PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Final results from the Betaseron® (interferon beta-1b) Pregnancy Registry: a prospective observational study of birth defects and pregnancy-related adverse events
AUTHORS	Coyle, Patricia; Roberts, Susan; Scheuerle, Angela; Thorp, John; Albano, Jessica; Rametta, Mark

VERSION 1 - REVIEW

REVIEWER	Yara Dadalti Fragoso Universidade Metropolitana de Santos, Brazil
REVIEW RETURNED	26-Dec-2013

GENERAL COMMENTS	The authors provide results from a prospective database on the safety of interferon beta 1-b used during pregnancy. Although the paper brings the novelty of a prospective database, it does not bring new data or new light on the matter. The sample is too small to provide conclusive information. The authors have not considered other confounding factors such as smoking, parity, other health conditions of the mother and other drugs that might have influenced the outcomes in pregnancy. In fact, the prospective database is just about the only one that could consider all these factors, as many retrospective, case- control studies always fall short of delivering this type of information. I have problems understanding the numbers: the database had 99 pregnancies, but four were lost to follow-up. This would leave the database with 95 pregnancies with sufficient information. However, 99 births were registered and there were three twins – which would account for six births, I suppose, but it is not clear. Then, three plus 95 (or 92, I am already lost in the calculations of how the twins were considered) would not amount to 99. Later on, in the Results, only three pregnancies were lost to follow-up. Table 2 states that the analyzed population was 96, but n=95 in the same table. There is also plenty of missing data in Table 2 for a prospective study. These data are very important and I do not think that cases with missing information should be included at all in the Results. I do not think this database can consider 95 or 96 cases if there are missing data. Of course, they have to be mentioned, but they must be excluded from Results. Why would information be missing in patients enrolled in a database especially set up for this purpose? There is no information on the duration of exposure to the drug in question, or to any other drugs. Antibiotics, analgesics, vitamins, nutritional supplements, regular medications (asthma, hypertension, digestive dysfunction) are not mentioned at all and may have an important influence on the outcome. In fact, birth defects were almost twice as frequent in children whose mothers had been exposed to Betaseron than the general population of mothers. However, the best comparison would be with mothers who had not been exposed to drugs, since MS itself may

	affect some aspects of pregnancy (spasticity, infections, bladder disorders, and other disabilities). The authors explain that this value of birth defects has a p=0.09 in the Fisher exact test. It is always important to remember that p=0.09 means that the statement has 91% of chance of the statement to be correct… I do not think a reassuring conclusion can be obtained from these numbers. Why was Fisher exact test chosen for the analyses? In any case, the numbers of the database do not have the power to allow for conclusions one way or another. I personally find the idea of a prospective database on MS drugs (and symptomatic drugs) in pregnancy very appealing. The results would be of great importance, highly needed for neurologists, obstetricians, patients and families alike. Partial results like the present ones do not add to the present knowledge, I am sorry to say.

REVIEWER	Mario Zappia Department "G.F. Ingrassia" - Section of Neurosciences University of Catania
REVIEW RETURNED	08-Jan-2014

GENERAL COMMENTS	In this prospective study, the Betaseron Pregnancy Registry prospectively monitored women exposed to interferon beta-1b during pregnancy to evaluate the rates of birth defects, spontaneous abortions (SABs), and other negative outcomes in the study population. The results of the current study showed that rates of birth defects and SAB in women exposed to interferon beta-1b during pregnancy were not significantly different from population comparators. The aim of the study is interesting and the results add some contributes to the topic. Nevertheless the authors should clarify some aspects related to their work. • In the Results the authors wrote “the birth defect prevalence estimated by the Betaseron Pregnancy Registry (5.8% [95% CI 1.9–13.0]) was not significantly different (note the overlapping confidence intervals in data from the Betaseron Pregnancy Registry) from that reported by MACDP (2.78%, P=.092, Fisher’s exact test)”. Although the results were not significant, the percentage of birth defects in women exposed to interferon beta-1b was more than the double compared with the population comparison, and it could have a relevance related to the exposition to the drug during pregnancy. This result should be adequately discussed. • In the Article Summary section the authors stated “the smaller than expected sample size limits the ability to draw definitive conclusions”. What was the sample size expected by the authors and how was it calculated? Moreover, considering that also in the Discussion they claimed “sample size was still smaller than necessary to have sufficient statistical power to draw definitive conclusion”, what is the study population number necessary to increase the statistical power of the study? • The authors specified that all codes were sorted into the appropriate organ system classes by an expert in dysmorphology (AES) who evaluated the potential temporal relationship with exposure to interferon beta-1b. Please, the authors should specify how the expert in dysmorphology have evaluated and classified the potential temporal relationship with exposure to interferon beta-1b in the current study. • The authors divided the exposure time to interferon beta-1b in first, second and third trimester, however it would be interesting to show

	the exposure time in days. Please add in the table 2 a line with the days of drug exposure during pregnancy. Moreover, it would be interesting to evaluate the association between duration time (days) of exposure to the interferon beta-1b and stillbirths, birth defects and spontaneous abortions. Did the authors investigate these associations?

REVIEWER	Kerstin Hellwig St. Josef Hospital Bochum Germany
REVIEW RETURNED	13-Jan-2014

GENERAL COMMENTS	This paper is intresting and should be published, although it is very long, a little hard to read. There are some problems with the numbers in the abstract 4 lost to follow up, then only 3 in the result section. Also some inconsitencies Table 2 are there 95 or 96 pregnancies? The authors mix parts of the discussion in the result section , I would suggest to separat this (eg prevalence of misscariage in the US). Where all the women with prenantal testing befor inclusion frist trimester exposers? Are there differences in the misscariage rate between the group without prenatal testing? If this was a prospective cohort study do you mean with the outcome incidence instead of prevalence?

VERSION 1 – AUTHOR RESPONSE

Reviewer #1
Dr. Fragaso called into question the clinical value of the data being presented given the small sample size and the fact that some cases had missing data. While we agree that a larger sample size with a more comprehensive assessment would have been ideal, we would argue that these data are of great value because they represent the only prospectively-collected evidence currently available on interferon beta-1b exposure during pregnancy. They also involve the largest number of interferon beta-1b exposures reported to date. There is a clear need for more information to guide decision-making for women with MS who are or want to become pregnant and these data can help in that process.

Dr. Fragaso also provided several comments requesting additional information or considerations in the interpretation of the data. Unfortunately, these are not possible with the Betaseron Pregnancy Registry. In fact, they are not available in any of the prior pregnancy registries for other MS disease modifying therapies. The amount of information that is captured using the spontaneous reporting methods of the present registry is always limited so as not to create such a burden that recruiting for the registry becomes impossible. The potential confounding factors (eg, smoking, duration of drug exposure) that Dr. Fragaso mentioned in her comments simply could not be collected in this registry. Some missing data also cannot be avoided using this reporting method. The last 2 paragraphs of the Discussion section address these issues, but we have made some additions to make the limitations in data capture that are inherent in a registry more clearly stated. It is also important to point out that cases reporting birth defects were reviewed for other factors that may have influenced the pregnancy outcome, and the relevant information is presented in Table 5. An additional statement has been added to the Methods section to clarify this point.

We agree completely with Dr. Fragraso's assertion that pregnant women with MS who did not have drug exposure would have been the ideal comparator population in this study. Unfortunately, reliable data on this population are not available for comparison and therefore population estimates like the ones used in the present study are the standard for pregnancy registries in MS (for examples, see the Avonex, Gilenya, and Tysabri pregnancy registries, all of which were presented at the 2012 ECTRIMS conference). The difficulties of selecting the ideal population for a birth defect prevalence registry have been described in an earlier publication (Scheuerle A., Vannappagari V.X., Miller M.K. Birth Defects Res A Clin Mol Teratol. 2009;85:611-620) in which the authors concluded that no ideal comparator is available.

With regard to the statistical methods, a detailed a priori analysis plan was created at the start of the study which stipulated the use of the Fisher's exact test. This methodology is consistent with that of other pregnancy registries. Appropriate references are included in the Methods section of the manuscript and some additional details have been added to clarify anything that might be potentially unclear.

Lastly, Dr. Fragaso pointed out a few instances where the data could be clarified with regard to the number of patients in the analysis. We welcome these comments as these issues are likely to arise with any potential reader and should be addressed to improve the clarity of the manuscript. Several changes have been made to improve the clarity of the manuscript, including the addition of a footnote to Table 2 and a correction in the abstract.

Reviewer #2

Dr. Zappia astutely pointed out in his comments that the percentage of birth defects in women exposed to interferon beta-1b was almost twice that of the population comparator but the difference was not statistically significant, and we agree that this should be adequately discussed in the manuscript. Therefore, we have added some language to the Discussion explaining the lack of a pattern of birth defects and how this should be interpreted as no signal for interferon beta-1b-induced birth defects. Dr. Zappia also requested some additional details in the Methods, so we have added a paragraph on the expected sample size and a sentence on assessing the temporal relationship between exposure and birth defects.

Dr. Zappia's final comment related to the potential effects of exposure duration on pregnancy outcomes. Unfortunately this analysis cannot be conducted because exposure data were not prospectively collected.

Reviewer #3

Dr. Hellwig pointed out some of the same issues related to clarity which were mentioned by Dr. Fragaso and, as previously stated, changes have been made to the manuscript to clarify how many patients were included in each stage of the analysis. With regard to her comment on the comparisons with population data belonging in the Discussion section, we believe these data should be in the Results section because the published rates from the MACDP serve as the population-based external comparison group and a direct statistical comparison was made between data from the Betaseron Pregnancy Registry and MACDP population data. Further this estimate was used in sample size calculations for the primary objective. Therefore, published MACDP data also belong in the Methods section as they are an essential part of the study methods. Lastly, Dr. Hellwig commented that we may wish to change "prevalence" to "incidence" when discussing birth defects. Because this manuscript draws comparisons with population data, we feel the term "birth defect rate" would be more appropriate and we have made that change throughout the manuscript.

VERSION 2 – REVIEW

REVIEWER	Prof. Mario Zappia Department GF Ingrassia, Section of Neurosciences, University of Catania, Catania, Italy
REVIEW RETURNED	11-Mar-2014

GENERAL COMMENTS	The authors have exhaustively answered to the questions requested. According to the reviewer this article do not need further revision.

REVIEWER	Kerstin Hellwig St. Josef Bochum
REVIEW RETURNED	19-Mar-2014

GENERAL COMMENTS	I have 2 more questions or coments: 1. what is the percentage of premature bron babies? The median was gw 39 which is ok, but as IFn were attributed to prematurity this should be pointed out. 2. Women were included with oprenatal testing if this was whithour anomalies, how many were exlcuded then by having testing with anomalies? This can bias the the reuslst eg decreasing misscarriage rates, malformation?

VERSION 2 – AUTHOR RESPONSE

In response to Reviewer #3's new comments, we have added some new data to Table 4 to provide information on the percentage of premature babies. Regarding the second comment, we are unable to provide the number of women who had prenatal testing that identified an anomaly. Per the protocol of the registry, these women were excluded from enrollment and therefore no information is available. The enrollment criteria are explained in the manuscript in greater detail in the second paragraph on page six.