Author's response to reviews

**Title:** Differential glatiramer acetate treatment persistence in treatment-naive patients compared to switchers from interferon.

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Article title: Differential glatiramer acetate treatment persistence in treatment-naive patients compared to switchers from interferon.

Former article title: Analysis of treatment persistence with Glatiramer Acetate in treatment-naive patients and after treatment failure with Interferons.

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Authors: Mireya Fernández-Fournier, Antonio Tallon Barranco, Beatriz Chamorro, Patricia Martínez-Sánchez, Rosario Madero and Inmaculada Puertas.

Journal: BMC Neurology

21 January 2015

Dear Mr Jhonell De Los Santos and Dr Roberto Bergamaschi,

Please find enclosed our revised manuscript entitled “Differential glatiramer acetate treatment persistence in treatment-naive patients compared to switchers from interferon” by Fernandez-Fournier et al, currently under consideration for publication as a Research article in BMC Neurology.

We are very grateful to you for the opportunity to send back the revised version of the manuscript, as well as to the reviewers for their comments, as it has helped to improve the text. We have carefully studied all points addresses by reviewers and major changes have been made to the manuscript. As requested, we are providing a revised manuscript and a full covering letter with a point-by-point response to the reviewers’ comments.

We look forward to hearing from you at your earliest convenience. We are willing to make further revisions if deemed necessary. Please address all correspondence to: fernandezfournier@hotmail.com

Yours faithfully,

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Major changes have been made to the text. The manuscript has been revised and edited for the following:

Reviewer 1: Tomas Kalincik

A. MAJOR REVISIONS:

First of all we want to thank the reviewer for his helpful comments in ameliorating the quality of this manuscript.

1) The description of the used statistical methodology is rather brief (p.6-7, l.143-150). Given that the study describes treatment persistence in a retrospective analysis of observational data, which also comprises a comparative component, a great attention should be paid to the elimination of potential confounders. To my understanding and based on the provided description, the proportional hazards model was only adjusted for age; however, numerous other confounders, including demographic and clinical information, should have been taken into account and adjusted for. Also the lack of statistical significance of the described difference in the sex ratio between the groups (with females being more likely to initiate GA as their first disease modifying therapy) does not justify the lack of the adjustment for sex, as the lack of statistically significant difference could also be due to the lack of power for the purpose of comparison of sex ratios between the groups. The authors indicate that a value “lost to follow-up” was allowed as a reason for treatment discontinuation. Figure 1 indicates that those lost to follow-up while treated with GA were excluded from the analysis (this should be stated explicitly in the Methods); however, it would be appropriate to include these patients in the analysis, indicating their censoring as per survival analysis. The same applies to the trend for the EDSS to be lower in the treatment-naïve cohort. On page 7 lines 155-156, the authors report average patient age in October 2013. However, if age is considered as a potential confounder of treatment persistence, it should be reported at the time of treatment initiation and summary statistics for both groups should be shown.

Lines 51, 142 - 143 and table 1: Regarding potential confounders, we have adjusted for both sex and age and we have specified this in the description of statistical methodology/data analysis
“…estimated using a Cox regression model, with and without adjusting for sex and age at the time of GA initiation.”
And in the Results section of the Abstract: “…after adjusting for sex and age.”
As suggested, we have calculated age at the time of treatment initiation and used this for summary statistics and when adjusting for age in the Cox regression model.

Lines 232 - 233 and table 1: We agree that adjusting for clinical data as measured by the EDSS scale would be desirable, however, due to the fact that this is a retrospective study we are missing data regarding the EDSS at specific time-points. We have specified this: “Missing data do not allow us to conclude if EDSS is predictive of treatment discontinuation in our cohort.” Even if EDSS is important, in this cohort no differential effect is seen between the two groups. We will take this into account in the design of future studies.
Lines 134 - 148: The description of the used statistical methodology has been rewritten and expanded. It now includes a description of the principal variable as well as all of the above. We have specified censored data for survival analysis and the use of Estimated Hazard ratios with their 95% confidence intervals. Also, we have changed the subtitle from “Statistical analysis” to “Data analysis”. This section now reads:

“Data analysis

Statistical analysis was performed using SAS 93 (SAS Institute, Cary, NC, USA). Quantitative data are described using the mean ± standard deviation (± s.d.), median and range. Qualitative data are described using absolute frequencies and percentages. The homogeneity of the groups was analysed using Fisher's exact test for categorical data and Mann-Whitney’s test for quantitative data. Treatment time on GA was studied. The Kaplan Meier method was used to estimate time to treatment discontinuation. The Logrank test was used to test for differences between groups. Risk of treatment discontinuation was estimated using a Cox regression model, with and without adjusting for sex and age at the time of GA initiation. The independent variable of this study was time to GA treatment discontinuation, defined as the time lapse between treatment onset and treatment discontinuation in months. Patients who were still treated with GA on October 1st 2013 and those lost to follow up on treatment with GA have been considered censored values as per survival analysis. Estimated Hazard ratios with their 95% confidence intervals are provided.”

Figure 1 and lines 145 - 147: Patients lost on follow-up are included in the statistical analysis and these cases are treated as censored values as per survival analysis; we have specified this in the methods section: “Patients who were still treated with GA on October 1st 2013 and those lost to follow up on treatment with GA have been considered censored values as per survival analysis.” Figure 1 has been modified for clarification.

2) **The authors should clearly indicate the criteria for previous treatment with GA.**

Was any pre-GA exposure to IFN considered or were only patients immediately switching therapy from IFN to GA included? If the latter is the case, what was the definition of treatment switch and was a maximum “inter-treatment gap” defined for this purpose?

Lines 97 - 99: Regarding previous treatment exposure, all patients who were prescribed GA and had previously been treated with IFN were considered. We did not define a concrete “inter-treatment gap” for this in the study design and all RRMS patients who had been treated with IFN and had later been treated with GA are included and considered as switchers.

In the text, the following sentence has been added: “All RRMS patients who had been treated with IFN before initiating treatment with GA were included and considered as switchers.”

3) **The authors should provide the descriptive statistics of the available on-study Follow-up (from GA initiation to the end of the available follow-up regardless of Treatment status).**

Lines 174 - 175: This information has been included. “Overall, patients in our cohort were treated with GA an average of 39 months (s.d 30.0). Median follow-up was 34 months.”
**Lines 46 – 47:** The abstract has been updated accordingly: “ATT in our cohort was 39 months (s.d 30.0), median follow-up 34 months.”

4) *What was the fate of the patients who discontinued therapy? The authors should provide information about the further treatment status for the entire cohort as well as for the treatment groups.*

**Lines 159 - 167:** Information regarding the fate of all patients who discontinued GA therapy is provided. We have specified this information by treatment group. All other patients continued on GA to available follow up. The second paragraph in the Results section has been modified and now reads: “At the end of the study (Figure 1), from the 155 patients, a total of 76 patients (49.0%) were still treated with GA. Prescription had been withdrawn in 50 cases (32.3%), 3 patients (1.9%) had temporarily stopped GA for pregnancy planning and 26 patients (16.8%) had been lost to follow-up (Figure 2). From the 50 patients who stopped GA, most (n=31 (62.0%); 93.5% naive, 6.5% switchers), were switched to second-line drugs, but also to IFNs (n=14 (28.0%); 100% naive) and immunosuppressive drugs (n=2 (4.0%); 100% naive). Treatment was sometimes stopped altogether following patients’ demands (n=2 (4.0%); 100% naive) or conversion to secondary progressive MS (n=1, naive).”

5) *p.7, l.162-163: Reporting the proportion of patients who discontinued therapy at a particular date (as of study end-point) does not provide the reader with any useful information. The proportions of discontinuers should be reported in relation to the time on-treatment (i.e. at defined time points post treatment initiation, e.g. using visualisation with Kaplan-Meier curves for the evaluation of total treatment persistence).*

**Lines 175 - 180:** We have calculated, using the Kaplan-Meier method, the proportion of discontinuers at different, defined, time points post treatment initiation and this information has been included in the Results section, by treatment groups: “Six months after GA treatment initiation, probability of continuing on GA was 91.4% (88.6% for treatment-naive patients and 96.3% for switchers). Twelve months after treatment initiation, probability of continuing on GA was 82.5% (76.8% for treatment-naive patients and 92.5% for switchers). Two years after treatment initiation, probability of continuing on GA was 72.5% (62.3% for treatment-naive patients and 90.6% for switchers).”

**Lines 47 - 49:** The abstract has been updated accordingly: “Six months after glatiramer acetate initiation, probability of persisting on GA was 91.4%, 82.5% after 12 months and 72.5% after 2 years.”

6) *p.8, l.172-176: The break-down of patients who stopped therapy provides information about 51 patients, which is not in agreement with the 50+3 patients reported on p.7, l.163-164. According to the break-down, 4 patients stopped their therapy and 47 switched to other agents, whereas the break-down of the switchers by the reason for switching adds-up to 50 patients. This information should be rectified and should also be provided per treatment group (naive vs. post-IFN); e.g. it can only be assumed that the 14 patients switching from GA to IFN were presumably from the treatment-naive cohort. Finally, the information*
about the reason for switching therapy from GA would more logically follow after the overview of the number of patients undergoing the switch.

**Lines 165 – 167:** The total number of patients who stopped therapy altogether is 3 and not 4 (in 2 cases following patients’ demands and in 1 case for conversion to a secondary progressive form of the disease). This has been corrected and provided by treatment group (naive vs post-IFN) “Treatment was sometimes stopped altogether following patients’ demands (n=2 (4.0%); 100% naive) or conversion to secondary progressive MS (n=1, naive)” so that both break-downs of patients add up to 50 patients which is correct.

**Lines 159 - 173:** The fate of all patients who discontinued GA therapy is provided. We have now included information specified by treatment group (naive vs. post-IFN). Also, the Results section has been modified to follow the suggested order and figures 3 and 4 have therefore changed places. The **second and third paragraph of the results** section read:

“At the end of the study (Figure 1), from the 155 patients, a total of 76 patients (49.0%) were still treated with GA. Prescription had been withdrawn in 50 cases (32.3%), 3 patients (1.9%) had temporarily stopped GA for pregnancy planning and 26 patients (16.8%) had been lost to follow-up (Figure 2). From the 50 patients who stopped GA, most (n=31 (62.0%): 93.5% naive, 6.5% switchers), were switched to second-line drugs, but also to IFNs (n=14 (28.0%); 100% naive) and immunosuppressive drugs (n=2 (4.0%); 100% naive). Treatment was sometimes stopped altogether following patients’ demands (n=2 (4.0%); 100% naive) or conversion to secondary progressive MS (n=1, naive).

In our cohort, reasons for switching from GA to another DMD were: (a) lack of efficacy, including relapses (22 patients, 14.2%), disability accrual (3 patients, 1.9%) or evidence of disease activity on follow-up MRI (2 patients, 1.3%); (b) injection intolerance (20 patients, 12.9%): mainly injection pain and mild-moderate skin reactions such as redness or itching; (c) SAEs (3 patients, 1.9%): injection associated unremitting chest pain, allergic reactions or severe local reactions such as marked lipoatrophy.”

7) p.8-9, l.194-196: Treatment persistence among 1113 patients in relation to several examined potential predictors, including the pre-treatment status and the preparation (incl. GA) was reported by Jokubaitis et al. 2013, PLoS One 8:e59694.

**Lines 197 – 199:** This paper is acknowledged “This is the second published study that aims to describe differences in treatment persistence with GA in treatment-naive patients with respect to patients previously treated with IFNs, and the first to do so in Europe.”

**Lines 217 - 233:** We have taken into account their results and compared them to our results in the Discussion section. The fourth paragraph of the reads:

“In contrast to the situation in clinical trials, switching DMDs is common in daily practice. Studying treatment use and response in ‘real-world/clinical practice’ is relevant for the future care of RRMS. It is important to address questions not answered by clinical trials regarding not only efficacy, but also tolerability and treatment persistence[21]. Treatment persistence varies with country of residence[22]. Jokubaitis
et al. compared treatment persistence on GA and IFN in Australian patients and report that, in their cohort, patients receiving IFN β-1a as a first DMD persisted longer on treatment than those treated with GA as a first DMD[22]. Similarly, Kleinman et al. found lower discontinuation rates on IFN β than on GA in the US[23]. On the other hand, data from the REGARD clinical trial do not show significant differences in treatment persistence between IFN β-1a and GA[24]. In the Australian cohort, higher EDSS and younger age at DMD initiation were predictive of treatment discontinuation, however in our cohort, adjusting for age hardly modified the relative risk of GA discontinuation. Another study on DMD use reported female sex as a predictive factor of treatment discontinuation[25], but this was not reproduced in the Australian study or in our cohort. Missing data do not allow us to conclude if EDSS is predictive of treatment discontinuation in our cohort.”

8) p.9, l.219: It is unclear where the proportion of patients (17.4%) with break-through activity is derived from. I assume that this is the sum of the patients who switched from GA to another drug due to previously identified relapses, progression of disability or MRI activity (p.7, l.167-168). However, this is most probably an underestimation of the real break-through activity, as it does not include the patients who remained on GA despite break-through activity. Also, reporting of on-treatment disease activity should always be related to treatment duration (see above).

Lines 234 – 236: Yes, the proportion of patients with break-through activity is derived from adding the number of patients changed from GA to another DMD due to relapses, disability accrual or disease activity on follow-up MRI, as specified in the results section. We now specify this, at the line indicated by reviewer 1 in the Discussion section: “with only 17.4% of patients presenting breakthrough disease (relapses, progression of disability or MRI activity)”

Lines 100 – 104: This is now also specified in the Methods section, subsection Study design, second paragraph:
“Treatment failure was defined as reasons given by the treating neurologist for GA discontinuation. The following reasons were considered: lack of efficacy (including relapses, progression of disability or magnetic resonance imaging (MRI) activity), occurrence of serious adverse event (SAE), injection site reactions, pregnancy or pregnancy planning at study end-point and loss to follow-up.”

Lines 244-246: We have included the comment suggested by reviewer 1 that this may be an underestimation of the real break-through activity, as it does not include the patients who remained on GA despite break-through activity. The fifth paragraph of the Discussion now reads:
“With respect to efficacy, GA was useful in our ‘real world’ setting, with only 17.4% of patients presenting breakthrough disease (relapses, progression of disability or MRI activity), which is lower than that of clinical trials and on the low end of reported breakthrough disease for clinical practice, which is of 18 - 30% in 5 years for first-line DMDs[19]. This difference might be explained by clinical trials’ selective inclusion criteria that tend to include only highly active patients with a greater probability of break-through disease than that of “the average MS patient”. It may also result from underestimating real breakthrough activity, as patients who might have remained on GA despite disease activity are not included.”
9) p.10, l.225: Is the reported average time on GA for those escalating therapy (29.2 months) merely the time on GA for those who discontinued therapy due to the lack of treatment efficacy (l.177-178) or was this calculated specifically for those who escalated therapy?

Lines 187 - 188 and 277 - 278: The reported time on GA (29.2 months) is the time on GA for those who discontinued therapy due to the lack of treatment efficacy as stated in lines 187-188. We have rephrased this in the Discussion as follows: “patients who discontinued GA for lack of treatment efficacy were on GA for over two years (29.2 months (±17.5)).”

10) The Discussion of study limitations should be more comprehensive and should discuss some of the issues raised above, as well as the limitations concerning the cohort size and the statistical power, and the generalizability of the observations.

The discussion has been rewritten following comments from both reviewers. We have commented on the cohort size and the retrospective study design which limits study variables, such as EDSS at defined time-points but also other variables which were not studied, such as prevalence of neutralizing antibodies or patient satisfaction. Other limitations are discussed in this paragraph. Regarding the generalizability of our observations, we comment on the fact that the time frame of the study influences treatment persistence; with changing availability of different treatments for MS over the last decade, as well as changing criteria for definition of a suboptimal treatment response as seen on MRI scans.

Lines 269 – 284: Paragraph 9 in the Discussion section now reads “Limitations to our study include the retrospective design that limits study variables. The prevalence of IFN neutralizing antibodies was not consistently checked, treatment compliance was not well recorded and no questionnaire addressing patients’ perception of treatment was administered. Other limitations of this study are cohort size and loss to follow-up, with 26 patients (16.8%) lost to follow-up whilst on treatment with GA, however this seems reasonable taking into account the 10 year time-span of the study. This pilot study was designed as an exploratory study so we were not able to conduct certain sub-analysis. However, despite the limited sample size significant results are obtained in treatment persistence between treatment naïve patients and switchers. Regarding clinical applicability of these results, the time frame for this study must be taken into consideration as it might influence treatment persistence. In our cohort, patients who discontinued GA for lack of treatment efficacy were on GA for over two years (29.2 months (±17.5)). Patients underwent regular follow-up visits at least twice per year, so GA might be seen a useful initial treatment even if long-term response was suboptimal in some cases. However, patients might have remained on GA waiting for the arrival of new therapies. Between 2004 and 2013 progress in the treatment of MS was remarkable, with new second-line drugs being licensed. At the same time, MRI criteria for early identification of non-responders were developed and progressively incorporated into clinical practice. With regard to the future, the situation is one of an increasing complexity, as new oral drugs, available as first-line therapies, arrive[20, 29].”

The fact that this study may be underestimating real breakthrough activity is stated in the fifth paragraph, when discussing efficacy results of this study:
“With respect to efficacy, GA was useful in our ‘real world’ setting, with only 17.4% of patients presenting breakthrough disease (relapses, progression of disability or MRI activity), which is lower than that of clinical trials and on the low end of reported breakthrough disease for clinical practice, which is of 18 - 30% in 5 years for first-line DMDs[19]. This difference might be explained by clinical trials’ selective inclusion criteria that tend to include only highly active patients with a greater probability of break-through disease than that of “the average MS patient”. It may also result from underestimating real breakthrough activity, as patients who might have remained on GA despite disease activity are not included.”

Also, we have specified in the manuscript that treatment persistence varies with country of residence on line 221 “Treatment persistence varies with country of residence[22].”

B. MINOR AND DISCRETIONARY REVISIONS:

11) p.5, l.120: Were also patients who received therapy other than IFN or GA after discontinuing GA excluded from the study?

No, these patients were not excluded from the study. Treatment times were calculated, in months, as the time lapse between GA treatment initiation and the last documented injection, independently of the treatment patients may have received after GA. Information regarding therapy after GA is given for all patients who discontinued GA for information purposes only.

12) p.5, l.127-128: How were the criteria of MRI activity defined?

We have defined in the Methods section what has been considered MRI activity for treatment discontinuation “A lack of efficacy or suboptimal response to GA was considered the reason for a treatment change based on the clinical history, considering the occurrence of relapses (≥2 relapses or 1 severe relapse), accrual of disability or disease activity on follow-up MRI (presence of gadolinium-enhancing lesions or increased T2-lesion load).”

13) p.7, l.162: While Figure 1 states that 74 patients who continued therapy were included in the analysis, the text reports 75 patients.

Figure 1: Figure 1 has been corrected and also modified for clarification purposes.

14)p.8, l.177-183: The incidence of reasons for treatment discontinuation seems to vary with treatment duration (e.g. serious adverse events tend to occur earlier than injection-site reactions). It would be of interest to show whether these differences reach the level of statistical significance in the survival analysis and visualise these in survival curves.

The independent variable of this study was time to GA treatment discontinuation, defined as the time lapse between GA treatment onset and treatment discontinuation in months, independently of the reason for treatment discontinuation. This is now specified as such in the Methods section. We have included figure 4 to illustrate the point that secondary effects (SAEs and injection site reactions) limited GA use before
ineffectiveness, in our cohort. We have specified that time to GA treatment discontinuation is the independent variable:

**Lines 143 – 145:** “The independent variable of this study was time to GA treatment discontinuation, defined as the time lapse between treatment onset and treatment discontinuation in months.”

**Lines 182 – 184:** The sentence in the Results section has been rephrased so that the interval estimators are clarified: “The risk of GA discontinuation was 2.8 \([\text{C.I.} 95\%: 1.7 – 4.8]\) times greater for treatment-naive patients and this was hardly modified after adjusting for sex and age (3.0 \([\text{C.I.} 95\%: 1.8 – 5.2]\)).”

**Lines 49 - 51** in the Abstract have also been rewritten for clarification: “The risk of glatiramer acetate treatment discontinuation was 2.8 \([1.7 – 4.8]\) times greater for treatment-naive patients than for interferon-switchers and this was hardly modified after adjusting for sex and age.”

**Lines 147 – 148:** The use of estimated Hazard ratios with their 95% confidence intervals is specified in the Methods section: “Estimated Hazard ratios with their 95% confidence intervals are provided.”

We provide both the adjusted and the unadjusted hazard ratios just for information. It is interesting that they hardly vary as a previous study has reported that age can influence treatment persistence. The following comment has been included in the Discussion,

**Lines 227 – 232:** “…In the Australian cohort, higher EDSS and younger age at DMD initiation were predictive of treatment discontinuation, however in our cohort, adjusting for age hardly modified the relative risk of GA discontinuation. Another study on DMD use reported female sex as a predictive factor of treatment discontinuation[25], but this was not reproduced in the Australian study or in our cohort.”

**16) In the Introduction (p.4, l.81-83), the authors state that there is a need to carefully evaluate the reasons for treatment failure, including adherence, neutralising antibodies or adverse events. This statement is somewhat out of the context of the reported analysis, as of the three given reasons for treatment failure, the study only evaluates one -the serious adverse events.**

The introduction has been rewritten and this point has been moved to the Discussion section.

In theory, it is important to evaluate all reasons for treatment failure,

**Lines 208 – 213** “…first-line treatment failure with IFNs may be due to the existence of neutralizing antibodies[12], poor tolerance or occurrence of adverse events, and may not reflect the need to escalate to second-line therapies such as Fingolimod or Natalizumab…”

As seen in this study, in our routine clinical practice no detection of neutralizing antibodies was performed and treatment decisions were made on based on clinical
information regarding disease activity and medication side-effects alone. Treatment adherence encompasses both compliance and persistence. Compliance was poorly evaluated and recorded. Reasons for treatment failure can potentially include poor compliance or neutralizing antibodies. The limitations of not having addressed these points, due to the retrospective design of this study, is now discussed in paragraph 9 of the Discussion section.

**Lines 266 – 271:** “Limitations to our study include the retrospective design that limits study variables. The prevalence of IFN neutralizing antibodies was not consistently checked, treatment compliance was not well recorded and no questionnaire addressing patients’ perception of treatment was administered.”

We have included these comments in the Results section because we believe that this needs to be studied in future prospective studies, as we say in the last paragraph of the Discussion section:

**Lines 285 – 290:** “...even if safety and efficacy parameters of both drugs are similar, patient needs, such as different levels of tolerability, need to be addressed and taken into account for treatment choice[30]. A prospective study, with questionnaires specifically addressing subjective treatment perception as well as treatment adherence, in both treatment-naive patients and switchers might shed light on the differentiation in treatment persistence.”
Reviewer 2: Alessandra Lugaresi

Thank you for your comments that allow us to improve the manuscript.

B. MINOR REVISIONS:

*There are several mistakes to be corrected, highlighted in the attached pdf e.g. the use of end-point to indicate end of observation, loss to follow-up instead of lost to follow up (highlighted in the revised text). Etc*

**Lines 1 – 3:** The title of the article has been changed as suggested: “Differential glatiramer acetate treatment persistence in treatment-naive patients compared to switchers from interferon.”

**Line 28:** This sentence has been rephrased as suggested. It now reads: “Although disease modifying drugs’ (DMD) efficacy and side effects have been fully analysed in clinical trials, the effects of therapy switch are less well studied.” **Lines 80 – 81** in the Background section have been changed accordingly and now read: “Although DMD efficacy and side effects have been fully studied in clinical trials, effects of therapy switch are less well known.”

**Line 35 and figures 1-3:** The term lost to follow-up has been corrected as indicated.

**Lines 41 - 42:** “At study end-point” has been replaced by “at the end of the study” as indicated.

**Line 51:** The independent variable of this study was time to GA treatment discontinuation, defined as the time lapse between treatment onset and treatment discontinuation in months, independently of the reason for treatment discontinuation. This is now specified as such in the Methods section. **Lines 143 – 145:** “The independent variable of this study was time to GA treatment discontinuation, defined as the time lapse between treatment onset and treatment discontinuation in months.”

**Line 53:** We have better characterized patients in the paper:

--- **Lines 142 - 143 and table 1:** Regarding potential confounders, we have adjusted for both sex and age and we have specified this in the Methods section, in the description of statistical methodology/data analysis “with and without adjusting for sex and age at the time of GA initiation.” As suggested by reviewer 1, we have calculated age at the time of treatment initiation and used this new variable for summary statistics and when adjusting for age in the Cox regression model.

--- **Lines 232 - 233 and table 1:** Adjusting for clinical data as measured by the EDSS scale would be desirable, however, due to the fact that this is a retrospective study we are missing data regarding the EDSS at specific time-points. We have specified this: “Missing data do not allow us to conclude if EDSS is predictive of treatment discontinuation in our cohort.” Even if EDSS is important, in this cohort no differential effect is seen between the two groups.

--- **Lines 159 - 167:** Information regarding the fate of all patients who discontinued GA therapy is provided. The break-down, of patients who stopped their therapy is now provided per treatment group (naive vs. post-IFN). The second paragraph in the discussion has been modified and now reads: “At the end of the study (Figure 1), from
the 155 patients, a total of 76 patients (49.0%) were still treated with GA. Prescription had been withdrawn in 50 cases (32.3%), 3 patients (1.9%) had temporarily stopped GA for pregnancy planning and 26 patients (16.8%) had been lost to follow-up (Figure 2). From the 50 patients who stopped GA, most (n=31 (62.0%): 93.5% naive, 6.5% switchers), were switched to second-line drugs, but also to IFNs (n=14 (28.0%): 100% naive) and immunosuppressive drugs (n=2 (4.0%); 100% naive). Treatment was sometimes stopped altogether following patients’ demands (n=2 (4.0%); 100% naive) or conversion to secondary progressive MS (n=1, naive).”

Lines 174 - 175: The descriptive statistics of the available on-study follow-up has been provided: “Overall, patients in our cohort were treated with GA an average of 39 months (s.d 30.0). Median follow-up was 34 months.”

Lines 175 - 180: The proportions of discontinuers are reported in relation to the time on-treatment. We have calculated, using the Kaplan-Meier method, the proportion of discontinuers at different, defined time points post treatment initiation and this information has been included in the Results section, by treatment groups: “Six months after GA treatment initiation, probability of continuing on GA was 91.4% (88.6% for treatment-naive patients and 96.3% for switchers). Twelve months after treatment initiation, probability of continuing on GA was 82.5% (76.8% for treatment-naive patients and 92.5% for switchers). Two years after treatment initiation, probability of continuing on GA was 72.5% (62.3% for treatment-naive patients and 90.6% for switchers).”

Lines 54 - 55: This has been developed in the Discussion section:

Lines 221 – 233: We compare our results to published studies: “Treatment persistence varies with country of residence[22]. Jokubaitis et al. compared treatment persistence on GA and IFN in Australian patients and report that, in their cohort, patients receiving IFN β-1a as a first DMD persisted longer on treatment than those treated with GA as a first DMD[22]. Similarly, Kleinman et al. found lower discontinuation rates on IFN β than on GA in the US[23]. On the other hand, data from the REGARD clinical trial do not show significant differences in treatment persistence between IFN β-1a and GA[24]. In the Australian cohort, higher EDSS and younger age at DMD initiation were predictive of treatment discontinuation, however in our cohort, adjusting for age hardly modified the relative risk of GA discontinuation. Another study on DMD use reported female sex as a predictive factor of treatment discontinuation[25], but this was not reproduced in the Australian study or in our cohort. Missing data do not allow us to conclude if EDSS is predictive of treatment discontinuation in our cohort.”

Lines 254 – 258 We interpret these results and give a possible explanation: “The overall risk of GA treatment discontinuation was almost 3 times greater for treatment-naive patients than for IFN-switchers. Similarly, Jokubaitis et al. report lower treatment persistence on GA than on IFN only for treatment-naive patients but not when these drugs were used as second therapies[22]. We believe this might be influenced by non-naive patients being used to self-injections.

Lines 266 - 273: We comment on other factors that should be studied for better conclusions: Limitations to our study include the retrospective design that limits study variables. The prevalence of IFN neutralizing antibodies was not consistently checked, treatment compliance was not well recorded and no questionnaire addressing patients’ perception of treatment was administered. Other limitations of this study are cohort size and loss to follow-up, with 26 patients (16.8%) lost to follow-up whilst on treatment with GA, however this seems reasonable taking into account the 10 year time-span of the study. This pilot study was designed as an exploratory study so we were not able to
conduct certain sub-analysis. However, despite the limited sample size significant results are obtained in treatment persistence between treatment naïve patients and switchers. Regarding clinical applicability of these results, the time frame for this study must be taken into consideration as it might influence treatment persistence… patients might have remained on GA waiting for the arrival of new therapies…. …A recent review comparing efficacy of IFN with GA for RRMS concludes that, even if safety and efficacy parameters of both drugs are similar, patient needs, such as different levels of tolerability, need to be addressed and taken into account for treatment choice[30]...“

Line 111 - 113: The word on-going has been eliminated as suggested and inclusion criteria have been rephrased as follows: “In total 155 patients met inclusion criteria: age ≥18 years, RRMS diagnosis according to 2010 revised McDonald criteria[9] and treatment with GA between January 1st 2004 - October 1st 2013.”

Line 139: Spelling has been corrected.

Lines 159 and 186: “At study end-point” has been replaced by “at the end of the study” as indicated.

References 6, 14 and 15: Capital letters have been inserted where they were missing.

  1. Is the question posed by the authors well defined? Partially: although the stated aim is persistence in GA treatment, effectiveness would me more relevant.

In this study we aimed to study medication persistence with glatiramer acetate. Comparative effectiveness of GA and IFN beta formulations has already been studied. Recent papers by Kalincik et al and by La Mantia et al. provide comprehensive reviews. We have acknowledged and referenced these papers.

Lines 202 – 207: “Clinical trials and follow-up studies have shown that GA reduces clinical and radiological disease activity, proves more effective than placebo and shows at least similar efficacy to subcutaneous IFN β-1a and IFN β-1b[12–18].”

Treatment persistence is relevant for treatment choice in MS:

Lines 286 – 289: “A recent review comparing efficacy of IFN with GA for RRMS concludes that, even if safety and efficacy parameters of both drugs are similar, patient needs, such as different levels of tolerability, need to be addressed and taken into account for treatment choice[30].”


Even if studying effectiveness is desirable, due to the retrospective design of this study and cohort size we do not have sufficient information on clinical evaluations at defined time-points to conclude on this in our cohort.

2. Are the methods appropriate and well described? Partially: the definition of treatment failure and partial response should appear in the methods.

Lines 100 - 104: A partial response is not a variable in this study; treatment failure encompasses all evidence of breakthrough disease that led to GA discontinuation. The methods section has been rewritten as follows for clarification:

“Treatment failure was defined as reasons given by the treating neurologist for GA discontinuation. The following reasons were considered: lack of efficacy (including relapses, progression of disability or magnetic resonance imaging (MRI) activity), occurrence of serious adverse event (SAE), injection site reactions, pregnancy or pregnancy planning at study end-point and loss to follow-up.”

3. Are the data sound? Yes, but giving a partial perspective. It would have been interesting both to know if differences between naive vs switchers are in the rate of discontinuations due to side effects, rather than lack of efficacy.

The independent variable of this study was time to GA treatment discontinuation, independently of reasons for GA discontinuation. In this study cohort size limits what we can study. We agree that this is an interesting point for future studies. We have however described that “amongst patients who discontinued GA due to injection intolerance, most (n = 19 patients, 95%) were treatment-naive” (Lines 184 – 185).

Further additions of data have been made:
We have also calculated the proportion of discontinuers at different, defined time points post treatment initiation and this information has been included in the Results section, by treatment groups: Lines 175 - 180: “Six months after GA treatment initiation, probability of continuing on GA was 91.4% (88.6% for treatment-naive patients and
96.3% for switchers). Twelve months after treatment initiation, probability of continuing on GA was 82.5% (76.8% for treatment-naive patients and 92.5% for switchers). Two years after treatment initiation, probability of continuing on GA was 72.5% (62.3% for treatment-naive patients and 90.6% for switchers).”

Also, information regarding the fate of all patients who discontinued GA therapy is provided for the entire cohort and also by treatment groups: **Lines 159 - 167:** “At the end of the study (Figure 1), from the 155 patients, a total of 76 patients (49.0%) were still treated with GA. Prescription had been withdrawn in 50 cases (32.3%), 3 patients (1.9%) had temporarily stopped GA for pregnancy planning and 26 patients (16.8%) had been lost to follow-up (Figure 2). From the 50 patients who stopped GA, most (n=31 (62.0%): 93.5% naive, 6.5% switchers), were switched to second-line drugs, but also to IFNs (n=14 (28.0%); 100% naive) and immunosuppressive drugs (n=2 (4.0%); 100% naive). Treatment was sometimes stopped altogether following patients’ demands (n=2 (4.0%); 100% naive) or conversion to secondary progressive MS (n=1, naive).”

4. Do the figures appear to be genuine, i.e. without evidence of manipulation? Not applicable

5. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes.

6. Are the discussion and conclusions well balanced and adequately supported by the data? Partially. Real conclusions have not be drawn. Authors should present a more articulate discussion about factors contributing to higher persistence. Are patients more readily shifted to a second first line drug, looking for NO DISEASE ACTIVITY, whereas subsequently a partial response is accepted, especially by patients scared by second line treatment risks? Might this change with the availability of new first line oral drugs? Are patients sticking to the safest drug waiting for new treatments not available at the end of the observation period?

The discussion has been modified following comments and suggestions from both reviewers.

**Lines 221 – 233 and 250 - 255:** Factors contributing to higher persistence are described and contrasted with published studies on the subject:

“Treatment persistence varies with country of residence[22]. Jokubaitis et al. compared treatment persistence on GA and IFN in Australian patients and report that, in their cohort, patients receiving IFN β-1a as a first DMD persisted longer on treatment than those treated with GA as a first DMD[22]. Similarly, Kleinman et al. found lower discontinuation rates on IFN β than on GA in the US[23]. On the other hand, data from the REGARD clinical trial do not show significant differences in treatment persistence between IFN β-1a and GA[24]. In the Australian cohort, higher EDSS and younger age at DMD initiation were predictive of treatment discontinuation, however in our cohort, adjusting for age hardly modified the relative risk of GA discontinuation. Another study on DMD use reported female sex as a predictive factor of treatment discontinuation[25], but this was not reproduced in the Australian study or in our cohort. Missing data do not allow us to conclude if EDSS is predictive of treatment discontinuation in our cohort.”

**Lines 254 – 258:** We conclude that the overall risk of GA treatment discontinuation was almost 3 times greater for treatment-naive patients than for IFN-switchers, acknowledge that a previous study addressing persistence shows the same trend, and
provide a possible explanation: The overall risk of GA treatment discontinuation in our cohort was almost 3 times greater for treatment-naive patients than for IFN-switchers. Similarly, Jokubaitis et al. report lower treatment persistence on GA than on IFN only for treatment-naive patients but not when these drugs were used as second therapies[22]. We believe this might be influenced by non-naive patients being used to self-injections.

**Lines 275 – 285:** The time frame of this study and the changing scenario regarding availability of new drugs is discussed in paragraph 9 together with other limitations of the study: “the time frame for this study must be taken into consideration as it might influence treatment persistence. In our cohort, patients who discontinued GA for lack of treatment efficacy were on GA for over two years (29.2 months (±17.5)). Patients underwent regular follow-up visits at least twice per year, so GA might be seen a useful initial treatment even if long-term response was suboptimal in some cases. However, patients might have remained on GA waiting for the arrival of new therapies. Between 2004 and 2013 progress in the treatment of MS was remarkable, with new second-line drugs being licensed. At the same time, MRI criteria for early identification of non-responders were developed and progressively incorporated into clinical practice. With regard to the future, the situation is one of an increasing complexity, as new oral drugs, available as first-line therapies, arrive[20, 29].”

**Lines 289 – 291:** We propose future studies to address questions posed by the reviewer and other questions so as to better understand patients point of view and factors contributing to higher persistence. “A prospective study, with questionnaires specifically addressing subjective treatment perception as well as treatment adherence, in both treatment-naive patients and switchers might shed light on the differential treatment persistence.”

**Lines 294 – 299:** A summary paragraph with conclusions is provided: “In summary, GA was safe and useful with low rates of serious adverse events, similarly to previously published data, and low rates of break-through disease. Injection intolerance proved a major limitation to GA use. We conclude that prior DMD use seems to influence persistence on GA; patients switching from IFN to GA presented a significantly lower probability of GA treatment discontinuation than treatment-naive patients.”

7. Are limitations of the work clearly stated? Not completely (see above): nothing is said about the changing panorama of second line and first line treatment from 2004 to 2013.

**Lines 269 - 284:** We have incorporated a paragraph on study limitations with comments on the changing panorama of MS treatment between 2004 and 2013. Paragraph 9 in the Discussion section now reads
“Limitations to our study include the retrospective design that limits study variables... Regarding clinical applicability of these results, the time frame for this study must be taken into consideration as it might influence treatment persistence. In our cohort, patients who discontinued GA for lack of treatment efficacy were on GA for over two years (29.2 months (±17.5)). Patients underwent regular follow-up visits at least twice per year, so GA might be seen a useful initial treatment even if long-term response was suboptimal in some cases. However, patients might have remained on GA waiting for
the arrival of new therapies. Between 2004 and 2013 progress in the treatment of MS was remarkable, with new second-line drugs being licensed. At the same time, MRI criteria for early identification of non-responders were developed and progressively incorporated into clinical practice. With regard to the future, the situation is one of an increasing complexity, as new oral drugs, available as first-line therapies, arrive[20, 29].”

8. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes, but useful ref.s might be added:

These recent and papers are now referenced and comments are provided as follows:

Lines 202 – 207: “Clinical trials and follow-up studies have shown that GA reduces clinical and radiological disease activity, proves more effective than placebo and shows at least similar efficacy to subcutaneous IFN β-1a and IFN β-1b[12–18].”

Lines 286 – 289: “A recent review comparing efficacy of IFN with GA for RRMS concludes that, even if safety and efficacy parameters of both drugs are similar, patient needs, such as different levels of tolerability, need to be addressed and taken into account for treatment choice[30].”


9. Do the title and abstract accurately convey what has been found? Not completely. It gives an hint on the aim, rather than the results

**Lines 1 – 3:** The title of the article has been changed as suggested: “Differential glatiramer acetate treatment persistence in treatment-naive patients compared to switchers from interferon.”

**C. DISCRETIONARY REVISIONS**

1. The manuscript might incorporate at least the hint that comparison to interferon beta might shed light on the differential persistence in treatment.

**Lines 289- 291:** The following lines have been incorporated and conclude the Discussion section:
“A prospective study, with questionnaires specifically addressing subjective treatment perception as well as treatment adherence, in both treatment-naive patients and switchers might shed light on the differential treatment persistence.”

2. Some speculations about effectiveness or the risk-benefit considerations made when proposing a shift to GA rather than to a second line drug might be added.

In the treatment of MS risk-benefit considerations are indeed essential in daily practice.

**Lines 208 - 216:** We introduce risk-benefit of switching between first-line agents and second-line agents in the third paragraph of the Discussion as follows:
“The current paradigm is to start RRMS treatment with injectable DMDs as a first-line therapy and then advance into the therapeutic pyramid until the disease is effectively controlled. However, first-line treatment failure with IFNs may be due to the existence of neutralizing antibodies[12], poor tolerance or occurrence of adverse events, and may not reflect the need to escalate to second-line therapies such as Fingolimod or Natalizumab. Second-line drugs offer potentially greater efficacy, but are associated with an increased level of risk [3, 20], also costs sometimes need to be taken into account when evaluating treatment options. Thus, changes between first-line agents may be, in some cases, a suitable option.”

**Lines 248 – 253:** We explain that inefficacy is not the only reason for first-line therapy discontinuation: “…our data show a relatively high treatment discontinuation rate due to injection intolerance (12.9%, mainly due to injection pain and mild-moderate skin reactions, including redness, itching, etc.), mainly amongst treatment-naive patients, and this was independent of gender. Along these lines, previous studies indicate that a lack of treatment tolerance is a major reason for DMD discontinuation[22, 27, 28].”
Lines 259 – 265: We discuss on risk-benefit ratios: “Patients who discontinue treatment with IFNs may do so because of side-effects or disease activity. With regard to risk-benefit ratios, MS disease activity of some patients may not justify assuming the potential risks of second-line drugs. This study argues in favour of incorporating GA in the MS therapeutic algorithm not only as an initial agent but as a second therapy for certain patients who discontinue treatment with IFNs. Further investigation regarding reasons for treatment discontinuation and comparisons between treatment-naive patients and switchers is required.”
OTHER MODIFICATIONS TO THE MANUSCRIPT

The Introduction and the Discussion sections have been rewritten following comments from both reviewers.

We clarify the aim of this study and the principal variable throughout the manuscript:

**Lines 28 - 30:** “We aimed to study medication persistence with glatiramer acetate in treatment-naive patients and in interferon-switchers.”

**Line 33:** “Treatment time on glatiramer acetate was analysed.”

**Lines 84 - 87:** “The aim of this pilot exploratory study was to describe GA use and treatment persistence, measured as time to GA treatment discontinuation, in a group of Relapsing Remitting MS (RRMS) treatment-naive patients and in a group of RRMS patients switching from IFNs to GA.”

**Lines 104 – 108:** “Time to treatment discontinuation was studied… Treatment time for each individual patient was calculated in months as the time lapse between GA treatment initiation and the last documented injection. Average treatment times (ATT) were calculated...”

**Lines 313 - 314:** Competing interests have been updated, “After peer review, TEVA was asked and agreed to pay for the article processing charges.”

**Lines 327 – 328:** Acknowledgements have been updated: “We thank the Neurology Department at La Paz University Hospital. We thank Rosario Madero from the Department of Statistics for technical help.”