of an allergic reaction, an additional sample taken as close to the event as possible, considering the safety of the patient.

All samples from all patients who experienced an allergic event were analyzed for anti-PEG antibodies. In addition, a percentage of samples from patients who experienced no allergic event were analyzed for comparison.

	"Near Allergy" sample received	No unscheduled sample received
Total Patients	10	106
Predose Positive	9	19
% Predose Positive	90.00%	17.92%
Postdose Positive	6	10
% Postdose Positive	60.00%	9.43%

EXAMPLE DATA FOR ALLERGIC EVENT PATIENT

Visit	Screening Mean OD	Plate-Specific Cutpoint	Screening Result	% Inhibition	Confirmatory Result	Titer Result (Dilution)
Pre-dose	2.962	0.233	Presumptive Positive	80.09	Positive	1280
Unscheduled (~ 30 min post-dose)	0.539	0.233	Presumptive Positive	44.04	Positive	109
90 min post dose	0.406	0.233	Presumptive Positive	29.02	Positive	119
20 hrs post dose	0.384	0.233	Presumptive Positive	39.03	Positive	74



6 CONCLUSIONS

Preexisting anti-PEG response was observed in 90% of patients who had allergic reactions, compared to 20% of patients who did not. It is postulated that a high level of pre-existing anti-PEG antibodies was a major factor causing first-exposure allergic reaction to pegnivacogin. A likely factor is the relatively large amount of PEG delivered in a single 1 mg/kg bolus dose of pegnivacogin, e.g. ~64 mg of PEG for an 80 kg patient.