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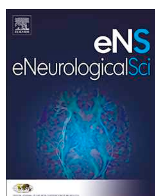

Letter to the Editor

Response to the Letter-to-the Editor by Cohen et al. concerning our eNeurologicalSci article, Melamed-Gal, et al. Physicochemical, biological, functional and toxicological characterization of the European follow-on glatiramer acetate product as compared with Copaxone. eNeurologicalSci 2018;12:19–30.https://doi.org/10.1016/j.ensci.2018.05.006

We appreciate the readers' interest in our paper [1] and we encourage the open scientific debate on the complexity of glatiramer acetate and the comparability of follow-on glatiramer acetate products. Below we provide responses to the points raised by the readers.

The authors wish to clarify that the clinical results from the GATE study were not dismissed, rather what was specifically highlighted was that the reported adverse event (AE) rate related to injection site reactions (ISRs) in the GATE study [2,3], is low in our opinion relative to other reported legacy Copaxone studies. The following sentence is from page 1437 of the Cohen et al. 2015 article [2] “Adverse events related to local injection site reactions occurred in similar proportions of participants treated with generic drug (22.9%[81of 353]) and brand drug (23.2% [83 of 357]) compared with 16.7% (14 of 84) of placebo participants and the same 22.9% and 23.2% percentages for AE ISRs were also provided in eTable 4, titled “Local injection site reactions summarized by MedDRA System Organ Class and Preferred Term (Safety Set)” [2]. Likewise for the open-label switch phase of the study, we reported the proportion of subjects with injection site reactions similar to the sentence on page 1911 of the Selmaj et al. 2017 article [3]: “Injection site reactions occurred in similar proportions in the GTR/GTR (1.2%) and GA/GTR (0.9%) groups versus 9.9% in the PLC/GTR group.” The reason for the differences in rate of ISRs reported as AEs between the GATE study and the Copaxone legacy clinical trials may be due to the different collection method of AEs used in the GATE study and the decision to present the ISRs captured in patient diaries separately from ISRs reported in the study periods in which diaries were not used, in contrast to Copaxone legacy trials which captured all ISRs as AEs regardless of the use of patient diaries. Please note in our article [1] we did not compare the rate of injection site adverse events in the GATE study to the GALA study as the dosing schedule for Copaxone 40 mg dose is different than for 20 mg dose.

To address the reader's comment regarding the representability of the Copaxone batches studied in our paper [1], we provide the below table (Table 1), which shows that the six randomly selected Copaxone product batches were produced from diverse drug substance batches, three of which mixed two glatiramer acetate drug substance batches and three from single glatiramer acetate batches. In total, eight different drug substance batches were used, spanning two years of manufacturing, therefore are fully representative of the process and consistent with regard to quality.

Drug Product Batch No	Drug Substance Batch No
P63950	242962914
	242962814
P63256	242951515
P63260	242951915
P63265	242953915
P63266	242953915
	242953815
P63275	242954315
	242954515

The readers indicate that the statistical analysis of the potency assay results is inappropriate, but they do not provide their arguments or rationale. As authors we are confident that the statistical approach we took is appropriate. As a result of the imbalanced sample sizes between available Copaxone lots and Synthon EU FOGA lots (Copaxone 231 samples, Synthon EU FOGA 6 samples) use of a standard statistical test would have been inappropriate. The simulation approach, in which six samples were randomly selected each time and their mean value calculated, enabled us to construct a distribution of mean values of Copaxone samples. Based on the Central Limit Theorem, the distribution of mean values follows a normal distribution regardless of the distribution of the raw data. Thus this method is suitable for calculating the probability of the observed mean value of Synthon EU FOGA samples to be considered part of that distribution.

The readers are correct, we provided the results of the TNFα secretion assay but inadvertently omitted it in Table 1 of our article, Melamed-Gal et al. [1]. Below is a revised Table 1 which includes the TNFα secretion assay and the adjusted percentage of methods showing differences between Copaxone and Synthon EU FOGA lots is 47%.

As the readers explain in their letter, we defined similar in our paper [1] as (1) within Copaxone specifications, (2) within inherent microheterogeneity ranges of tested Copaxone batches, or (3) not showing statistically significant differences. For CEX, Viscotek-TDAmax, RPLC 2D-MALLS and IMMS, which are high resolution research techniques, there are no official specifications and the reference ranges are defined by testing the Copaxone batches and the FOGAs batches concomitantly, as part of the same experiment. For these methods as outlined in our paper [1], we determined similarity based on whether the Synthon EU FOGA lots fell within the Copaxone microheterogeneity range tested at the same time as the Synthon EU FOGA lots. The dotted lines in Fig. 1C of our paper [1] delineate the Copaxone microheterogeneity range for the CEX, and show that the Synthon EU FOGA lots were not similar as there were larger negatively-charged subpopulation, a smaller weak positive-charged subpopulation, and larger (4 out of 6) strong positive-charged subpopulation compared to Copaxone lots. Likewise the dotted lines in Fig. 1D of our paper [1] illustrate the Copaxone microheterogeneity range for Viscotek and shows that the polypeptide chains of the Synthon EU FOGA showed different spatial arrangement compared with Copaxone, i.e. they exhibited higher effective molecular size

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Table 1
Copaxone product batches.

	Method	Attribute Studied	Synthon EU
Physicochemical	Molecular Weight Distribution	Molecular weight distribution	1/6 lots atypically high
	Coomassie CBBG-250	Molecular charge	
	Cation Exchange Chromatography	Charge distribution	
	Atomic Force Microscopy	Aggregate morphology and charge	
	Viscotek-TDAmax	Molecular size distribution	
	RPLC-2D-MALLS	Molecular weight hydrophobicity correlation	
	Ion Mobility Mass Spectrometry	Amino acid sequence, size, charge and shape	
Biological	Potency	Biological functionality-cytokine release	Significantly higher potency profile within Copaxone specifications
	Cytotoxicity	Cytotoxicity	
	Biological Activity (EAE)	Animal model for MS	
	GA specific mAb	Immuno-recognition	
	GA specific PAb	Immuno-recognition	
	Cell-based in vitro assay	Inhibition of TNFα secretion	
	Gene Expression/MOA	Modulation of genes	
	90 day in vivo rat toxicity study (daily dosing)	Local toxicity at injection site	

= similar = different

Adapted from Melamed-Gal et al. [1]

(higher Rh and IV values). The definition of the min-max range for IMMS was provided and we explain in our article [1] that the frequencies of results that were outside the range of Copaxone tested batches were about 50%, with about 30% being below that range and 20% above it. For RPLC-2D MALLS we indicate that most of the Synthon EU lots fell within the microheterogeneity range of the Copaxone lots, i.e. were similar except for a lot with the Remurel tradename.

The readers criticize the gene expression results by mentioning comments from the FDA concerning Teva's 8th Citizen Petition (FDA-2015-P-1050-0012) [4]. To remove all doubt, these mentioned FDA comments did not concern studies utilizing the Synthon EU product studied in our paper [1], nor is the Synthon EU FOGA product currently approved in the US. The FDA‘s comments regarding the gene expression methodology were addressed by Teva in a subsequent public docket submission to the FDA [5,6], which included further data to substantiate previous findings. The authors would like to note that similar gene expression studies have been reported in multiple peer reviewed publications [7–10] and include industry standard criteria.

Regarding the TNFα secretion results and the signaling pathway analyses, the gene expression results indicate differences in the Hallmark TNFα signaling via NF-kB pathway, defined as “genes regulated by NF-kB in response to TNF” (http://software.broadinstitute.org/gsea/msigdb/cards/HALLMARK_TNFA_SIGNALING_VIA_NFKB.html). These differences would not necessarily be anticipated to affect TNFα secretion, but rather signaling downstream of TNFα, hence the assertion of a discrepancy between the expression and secretion data is inaccurate. TNF production is controlled via multiple paths, including gene transcription, control of mRNA half-life and protein translation and secretion. Because cytokines commonly have complex cross-talk and overlapping functions with many other cytokines, examination of only one or a few molecules (by Western blot or ELISA, for instance) can give an incomplete picture of their roles in an experimental model. Moreover, in the case of inflammatory cytokines, their concentrations and fluctuations in concentrations following insults often fall below or around the technical limits of assays using current technology.

In our article [1] we reported on physicochemical and in vitro/ex vivo biological assays showing differences, beyond the intra-product lot to lot microheterogeneity ranges, that indicate that the amino acid antigenic sequences, length, and amount of peptides are not the same between the active substances in Synthon EU FOGA and Copaxone. The rat data demonstrate that these products show a difference in the response of an in vivo biological system on an endpoint of injection site reactions, the exact mechanisms leading to this difference and relevance of these differences to clinical endpoints are unknown. It is also unknown whether the physicochemical and biological differences that we report on, may be amplified under conditions not tested thus far in clinical studies, such as at a higher dose level or upon situations of (repeated) substitution.

In reference to readers' comment about determining similarity between random complex protein mixtures, we provide the following points. First, it should be noted that the complex immunogenic peptide mixtures are not entirely random, but rather the composition and the microheterogeneity (specifically defined in our paper [1]as intra-product lot-to-lot variability) of GA is controlled by the corresponding manufacturing reaction conditions. Indeed, ‘similar’ does not mean ‘identical’, but importantly, the differences in FOGA batches reported in our paper [1] are beyond the differences noted between batches of Copaxone, i.e. the defined microheterogeneity range. In this context it is confusing that the readers in their letter relate to the differences noted between the products as “microheterogeneities”, since this term was defined differently in our article.

Second, the GATE study established the therapeutic equivalence between the 20 mg/ml QD Synthon EU FOGA and Copaxone. As defined by the EU regulator, therapeutic equivalence means that the efficacy and safety of the hybrid formulation is similar to the efficacy and safety of the reference product [11]. However, pharmaceutical equivalence of active substances was not established, and cannot be concluded from therapeutic equivalence. In line with this, the demonstrated physicochemical and biological differences between these immunogenic products, as reported in Melamed-Gal et al. [1], should be further studied, particularly in the context of conditions of use that have not been researched in clinical trials.

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Sigal Melamed-Gal[a], Pippa Loupe[a,*], Bracha Timan[a], Vera Weinstein[a], Sarah Kolitz[b], Jenny Zhang[b], Jason Funt[b], Arthur Komlosh[a], Nurit Ashkenazi[a], Oren Bar-Ilan[a], Attila Konya[a], Olga Beriozkin[a], Daphna Laifenfeld[a], Tal Hasson[a], Benjamin Zeskind[b], Michael Hayden[a], Steffen Nock[a], Iris Grossman[a]

[a] *Global Research and Development, Teva Pharmaceutical Industries, Netanya, Israel*

[b] *Immuneering Corporation Boston, MA, USA*

E-mail address: pippa.loupe@tevapharm.com (P. Loupe).

* Corresponding author.