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Rapid Communication

Subvisible Particle Content, Formulation, and Dose of an Erythropoietin Peptide Mimetic Product Are Associated With Severe Adverse Postmarketing Events

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ABSTRACT

Peginesatide (Omontys®; Affymax, Inc., Cupertino, CA) was voluntarily withdrawn from the market less than a year after the product launch. Although clinical trials had demonstrated the drug to be safe and efficacious, 49 cases of anaphylaxis, including 7 fatalities, were reported not long after market introduction. Commercialization was initiated with a multiuse vial presentation, which differs in formulation from the single-use vial presentation used in phase 3 studies. Standard physical and chemical testing did not indicate any deviation from product specifications in either formulation. However, an analysis of subvisible particulates using nanoparticle tracking analysis and flow imaging revealed a significantly higher concentration of subvisible particles in the multiuse vial presentation linked to the hypersensitivity cases. Although it is unknown whether the elevated particulate content is causally related to these serious adverse events, this report illustrates the utility of characterizing subvisible particulates not captured by conventional light obscuration.

Introduction

The biopharmaceutical industry has capitalized on techniques such as random phage display peptide libraries and affinity purification methods to develop the next generation of therapeutics. These biobetters may have little or no amino acid sequence homology to the original molecule, making extrapolation of their safety profile from prior experience uncertain. Peginesatide (Omontys®; Affymax, Inc., Cupertino, CA) is one such product. An approved erythropoiesis-stimulating agent (ESA), it is comprised of a synthetic erythropoietin (Epo) peptide mimetic covalently dimerized and linked to polyethylene glycol (PEG). Peginesatide has no amino acid homology to Epo but shares a short Epo receptor (EpoR)-binding motif with other Epo peptide mimetics.[1] Remarkably, this drug is an effective ESA in patients with pure red cell aplasia who have anti-Epo antibodies.[2] Approved by the US Food and Drug Administration (FDA) in March 2012, it was voluntarily withdrawn from the market on February 23, 2013, following an unexpected rise in severe adverse events and associated fatalities on first drug exposure.

An FDA wide task force analyzed these adverse events using both data supplied by the drug manufacturer and generated by the agency. The FDA identified 49 related cases of anaphylaxis (including

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7 fatalities) based on the National Institute of Allergy and Infectious Diseases consensus case definition,[3] yielding an anaphylaxis rate of 1.8 per 1000 exposed patients, which is similar to the rate recently reported in this population.[4] The corresponding hypersensitivity rate was 3.5 cases per 1000, a rate that is considerably higher than the premarket clinical trials' experience of 0.84 hypersensitivity cases per 1000 exposed patients (with no fatalities), which is similar to the postmarket rate reported to the FDA for epoetin alfa.

As postmarket safety reports generally do not include as much information as the safety reports collected during clinical trials, analysis of the potential risk factors for anaphylaxis and death was limited. Prior ESA exposure was reported in 30 of 32 anaphylaxis cases (information was not available for the other 17 anaphylaxis cases), suggesting that these events are unique to peginesatide and not an ESA class effect. There was neither apparent geographic or demographic association nor a clear association with a particular drug lot. However, in 90% of the evaluable cases identified by the FDA, anaphylaxis occurred within 10 min of first drug exposure, and most fatal cases lacked the typical clinical manifestations associated with IgE-mediated type I hypersensitivity. Although most patients received a dose $\leq$10 mg, 4 of the 7 fatalities were associated with a dose >10 mg. Three of the severe cases ($n = 17$) had penicillin sensitivity, but no other drug sensitivities occurred in more than 1 severe case, and none of the reported fatalities shared a drug allergy. However, comorbidities such as left ventricular dysfunction, respiratory disorders, and hypertension were frequent and severe among the fatal cases.

The observed disparity in the pre- and postmarketing incidence of anaphylaxis focused attention on possible product quality differences between the drug product used during clinical trials and that introduced into the market. Peginesatide was manufactured and approved as both a single-use vial (SUV) and a multiuse vial (MUV), which differed in their formulation. Clinical trials primarily used the SUV formulation, but only the MUV formulation was marketed. Although the constituents of the MUV formulation are all generally recognized as safe, formulation composition is widely understood as having the potential to alter the properties of biological therapeutics, including the subvisible particulate (SVP) profile. SVPs are known to impact immunogenicity and have also been shown to promote inflammation.[5] Although control of SVPs in therapeutics has focused on particles $\geq$10 microns, numerous studies have shown that the overwhelming majority of SVPs are <10 microns. Advanced methods have been developed to provide morphological information for particle sizes in the micron range (e.g., flow imaging) and to quantify SVP in the submicron size range (e.g., nanoparticle tracking analysis [NTA]). These emerging techniques currently lack validated protocols, but their enhanced capacity to characterize and quantitate SVP may yield valuable insights into changes in product quality. For an in-depth discussion of the strengths and limitations of the techniques, see the reviews by Zolls, Narhi, and Filipe et al.[6-8]

As part of its investigation, the Task Force also reviewed batch records and Certificates of Analysis for all released lots, inspected drug manufacturing sites, and conducted release and extended characterization testing of undistributed MUV, as well as MUV from clinics where serious adverse events had occurred. In addition, experimental studies on the biological activities of peginesatide were performed by FDA laboratories and their consultants at the National Institutes of Health. Here we report, in part, the results of those investigations.

Results and Discussion

Product Quality Release Testing

After withdrawal of peginesatide (CAS #913976-27-9) from the market, extensive testing of MUV samples was independently conducted by the FDA and the manufacturer. All testing confirmed that the drug product met approved release specifications, including United States Pharmacopeia (USP) <788> limits for SVP (proprietary data not shown). Furthermore, the results were comparable to analysis of lots performed at the time of release, and no new impurities or contaminates were identified. Similarly, the results of limited FDA testing of unopened MUV returned from clinics where adverse events had occurred met the release specifications for content and impurities.

Extended Characterization of SVPs

Particle concentrations within the size range of 50 nm-1 μm were evaluated using NTA. For particles >100 nm, significantly higher concentrations were found in MUV lots compared with SUV lots (Table 1). Additionally, a significant difference ($p < 0.05$) in particle concentration was found between MUV lots C18881 and C19258 (Fig. 1, MUV-A) versus MUV lots C18686 and C18696 (Fig. 1, MUV-B). As revealed in Figure 1, the differences in particle concentration between MUV and SUV lots are largely attributable to the MUV-A lots. A similar trend among these MUV lots was also observed for Z average and polydispersity index by dynamic light scattering (see Supplementary Data and Table S1). The particulate content of the SUV lots examined was found to be independent of product concentration and product lot.

Flow imaging used to evaluate particles in the micron range captured mostly small and spherical images resembling silicon oil or air, particularly in the SUV samples. Thus, results were digitally filtered using particle libraries constructed to exclude such images and those of insufficient resolution (see Supplementary Data and Table S2 and Fig. S1). After applying these filters, the average particle concentration in MUV lots was dramatically higher than in SUV lots (Table 2). Although the detected concentration of particles >10 microns was low (<1000 particles/mL) compared with USP <788> limits for the light obscuration method, comparison of particle concentrations in SUV and MUV samples revealed significant ($p < 0.001$) differences between them in all the measured size ranges (Fig. 2). Furthermore, in each size range, the distribution of particle concentrations was significantly different between MUV lots and SUV lots ($p < 0.01$ by Kolmogorov–Smirnov test).

The consistency of the analytical results indicates that significant differences exist between the particulate concentration of the SUV and MUV lots, with the latter containing more particulates and greater variability in particulate load. We infer that these differences are most likely because of the MUV formulation but have not identified the specific factor that may be responsible. Although all

Table 1
NTA Peginesatide Median Particle Concentrations[a,b]

Hydrodynamic Diameter (nm)	SUV	MUV	p[c]
50-1000	9763	29,934	0.028
50-100	1610	1197	0.673
101-200	5176	14,426	0.022
201-300	1155	8526	0.001
301-400	286	2068	0.001
401-500	88	519	0.001
501-600	30	237	0.001
601-700	8	102	0.003
701-800	3	48	0.002
801-900	2	30	0.002
901-1000	2	18	0.008

[a] Particles/mL ($\times 10^4$).
[b] SUV and MUV were independently measured 6 (each SUV lot in duplicate) and 12 (each MUV lot in triplicate) times, respectively.
[c] Mann–Whitney test.