

1 ***Multiple sclerosis disease-modifying therapies and COVID-19 vaccines: A***
2 ***practical review and meta-analysis: Methods and Supplementary Figures***

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16 **1. Methods**

17 We hereby report our systematic review and meta-analysis study methods in
18 accordance with the Preferred Reporting Items for Systematic Reviews and Meta-
19 Analyses (PRISMA) statement (available from: <http://www.prisma-statement.org>).

20 **1.1. The Search**

21 Based on the objectives of the review, a comprehensive search of the MEDLINE,
22 Scopus, and Web of Science was performed with consideration of their specific
23 vocabulary and indexing approaches. To consider the unpublished data, the medRxiv
24 preprint server and Google Scholar were also searched as secondary sources. The
25 main backbone of the search consisted of, but was not limited to the following keywords:
26 “COVID-19 OR SARS-CoV-2 OR coronavirus”, “vaccine OR vaccination”, “multiple
27 sclerosis OR MS”, and “disease modifying therapies OR disease modifying drugs OR
28 DMT OR DMD”. As the COVID-19 vaccines were available after early 2021, the search
29 was restricted to the studies after January 2021 and was conducted on November 7,
30 2021. Furthermore, a Google Scholar weekly alert was set, enabling us to screen the
31 new results after the initial search date. The last screening of the new results was done
32 in January 27, 2022.

33 After conducting the searches independently, two review authors used the Mendeley
34 application for duplicate removal and screening of the titles and the abstracts of the
35 results one by one. The possibly eligible studies were sought for retrieval of the full texts.
36 Any errata or other linked citations were also retrieved. The reference lists of the
37 possibly eligible studies were also scanned for more possibly eligible studies. All of the
38 search results were archived in a reference manager file format, including a record of
39 the excluded studies along with the reasons for exclusion.

40 **1.2. Eligibility**

41 The exclusion of studies from syntheses was based on the following criteria and
42 priorities:

- 43 1. Not a primary investigation;

- 44 2. Retracted/withdrawn;
45 3. No eligible participants;
46 4. No eligible exposures; and
47 5. No eligible comparators.

48 Nine group of DMTs were considered as eligible exposures and receiving no DMTs –
49 either pwMS receiving no DMTs or healthy participants – was considered as the eligible
50 comparators. For the further syntheses addressing the effects of dosing intervals among
51 pwMS on BCDT, the fifth criterion was ignored. No restriction was set for language or
52 sample size of the studies.

53 The eligibility criteria for the participants were defined as:

- 54 1. No history/evidence of previous COVID-19; and
55 2. No history of corticosteroid administration within two months.

56 Three review authors independently assessed the full texts for eligibility. The papers
57 that at least two review authors consider eligible were included in the study, and others
58 were documented along with their reason for exclusion.

59 **1.3. Assessment of Risk of Bias**

60 The National Institutes of Health Study Quality Assessment Tools were used by three
61 review authors (raters) independently to assess the risk of bias in different levels of the
62 studies. The source of each risk-of-bias judgment of the raters, along with detailed
63 explanations and the final results of each assessment, is presented narratively and
64 summarized in a separate **Supplementary File**.

65 **1.4. Data Extraction**

66 Considering the heterogeneity of the used assays and their measuring units – which
67 was anticipated – in order to present more practical/translational syntheses and
68 facilitate the data extraction and synthesis processes, the data were extracted in a
69 dichotomized fashion based on the seropositivity cut-off indices of the assays used in
70 the studies. Hence, the number of seropositive and total participants were extracted,

71 stratified by DMT exposure status, and with the unexposed (UX) people (healthy
72 controls and/or pwMS on no DMTs) set as the comparator for all other DMTs. When
73 piloting the data extraction process, we noticed that in most studies, the number of
74 participants with negative post-vaccination serostatus would be zero for the UX cohorts
75 and most DMT cohorts, i.e., the “zero” cells will cause computational problems for
76 calculation of the effect measures. Hence, we decided to use the Peto method for
77 estimation of odds ratio (OR) and 95% confidence interval (95%CI), which avoids the
78 addition of a fixed continuity correction factor and has shown to feasibly provide
79 unbiased estimations for relatively balanced cohorts ¹. When neither the specific DMT
80 nor the UX cohorts contained seronegative participants, no relative effect measurement
81 was possible; therefore, those studies were only reported narratively. Additionally, due
82 to the usage of the Peto method, in order to prevent biased estimations, we decided to
83 exclude from quantitative synthesis and present narratively the measures calculated
84 from studies with arms containing less than five total participants and/or considerably
85 uneven arms. It should be pointed that the mentioned studies were not excluded from
86 the review, but only from the quantitative synthesis.

87 Furthermore, for extraction of further measures pertaining to the dosing intervals of
88 infused aCD20, unstandardized beta coefficient (B) along with 95%CI was calculated
89 manually by implementing a univariate logistic regression model on the descriptive
90 measures presented in the study.

91 Another issue identified when piloting the data extraction was that some studies
92 assessed their specified outcomes in multiple time points, including before the first dose,
93 before the second dose, and two to six weeks after the second dose. We only extracted
94 the measures pertaining to the latest timepoints after the second dose, limiting the
95 probability of missing the outcomes that took more time to occur. Data extraction
96 piloting also revealed inconsistent reporting of the effect of baseline CD19/CD20-
97 positive B-cell counts among pwMS on BCDT, preventing us from extracting and
98 synthesizing them.

99 In case the studies used more than one assay for detecting humoral responses to
100 SARS-CoV-2 – e.g., total anti-Spike (S) IgG, anti-S1 subunit IgG assays –including anti-

101 receptor-binding domain (RBD) assays, anti-nucleocapsid (N) IgG, or other
102 immunoglobulins than IgG, results were extracted regarding all assays, separately. The
103 anti-N IgG-positive participants who received mRNA vaccines were excluded from
104 synthesis as it indicated previous COVID-19 contraction.

105 **1.5. Syntheses**

106 After assessing heterogeneity using Cochran's Q and I^2 tests, the extracted results were
107 pooled using the Peto fixed-effects model. The funnel plots were assessed for small
108 study effects and possible publication bias. When the adequate number of studies were
109 available, we adopted an assay-specific fashion in the synthesis, i.e., measures of anti-
110 S1 (including RBD) IgG, anti-S (including trimeric S), interferon-gamma release, CD4+
111 and CD8+ activation-induced marker (AIM), and multiplex polymerase chain reaction
112 (M-PCR) assays were pooled and presented separately; otherwise, the T-cell assays
113 were presented narratively, and the B-cell assays were pooled all together.

114 The results of pooled analyses were presented in an extended forest plot, funnel plots,
115 and all outcomes which could not be entered into the pooled analysis were presented
116 narratively. A meta-regression analysis was planned. However, it was not conducted
117 due to several limitations, including our restrictions in obtaining data from the primary
118 investigators. Hence, the possible reasons behind heterogeneity were only discussed
119 narratively. We also found a few studies assessing the responses to boosters and
120 narratively synthesized them, although this was not planned in our initial protocol.

121 Sensitivity analyses by performing all analyses on the subgroup of outcomes from the
122 studies with "good" quality (least risk of bias) was done but was not presented as it did
123 not show any change in any analysis, except for lower statistical power. A meta-analysis
124 while adjusting the study weights based on the sensitivity of the used assays was
125 initially planned; however, as all the studies used assays with more than 90% sensitivity,
126 it was not conducted. The GRADE approach was used by two review authors to assess
127 the certainty of the evidence. The baseline certainty of evidence was considered as
128 moderate –due to observational nature of the synthesized studies, however, they were
129 upgraded to high in case they were completely supported by non-dichotomized analysis
130 in the individual studies. The judgments and arguments for down- or upgrading using

131 GRADE was presented and justified to ensure transparency. The final assessment of
132 certainty was presented in Table 2 (summary of findings), along with the effect
133 measures and CI of the outcomes.

134 **1.6. Software**

135 The Mendeley Desktop software version 1.19.8 for MacOS (Mendeley Ltd.) was used
136 for management and screening of the studies. The Review Manager (RevMan) software
137 version 5.4.1 for MacOS (The Cochrane Collaboration) was used for data extraction and
138 synthesis. The SPSS software version 23 for MacOS (IBM Inc.) was used for the
139 manual analyses.

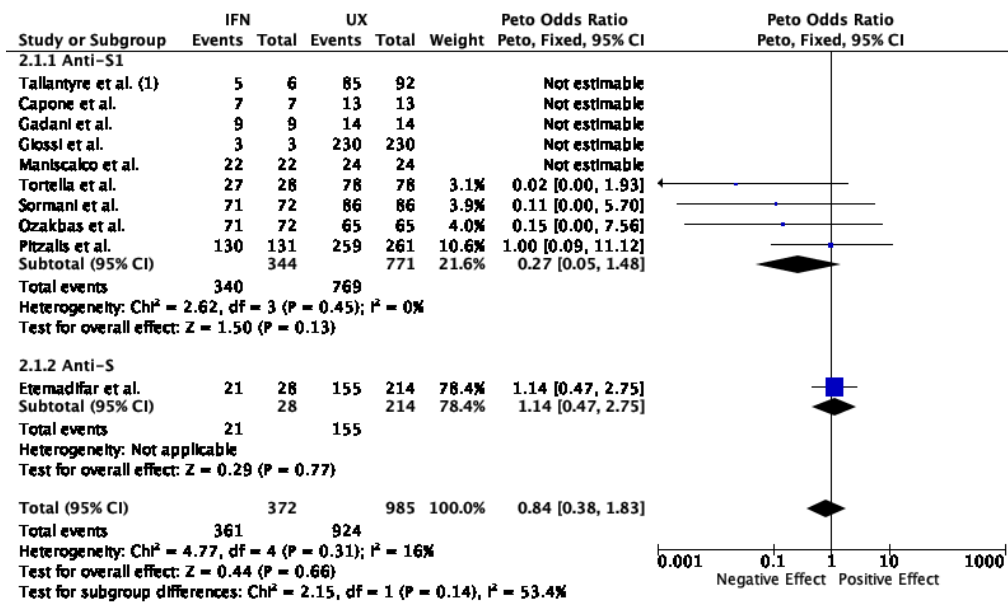
140 **1.7. Registration and Approval**

141 This study was registered in PROSPERO before initiation (id: CRD42021278107). No
142 Institutional Review Board / ethics committee approvals were required for this study
143 according to the national guidelines, as it did not involve human subjects.

144 **1.8. Availability**

145 All of the used materials for this review are available upon reasonable request from the
146 corresponding review author.

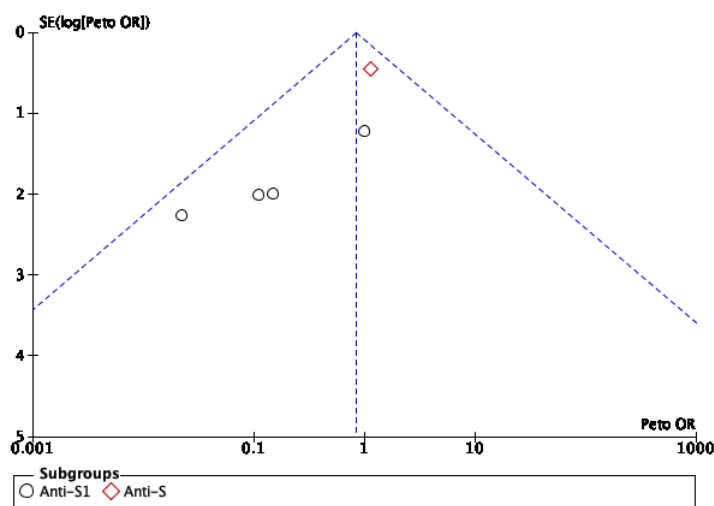
147 **2. Supplementary Figures**



Footnotes

(1) Excluded due to uneven arms.

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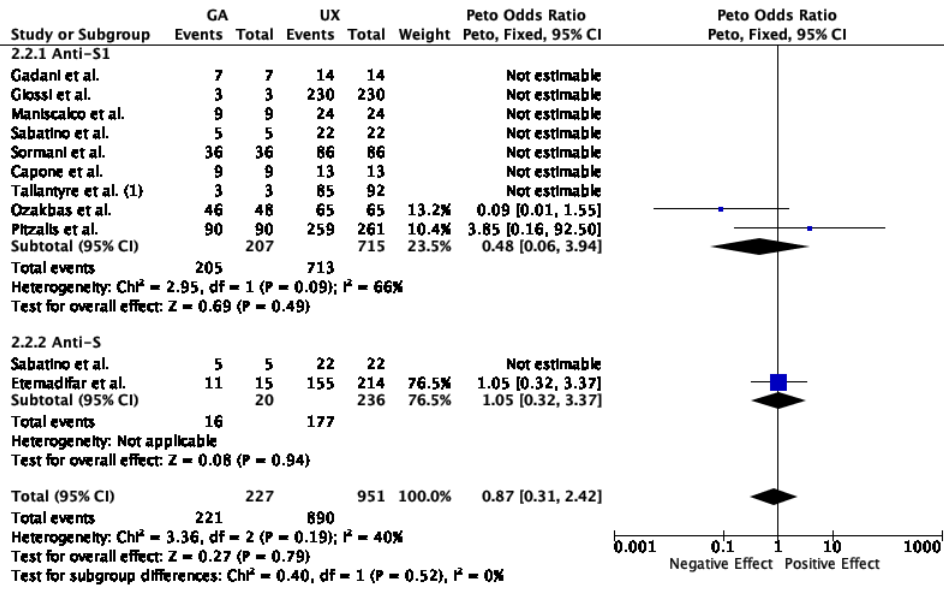
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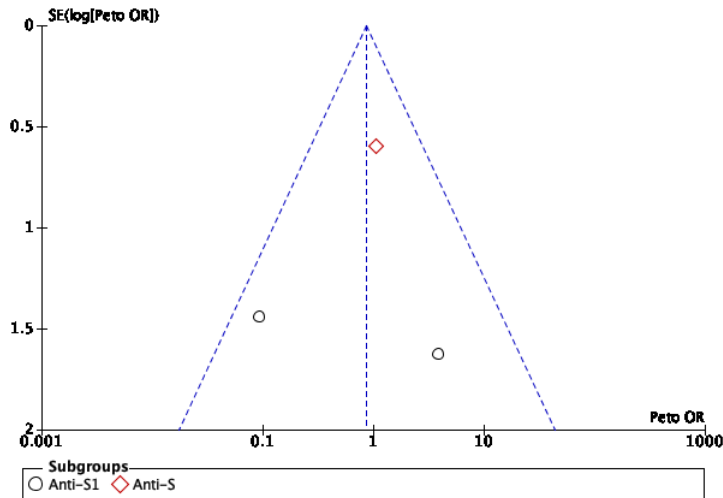
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Supplementary Figure 1; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring humoral response in pwMS on IFN



Footnotes
(1) Excluded as an arm contains less than five participants and the arms are uneven.



Supplementary Figure 2; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring humoral response in pwMS on GA

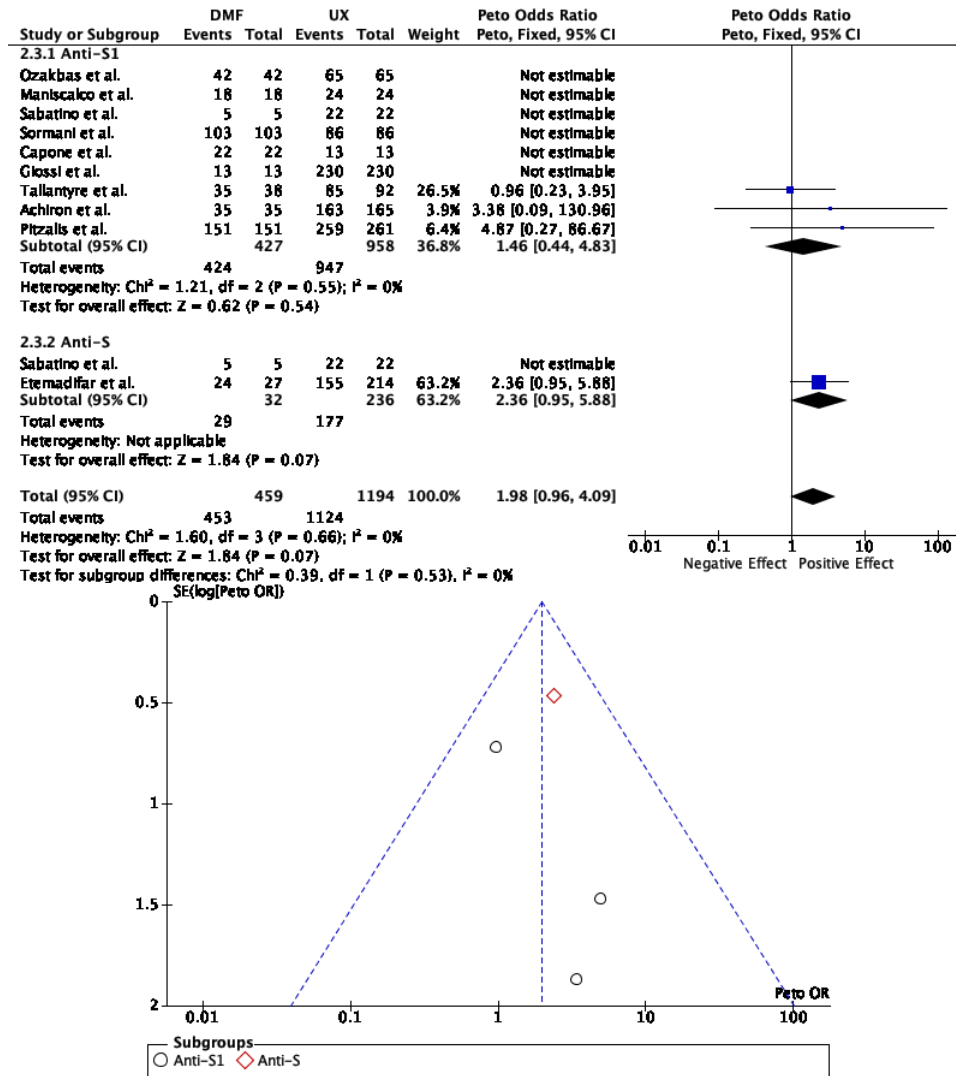
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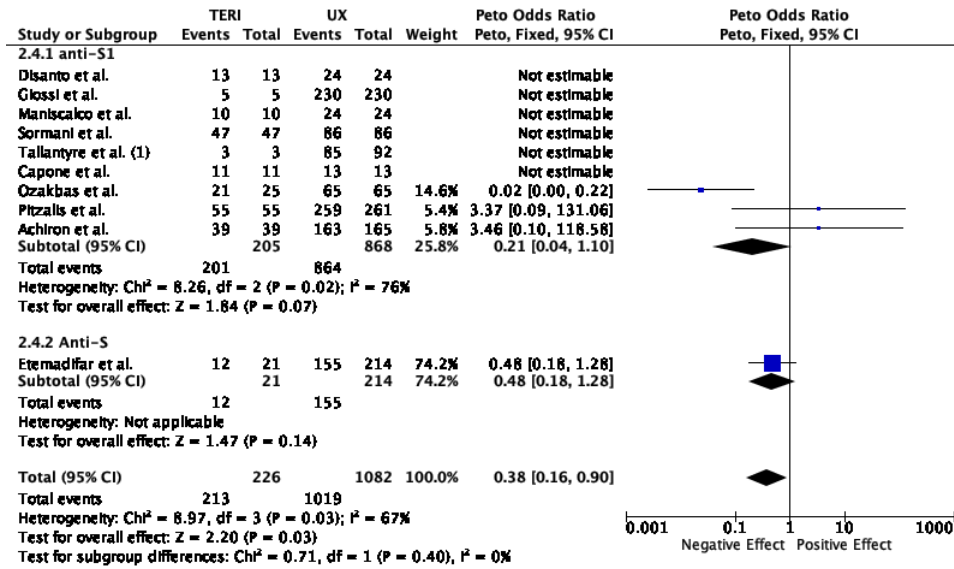
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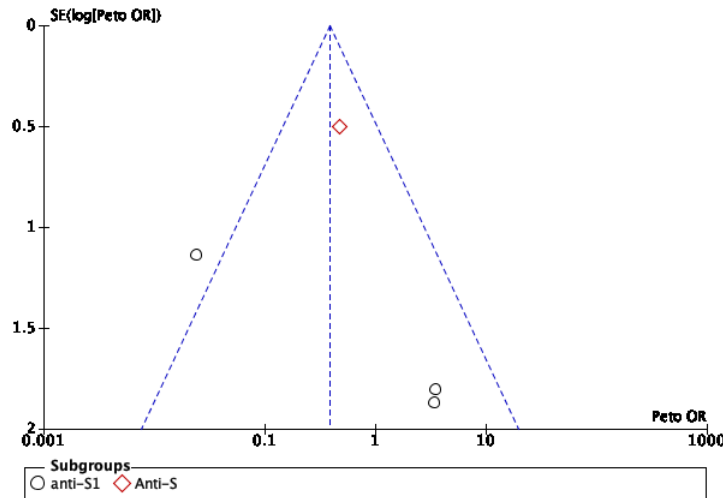
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Supplementary Figure 3; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring humoral response in pwMS on DMF



(1) Excluded as an arm contains less than 5 participants and the arms are uneven.



Supplementary Figure 4; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring humoral response in pwMS on TERI

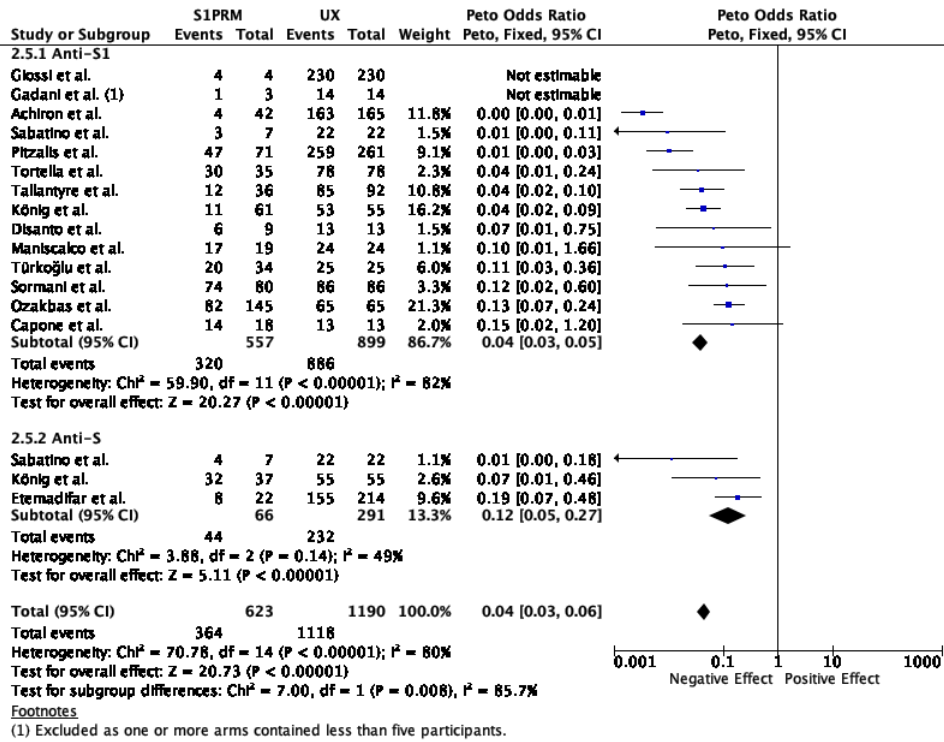
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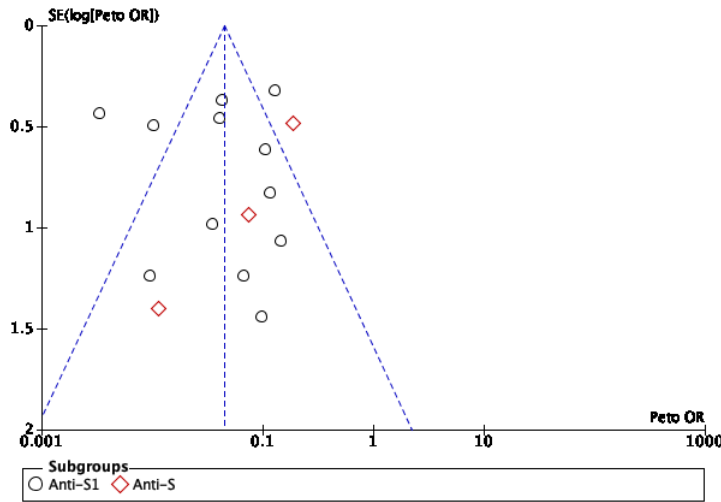
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Footnotes

(1) Excluded as one or more arms contained less than five participants.

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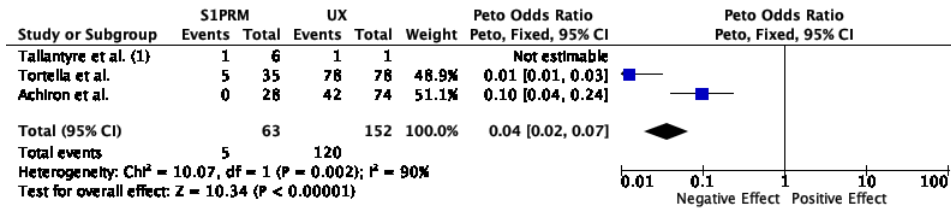
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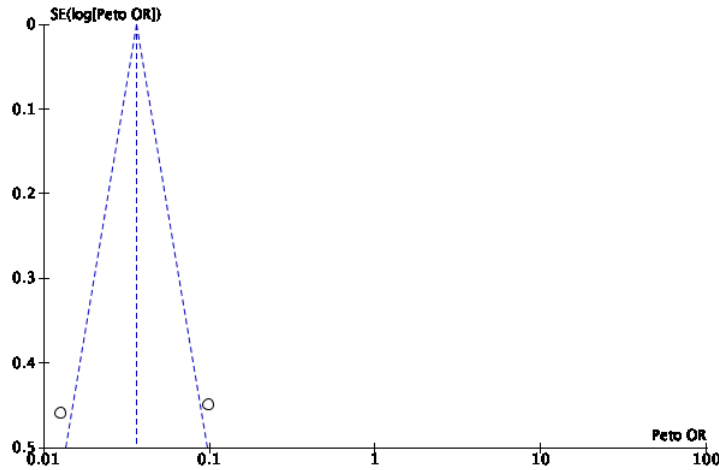
Supplementary Figure 5; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring humoral response in pwMS on S1PRM



Footnotes

(1) Excluded as one or more arms contain less than five participants.

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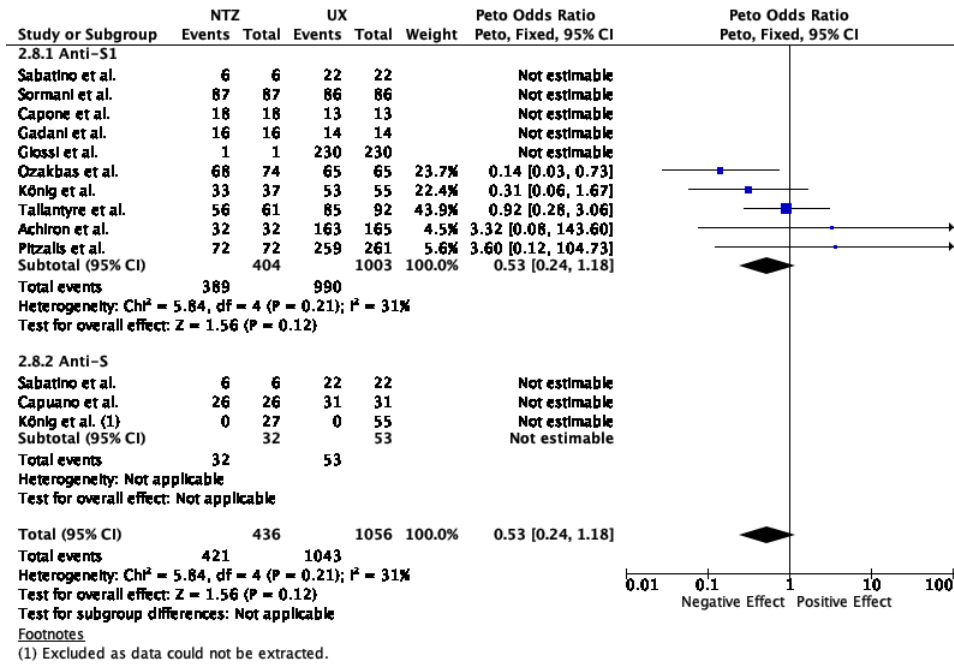
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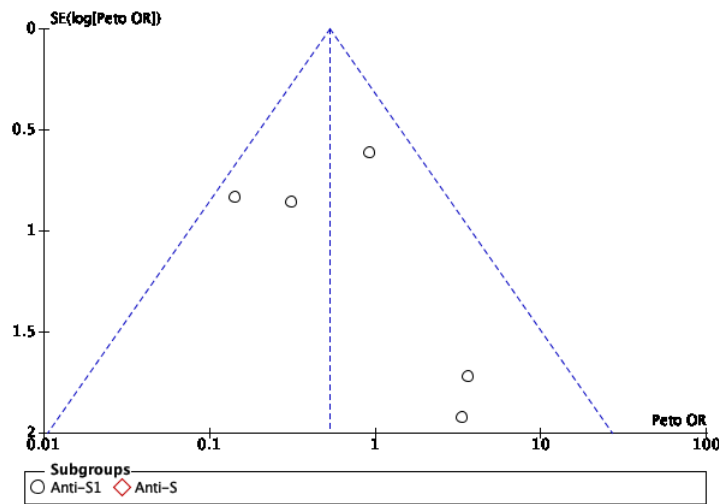
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Supplementary Figure 6; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring interferon-gamma release response in pwMS on S1PRM



Footnotes
 (1) Excluded as data could not be extracted.



Supplementary Figure 7; Results of individual studies, heterogenicity tests, forest and funnel plots of studies measuring interferon-gamma release response in pwMS on NTZ

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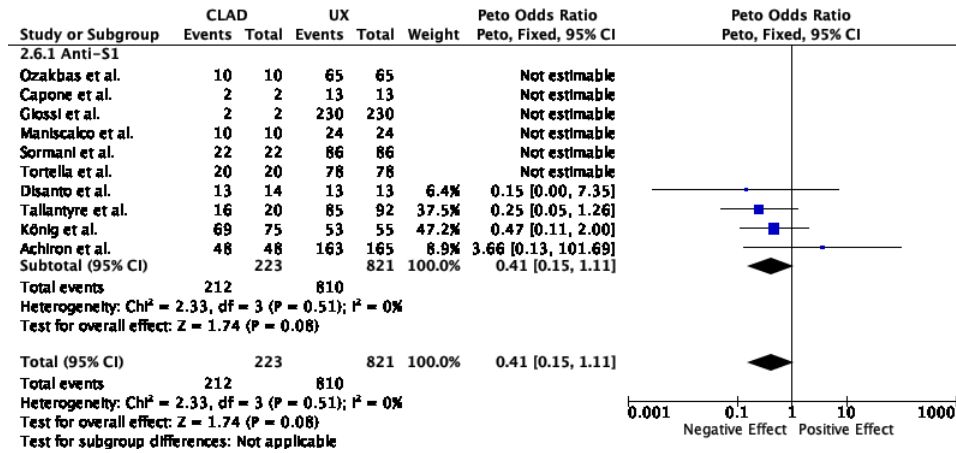
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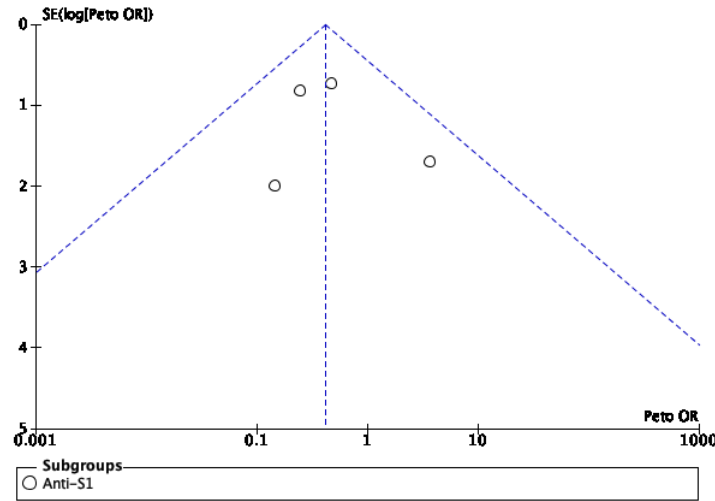
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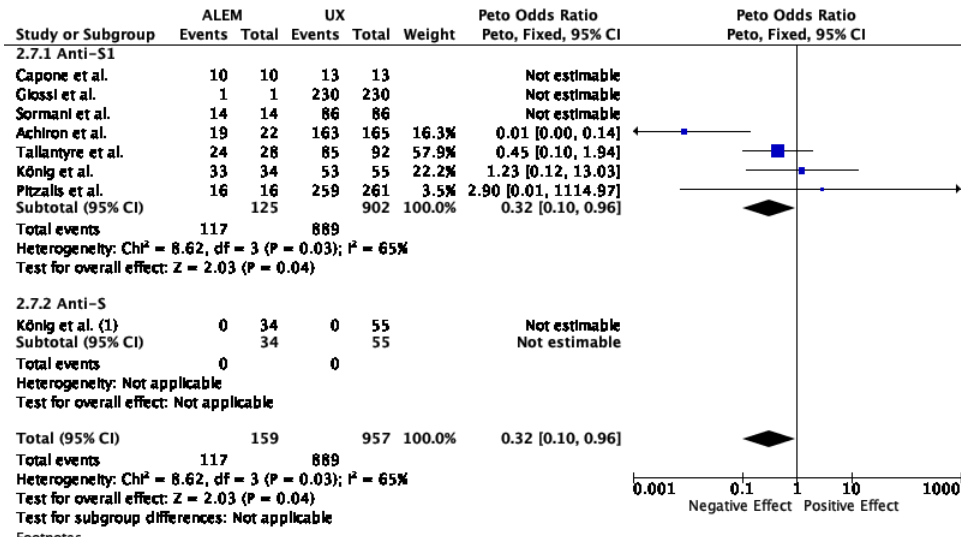
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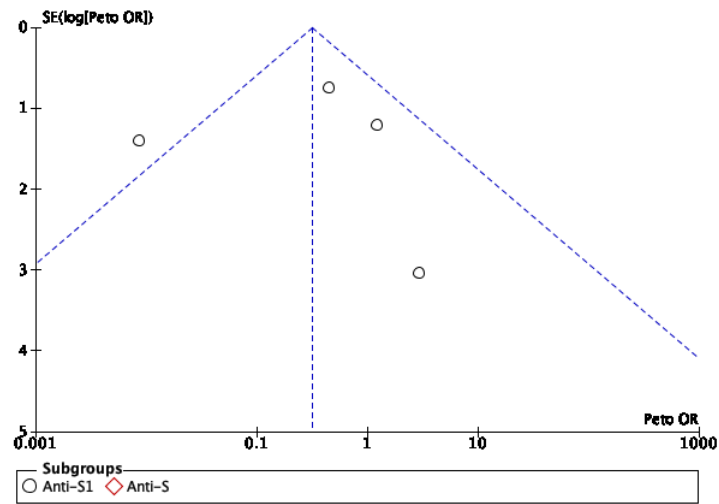
Supplementary Figure 8; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring humoral response in pwMS on CLAD

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Footnotes
(1) Excluded as no data could be extracted.

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Supplementary Figure 9; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring humoral response in pwMS on ALEM

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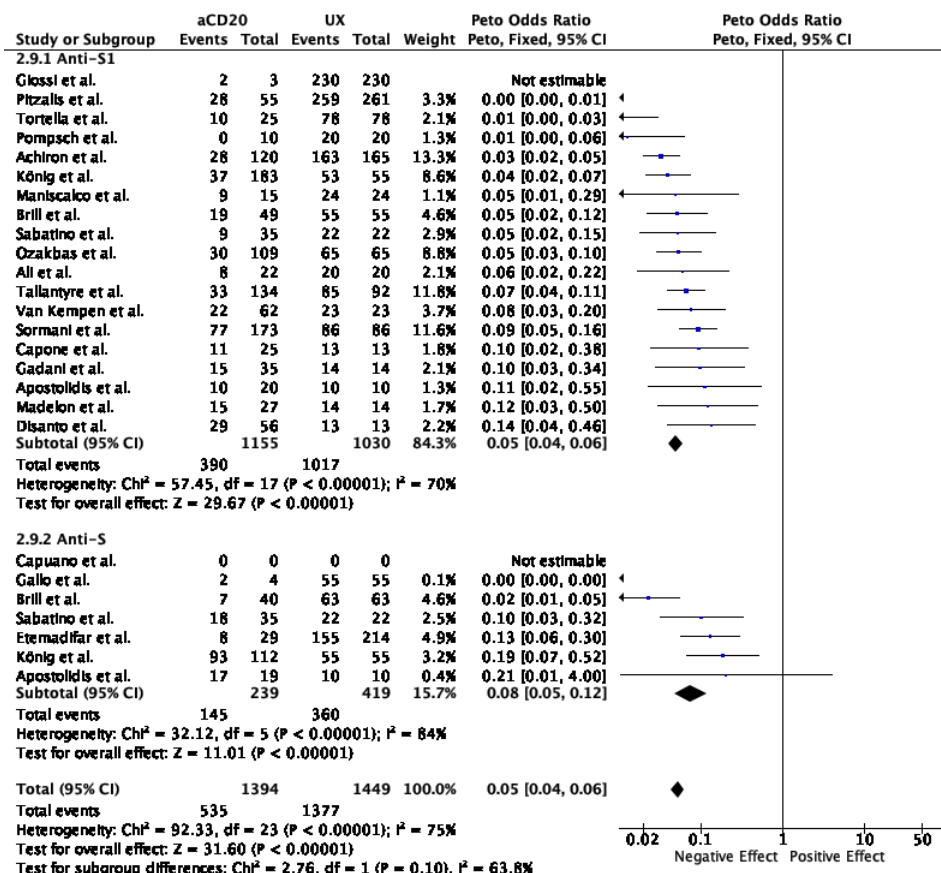
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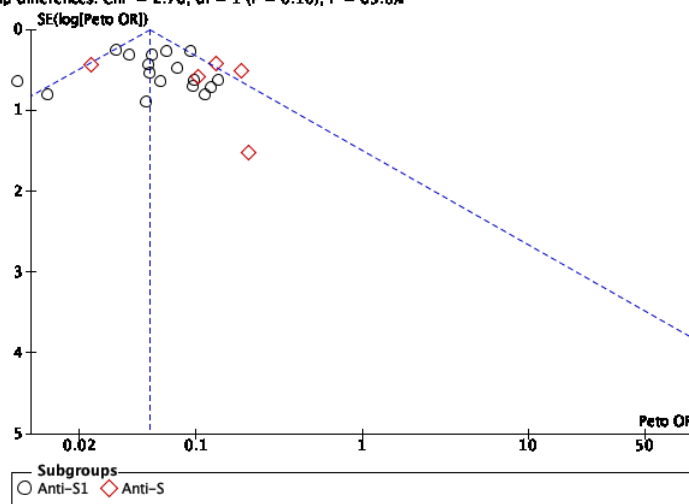
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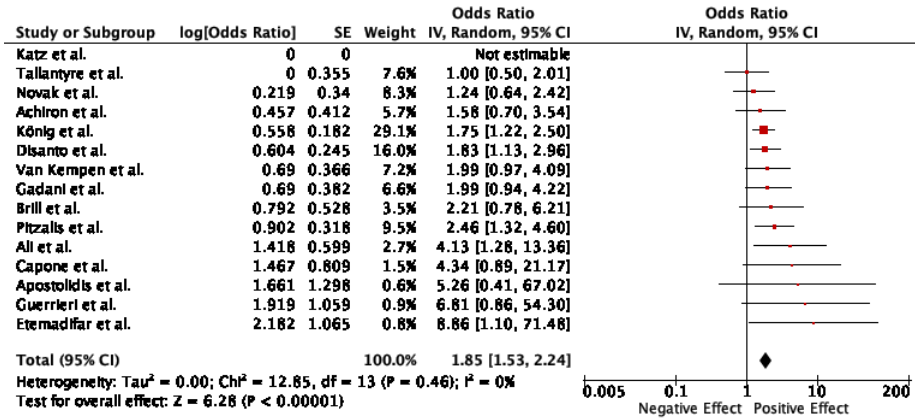
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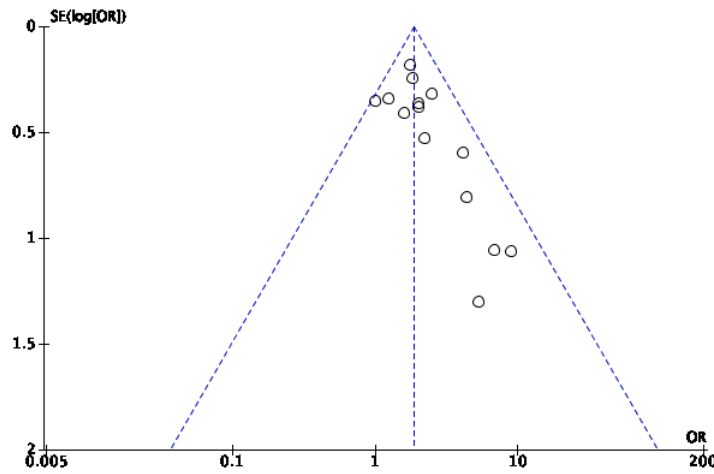
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Supplementary Figure 10; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring humoral response in pwMS on aCD20



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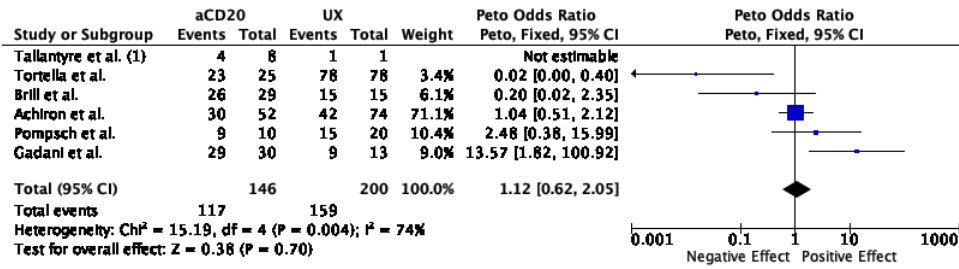
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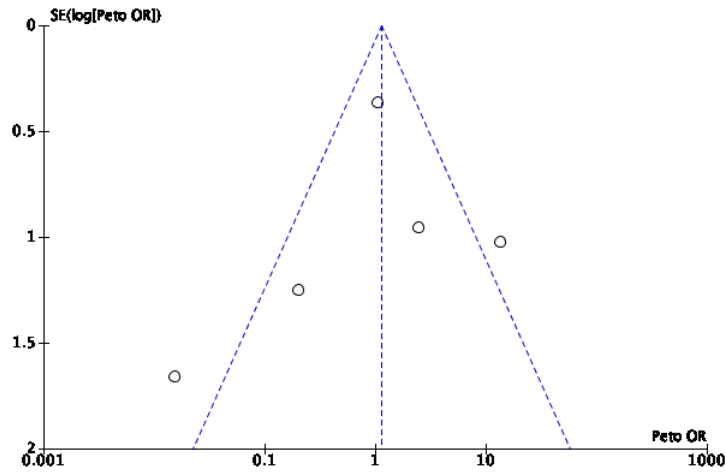
Supplementary Figure 11; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring the effect of aCD20-to-vaccine period on humoral response



Footnotes

(1) Excluded as an arm contains less than five participants

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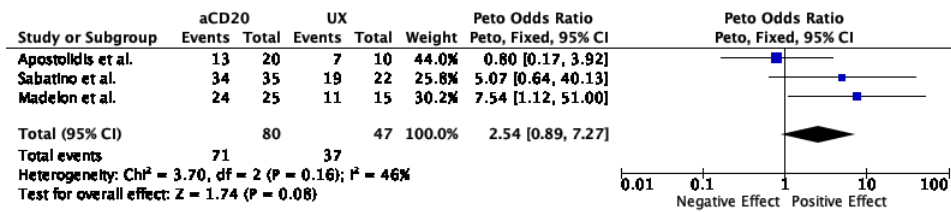
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Supplementary Figure 12; Results of individual studies, heterogeneity tests, forest and funnel plots of studies measuring interferon-gamma release response in pwMS on aCD20

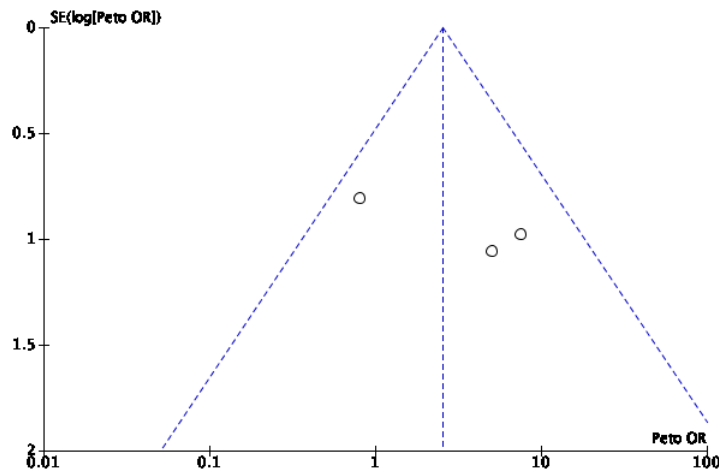
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218 *Supplementary Figure 13; Results of individual studies, heterogeneity tests, forest and funnel plots of studies*
 219 *measuring CD8+ AIM response in pwMS on aCD20*

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References

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