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RESEARCH LETTER

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Association between treatment effect on C-peptide preservation and HbA1c in meta-analysis of glutamic acid decarboxylase (GAD)-alum immunotherapy in recent-onset type 1 diabetes

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1 | BACKGROUND

Preservation of insulin secretion in type 1 diabetes (T1D) lowers the risk of complications such as retinopathy and severe hypoglycaemia.[1-3] C-peptide preservation—a biomarker for endogenous insulin secretion—is used as a surrogate endpoint in clinical trials assessing beta cell-preserving therapies. Showing efficacy on clinical outcomes such as severe hypoglycaemia has proven challenging given their rarity shortly after diagnosis. Here, we show that treatment effect on C-peptide correlates with treatment effect on HbA1c, a validated surrogate endpoint, in individuals treated with recombinant human glutamic acid decarboxylase 65 kDa in alum (GAD-alum).

A retrospective post hoc meta-analysis and prospective phase IIb trial, where the human leukocyte antigen (HLA)-specific analyses of the topline results were specified before database lock, have shown the efficacy of GAD-alum for stimulated C-peptide preservation in the genetic subpopulation of T1D patients carrying HLA DR3-DQ2.[4,5] Efficacy for reducing HbA1c and added benefit of more doses have also been observed in the HLA DR3-DQ2–carrying probable responder population. A phase III trial in recent-onset T1D patients carrying HLA DR3-DQ2 (DIAGNODE-3, NCT05018585) with the co-primary endpoints of C-peptide and HbA1c is ongoing.

Our research objective was to assess whether 15-month treatment effects on preservation of endogenous insulin production were correlated with treatment effects on blood glucose measured by HbA1c.

2 | METHODS

We carried out individual person meta-analysis of four phase II-III randomized controlled trials of subcutaneous or intralymphatic GAD-alum versus placebo in recent-onset T1D ($n = 627$).

All four studies were randomized placebo-controlled trials in recent-onset T1D patients receiving standard-of-care diabetes treatment including insulin replacement therapy. Study NCT00435981[6] was a multisite phase II clinical trial of subcutaneous GAD-alum including 70 patients in Sweden. Study NCT00529399[7] was a phase II clinical trial of subcutaneous GAD-alum conducted by Trialnet, which included 145 patients at 15 sites in the United States. Study

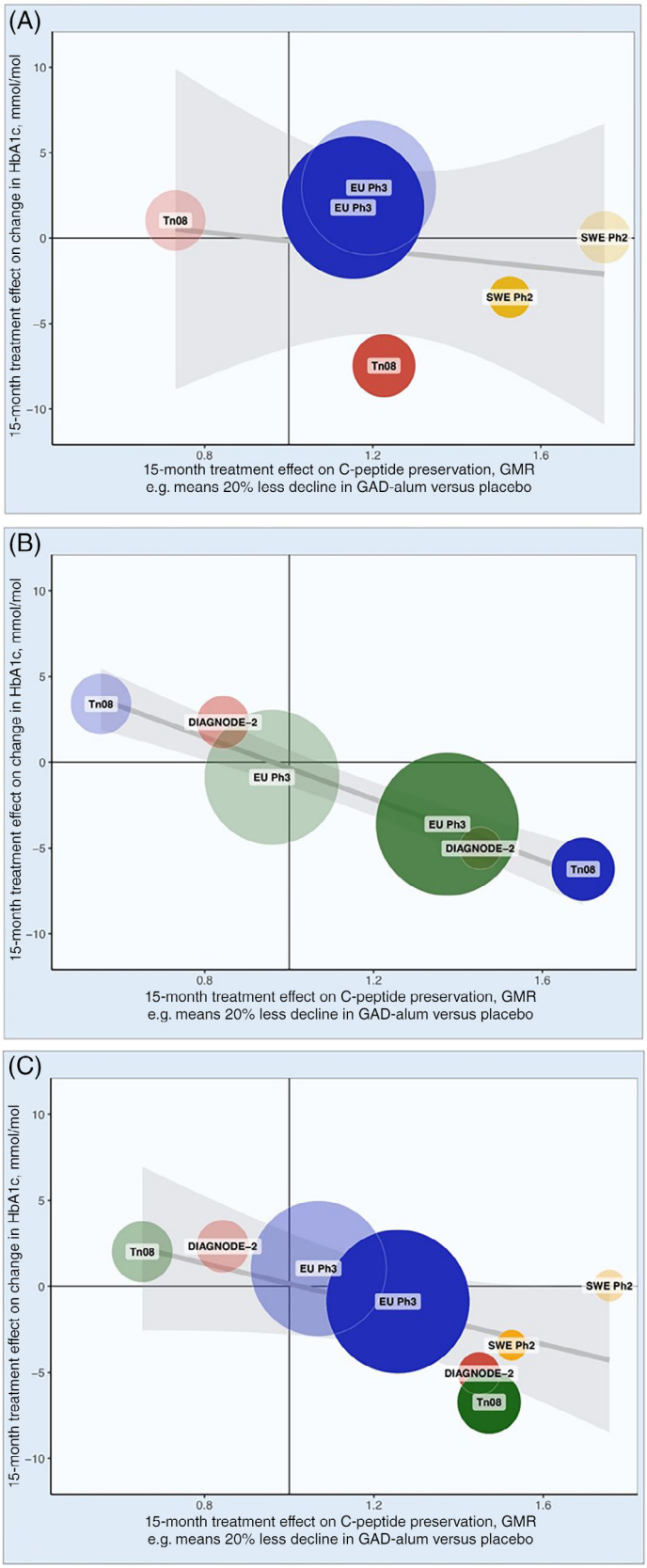

FIGURE 1 Scatter plots illustrating the association between treatment effect (change from baseline to month 15 compared with placebo) on C-peptide on the x-axis and HbA1c on the y-axis. The panels depict effect correlations for A, Two doses, B, Three to four doses, or C, Two to four doses. Values larger than 1 on the x-axis, and lower than 0 on the y-axis, indicate a beneficial treatment effect of GAD-alum compared with placebo. Circles represent randomized controlled trials with diameters proportional to sample size. Each trial was split into the probable responder population (present HLA DR3-DQ2, opaque circles) and non-responder population (absent HLA DR3-DQ2, transparent circles). The grey line represents the linear regression line and 95% confidence interval. Study labels: SWE Ph2—NCT00435981, Tn08—NCT00529399, EU Ph3—NCT00723411, DIAGNODE-2—NCT03345004. GAD, glutamic acid decarboxylase; GMR, geometric mean ratio

NCT00723411[8] was a phase III clinical trial of subcutaneous GAD-alum including 334 patients at sites in Finland, France, Germany, Italy, The Netherlands, Slovenia, Spain, Sweden and the UK. Study NCT03345004 (DIAGNODE-2)[4] was a phase IIb clinical trial of intralymphatic GAD-alum that included 109 patients from multiple sites in the Czech Republic, The Netherlands, Spain and Sweden.

For analysis, patients were classified according to HLA DR3-DQ2 presence, and as having received two injections or three to four injections of either GAD-alum or placebo. A covariate term for study was included in the analysis to account for minor differences in design. Stimulated C-peptide was assessed using the mixed meal tolerance test. Mean C-peptide area under the curve was calculated by the trapezoid rule and natural log-transformed. HbA1c was analysed at a central laboratory and not transformed. Mean change from baseline was analysed using a restricted maximum likelihood-based mixed model for repeated measures (MMRM) adjusted for the fixed effects of baseline C-peptide (or baseline HbA1c), study, treatment, HLA subgroup, visit, country, sex and age, as well as the interaction of baseline C-peptide (or HbA1c) by visit and treatment by HLA subgroup by visit. Patient identification number and country were included as categorical random effects. The primary comparison was the contrast between treatments at month 15 for active treatment versus placebo. The change from baseline to month 15 was chosen as 15 months was the longest follow-up available in all studies. Further details on the methods and the included studies are presented in the Appendix.

3 | RESULTS

Figure 1 shows trial-level scatterplots of the relationship between treatment effects of GAD-alum versus placebo on C-peptide (x-axis) and HbA1c (y-axis). For each trial, effects were estimated by MMRM analysis in probable responders (DR3-DQ2–positive) and non-responders (DR3-DQ2–negative), depicted in opaque and transparent circles, respectively, with diameters proportional to sample size. An association between effects on C-peptide and HbA1c is apparent for participants who had received three to four doses (B), while no association is apparent for two doses (A). The combined analysis of two to four doses (C) reflects the association driven by the three-to-four-doses group. The treatment effect of GAD-alum was foremost significant in DR3-DQ2 patients receiving three to four rather than two doses.[5] C-peptide preservation of 40% from three to four doses of GAD-alum corresponde to 3 mmol/mol lower HbA1c (regarding change from baseline compared with placebo) (Figure 1B). While the relationship between treatment benefit for C-peptide and HbA1c is apparent across trials and HLA groups, Figure 1 reinforces the previous findings of treatment benefit in those participants with HLA DR3-DQ2, while participants lacking DR3-DQ2 do not appear to benefit from GAD-alum.[4,5]

Several sensitivity meta-analyses were conducted and are reported in full in the Appendix. One analysis limited the age and country range of the four clinical trials in the meta-analysis to correspond to the planned confirmatory phase III trial (Figure 1). A second analysis used an alternative C-peptide treatment effect estimator (the quantitative response metric) (Figures A2 and A3).[9] A third sensitivity analysis additionally adjusted for insulin dose (Figure A4). These sensitivity analyses confirmed the associations and the treatment benefits seen in HLA DR3-DQ2–positive individuals who had received three or four injections.

We repeated the main analysis substituting HbA1c for the secondary endpoints of insulin dose-adjusted HbA1c (IDAA1c) and insulin dose to assess whether a similar association between treatment effects on C-peptide and these endpoints might exist. In both cases, there was a similar trend for an association between beneficial effects on C-peptide being associated with beneficial effects on lower IDAA1c and lower insulin dose, respectively, in HLA DR3-DQ2 individuals, and particularly in those who had received three or four doses (Figures A5 and A6).

4 | DISCUSSION

The correlation between 15-month effects on C-peptide and HbA1c suggests that therapeutically preserved C-peptide in recent-onset T1D might improve glycaemic control, probably at least for antigen-specific immunotherapies, as immunomodulatory drugs with consistent C-peptide effects have not shown convincing effects on HbA1c.[10-12] Whether this discrepancy is a result of disease heterogeneity requiring subgroup-targeted approaches, as for GAD-alum, or because HbA1c is affected by complex factors, remains open.

Continuous glucose measurement-derived variables, such as time in the glycaemic target range, are not accepted as primary endpoints by regulatory authorities, and serious clinical outcomes such as severe hypoglycaemia are (fortunately) rare in individuals with recently diagnosed T1D receiving standard-of-care treatment. This poses challenges for clinical trials with regard to the required sample size and follow-up to show efficacy. Therefore, many T1D trials assess a surrogate primary endpoint such as C-peptide, which is assumed to predict effects on clinical outcomes. Currently, neither the US Food and Drug Administration nor the European Medicines Agency accept C-peptide preservation as a single primary endpoint. Our findings provide important new evidence supporting the use of C-peptide as a surrogate endpoint in clinical trials in T1D. This is particularly poignant given current efforts by international consortia (such as the Critical Path Institute's Trial Outcome Markers Initiative, TOMI-T1D; https://c-path.org/programs/tomi-t1d/overview/tomi-t1d-team/) and key opinion leaders to convince the regulatory agencies to accept C-peptide as a surrogate primary endpoint. Both agencies have, amongst others, mentioned two aspects that have yet to be satisfactorily addressed: to show a quantitative relationship between the amount of preserved C-peptide and a clinical outcome or validated surrogate; and to show that therapeutic C-peptide preservation achieves clinical benefits (even although there is evidence based on naturally preserved C-peptide).[1-3] We believe that our novel findings address both aspects in that we show correlated benefits of therapeutically preserved C-peptide on the validated surrogate endpoint HbA1c. While it is not possible to say if there is a direct causal relationship between preserved C-peptide and lowered HbA1c (compared with placebo-treated patients) among individuals who received three to four injections of GAD-alum, our findings certainly suggest benefits of therapeutically preserved C-peptide on blood glucose control within about 2 years of diagnosis.

The current analysis has certain limitations. It is a post hoc exploratory analysis whose results are considered hypothesis-generating, not confirmative. No formal statistical significance testing was carried out and the observed associations should be considered preliminary until confirmation in a well-powered prospective trial. The association between the treatment effect has not been reported for other investigational treatments, such as for other antigen-specific therapies or immunosuppressive treatments. The findings may be specific to the antigen-specific treatment used in the trials, and while all available randomized controlled trials of GAD-alum with sufficient data have been included in the analysis, the findings still need to be independently validated in a separate dataset. The current meta-analysis of previously published clinical trials of rhGAD65 also provides a concise summary of efficacy results of this treatment modality.

Overall, we show that preservation of C-peptide using GAD-alum correlates with effects on HbA1c in individuals with recent-onset T1D carrying HLA DR3-DQ2. These findings will be evaluated in a confirmatory phase III trial (DIAGNODE-3) and support using C-peptide as a clinically relevant surrogate endpoint for endogenous insulin production-preserving therapies.

AUTHOR CONTRIBUTIONS

CN initiated, and CN, UH and JL planned and developed the work. CN carried out the analyses and wrote the first draft, and CN, UH and JL critically revised the manuscript. CN, UH and JL have verified the underlying data and all authors have had full access to the data.

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CONFLICT OF INTEREST

UH and CN are employed by Diamyd Medical and own stock in the company. JL has received unrestricted grants from Diamyd Medical, was earlier a member of Provention Bio, Inc.'s Advisory Council and is a present member of Dompé Farmaceutici S.p.A.'s International Advisory Board.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data are not available by open access since participants did not consent to making their data publicly available in that way. Data underlying the current manuscript will be made available after reasonable request via a data transfer agreement to those interested in collaborations on further research. Such requests should be addressed to the corresponding author.

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APPENDIX A: SENSITIVITY ANALYSIS 1

A.1 | Limited age and country range

A phase III clinical trial of GAD-alum in the genetically defined subpopulation of recent-onset T1D patients with HLA DR3-DQ2 is ongoing (DIAGNODE-3, EudraCT 2021-002731-32, NCT0501 8585; principal investigator is the senior author of the current manuscript, Professor Johnny Ludvigsson). DIAGNODE-3 is an international, multi-centre, randomized controlled clinical trial aiming to enroll 330 recent-onset T1D patients with GAD65 antibodies and the genetic HLA DR3-DQ2 haplotype who will be randomised in a 2:1 ratio to three monthly intralymphatic injections of 4 micrograms of Diamyd or placebo, and be followed for 24 months. The co-primary endpoint is the change from baseline to Month 24 in C-peptide and HbA1c. In order to assess whether the observed correlation between treatment effects on C-peptide and HbA1c holds in a population that is more similar to the target population in DIAGNODE-3, we repeated the meta-analysis as explained in the Methods section below but limited the age range to between ≥12 and < 29 years and included only countries that are currently (first half of 2022) planned to participate in the DIAGNODE-3 trial. Figure A1 below shows a summary of the changes.

The revised dataset included 405 individuals of whom 196 carried HLA DR3-DQ2. Figure 1 below shows the results from MMRM analysis. The pattern is essentially the same as in the main analysis—or be it with somewhat more variation (the overall sample size is reduced by one third). As in the main analysis, the correlation between the treatment effects is strongest in those individuals who received 3–4 injections (panel b) who are positive for HLA DR3-DQ2 (opaque circles). There is no apparent association for those treatment groups who received two injections (panel a), or who lack HLA DR3-DQ2 (transparent circles).

APPENDIX B: SENSITIVITY ANALYSIS 2

B.1 | Using the Quantitative Response (QR) metric to quantify C-peptide treatment effect

In order to assess the robustness of the findings regardless of analytical model, we repeated the analysis of the C-peptide effect using a modified version of the QR metric suggested by Krischer and Bundy (Endocrinol Diabetes Metab 2020, PMID 32704564). In brief, the original QR metric quantifies the treatment effect on C-peptide preservation as the difference between the observed C-peptide and the

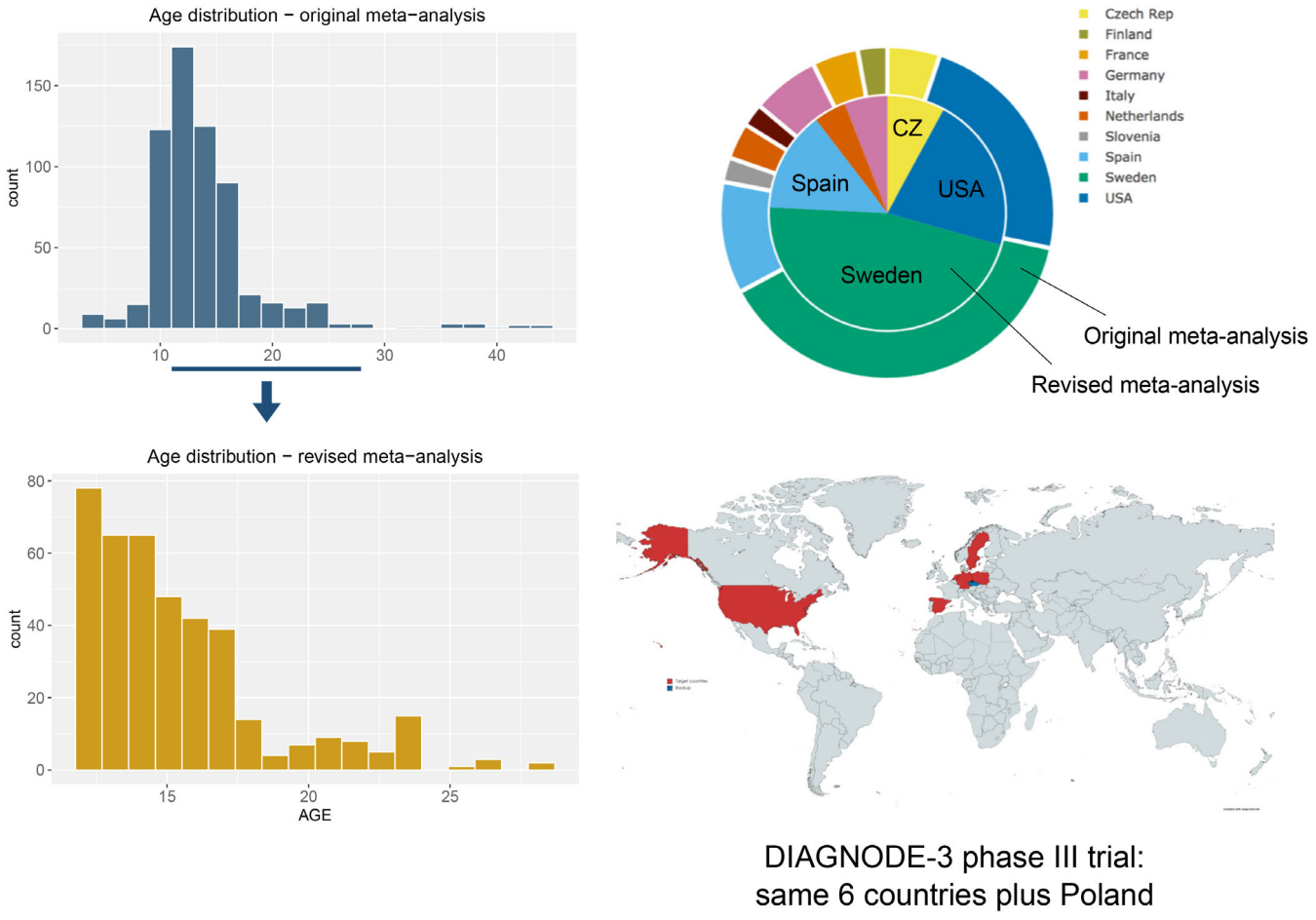
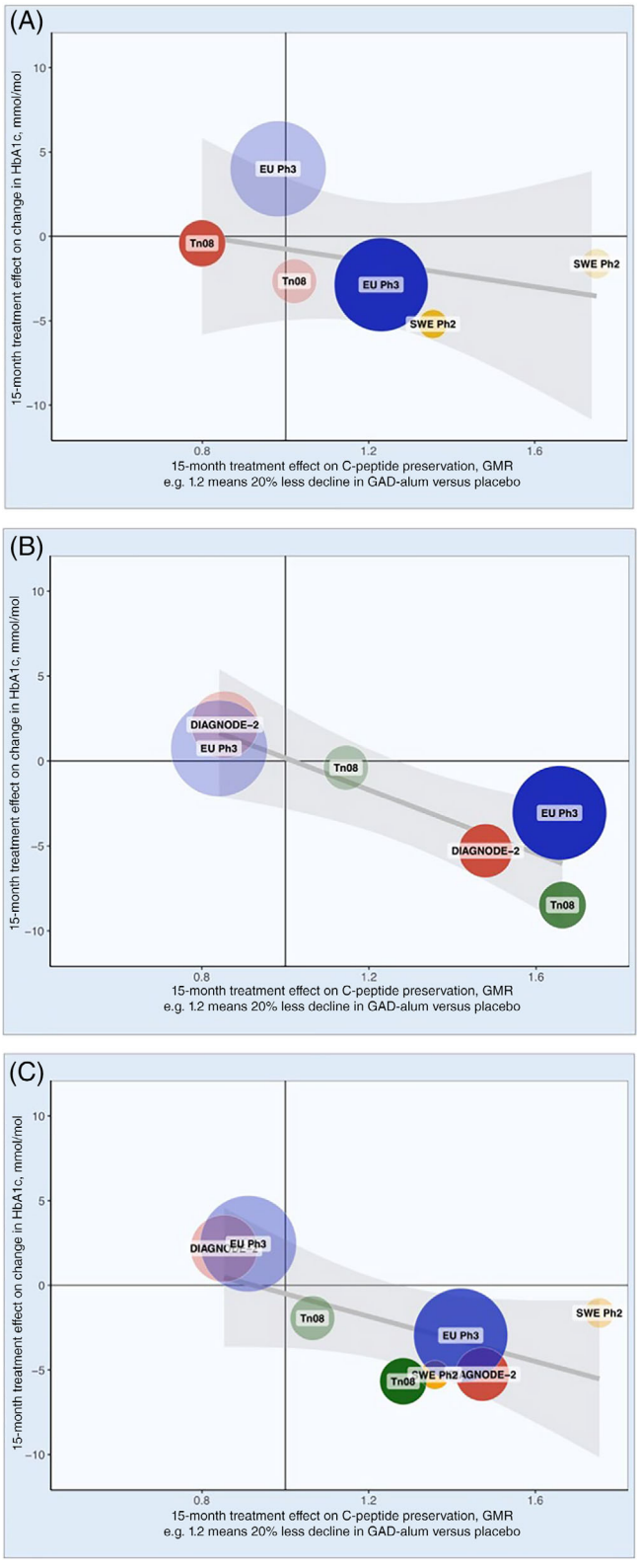


FIGURE A1 Overview of the revised meta-analysis dataset

predicted C-peptide after 12 months in recent-onset T1D patients. To predict 12-month C-peptide decline in the absence of a disease-modifying treatment, the authors built an ANCOVA model incorporating baseline C-peptide and age using placebo groups from Trialnet studies. In a first step, we modified the QR metric in that we recalibrated the ANCOVA model to predict 15-month C-peptide levels using individuals assigned to the placebo groups in three of the studies in the meta-analysis (NCT00435981, NCT00529399, NCT00723411). We then used the fourth study in the meta-analysis (NCT03345004) as a validation sample to assess how well the modified 15-month QR metric predicted 15-month C-peptide in subjects randomized to placebo. We found good agreement between predicted and observed values with an R-squared of 51.5% (Figure A2).

In a second step, we used the modified QR metric to estimate the 15-month treatment effect on C-peptide preservation in all individuals who had received active treatment in the four studies. Mean QR metrics per study and HLA group were calculated and used to replace the MMRM-estimated effects in the scatterplots. Since the QR metric's logic is based on the natural history of new-onset T1D with its steep decline in C-peptide in the first year, no equivalent alternative measure is available for HbA1c. Hence, the same MMRM-based estimates of treatment effects on HbA1c as before were used for plotting. Figure A3 illustrates the scatterplots with the modified QR-estimated C-peptide effect on the *x*-axis.



APPENDIX C: SENSITIVITY ANALYSIS 3

C.1 | Adjustment for insulin dose

Additional insulin dose adjustment of the main analysis presented in the main text and in Figure 1 of the manuscript had little effect on the results suggesting correlated treatment effects in HLA DR3-DQ2 individuals driven by those who had received the larger number of doses (3 or 4).

C.2 | Association between treatment effect on C-peptide and IDAA1c/insulin dose

In order to assess whether a similar association between treatment effects on C-peptide and HbA1c might exist for the secondary endpoints of insulin dose-adjusted HbA1c (IDAA1c) and insulin dose, we repeated the main analysis substituting HbA1c for these two endpoints. In both cases, there was a similar trend for an association between beneficial effects on C-peptide being associated with beneficial effects in lower IDAA1c and

FIGURE A2 Scatter plots illustrating the association between treatment effect (change from baseline to Month 15 compared to placebo) on C-peptide on the x-axis and HbA1c on the y-axis. Panels (a) 2 injections, (b) 3-4 injections, (c) 2-4 injections. Values larger than 1 on the x-axis, and lower than 0 on the y-axis, indicate a beneficial treatment effect of GAD-alum compared to placebo. Circles represent randomized controlled trials with diameters proportional to sample size. Each trial was split into the responder population (present HLA DR3-DQ2, opaque circles) and non-responder population (absent HLA DR3-DQ2, transparent circles). The grey line represents the linear regression line and 95% confidence interval. Study labels: SWE Ph2 - NCT00435981, Tn08 - NCT00529399, EU Ph3 - NCT00723411, DIAGNODE-2 - NCT03345004

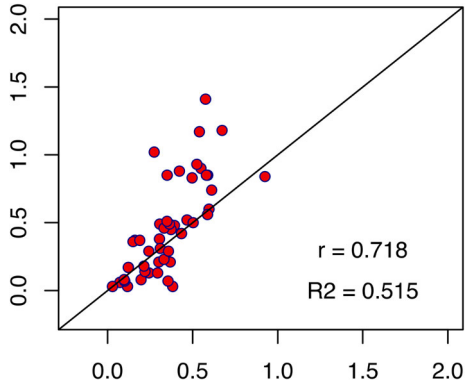


FIGURE A3 Scatterplot illustrating good agreement between observed C-peptide (*y*-axis) and modified QR-predicted C-peptide at Month 15 in study participants with recent-onset T1D assigned to placebo treatment in the DIAGNODE-2 trial

lower insulin dose, respectively, in HLA DR3-DQ2 individuals, and particularly in those who had received 3 or 4 doses.

C.3 | Methods

The primary objective of this post-hoc explorative meta-analysis was to assess the correlation between the estimated treatment effect (as the change from baseline to Month 15 compared to placebo) on C-peptide and HbA1c using individual-level data from four randomized controlled trials of either subcutaneous (3 studies) or intralymphatic (1 study) injections of 2 to 4 monthly doses of GAD-alum (recombinant human GAD65 in alum, Diamyd®). Individual-level data from four randomised, double blind, placebo-controlled clinical trials, clinical trial identifiers NCT00435981, NCT00529399, NCT00723411 and NCT03345004 that evaluated GAD-alum therapy (compared with alum placebo) in GAD autoantibody-positive individuals with recent-onset T1D were combined. These studies were selected because they represent placebo-controlled studies of the efficacy of Diamyd® therapy in individuals with recently diagnosed T1D where Diamyd® was the sole active study drug. Vitamin D was administered in NCT03345004 but is only considered a supplement.

- Study NCT00435981 was a two-arm Phase II clinical trial which included 70 patients in total at multiple sites in Sweden. Patients were aged 10-18 years. Study participants were administered either two subcutaneous injections of 20 µg Diamyd® on day 1 and 30 or two injections of alum alone on the same days.
- Study NCT00529399 was a three-arm Phase II clinical trial which included 145 patients at 15 sites in the USA. Patients were aged 3–45. Subjects were administered either three subcutaneous injections of 20 µg Diamyd® on day 1, 30 and 90, two subcutaneous injections of Diamyd® and one injection of alum only on the same days, or three injections of alum only.
- Study NCT00723411 was a three-arm Phase III clinical trial which included 334 patients at sites in Finland, France, Germany, Italy, The Netherlands, Slovenia, Spain, Sweden and the United

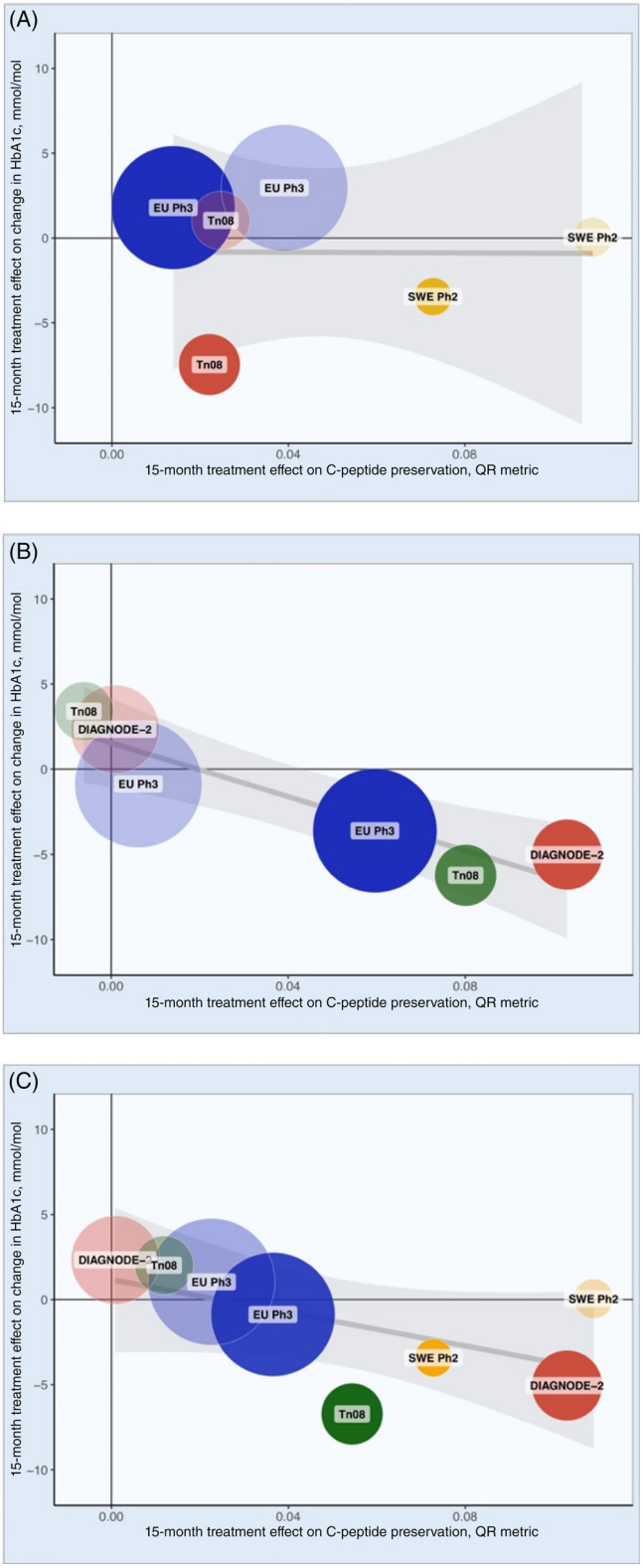


FIGURE A4 Scatter plots illustrating the association between treatment effect (change from baseline to Month 15 compared to placebo) on C-peptide on the *x*-axis and HbA1c on the *y*-axis. Panels (A) 2 injections, (B) 3–4 injections, (C) 2–4 injections. Similar to Figure 1 of the main text but C-peptide effects on the x-axis have been estimated using the modified QR metric instead of MMRM

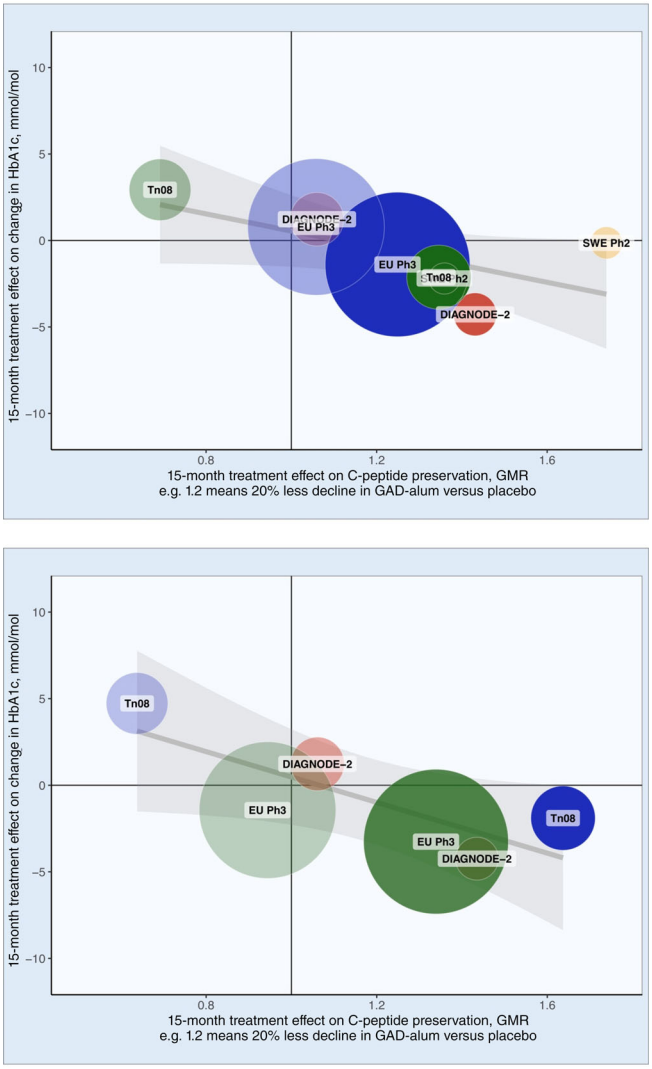


FIGURE A5 Scatter plots illustrating the association between treatment effect (change from baseline to Month 15 compared to placebo) on C-peptide on the *x*-axis and HbA1c on the *y*-axis; as in Figure 1 of the main text but with additional adjustment for insulin dose in the MMRM model. Upper panel: 2–4 injections. Lower panel: 3 or 4 injections

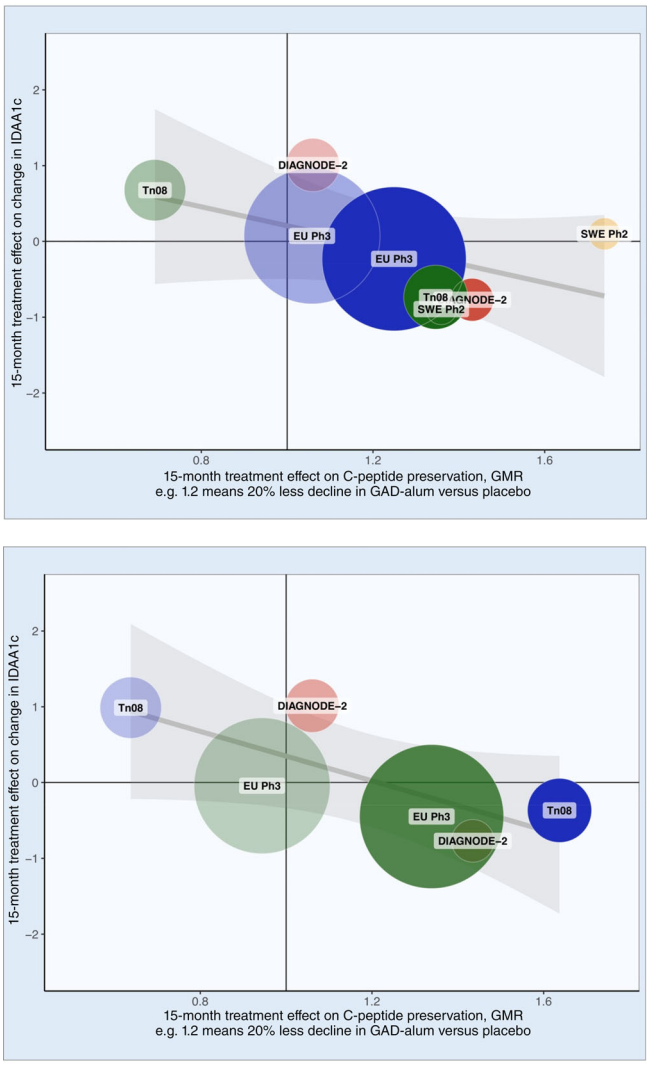


FIGURE A6 Scatter plots illustrating the association between treatment effect (change from baseline to Month 15 compared to placebo) on C-peptide on the *x*-axis and IDAA1c on the *y*-axis. Upper panel: 2–4 injections. Lower panel: 3 or 4 injections

Kingdom. Patients were aged 10–20. Subjects were administered either four subcutaneous injections of 20 µg Diamyd® on day 1, 30, 90 and 270, two subcutaneous injections of Diamyd® and two injections of alum only on the same days, or four injections of alum only.

- Study NCT03345004 (DIAGNODE-2) was a two arm Phase II clinical trial which included 109 patients from multiple sites in the Czech Republic, The Netherlands, Spain and Sweden. Patients were aged 12–24. Subjects were administered either three intralymphatic injections of 4 µg Diamyd® on days, 30, 60 and 90, or three intralymphatic injections of alum alone on the same days. Subjects treated with Diamyd® also received supplementation with 2000 IU/day of Vitamin D between day 1 and day 120, whereas placebo treated patients received Vitamin D placebo.

For the analysis, patients were classified as having received placebo, two injections, or 3–4 injections. Subjects were also coded according to if they were carriers of the HLA defined subgroup HLA DR3-DQ2-carrier or HLA DR3-DQ2-noncarrier. All included clinical trials were placebo-controlled with either a 1:1 or 1:1:1 randomisation ratio. The placebo treatment was administered in the same way as active treatment and was indistinguishable from the active drug. In three of the four trials, patients were treated with 20 µg per injection administered subcutaneously, whereas in one of the trials (DIAGNODE-2, NCT03345004), participants were treated instead with 4 µg per injection administered intralymphatically. There were slight differences between trials regarding the days on which the injections were administered due to minor variations in trial design. In addition, there were also differences in the time from T1D diagnosis allowed for inclusion in the different studies ranging from 3 to

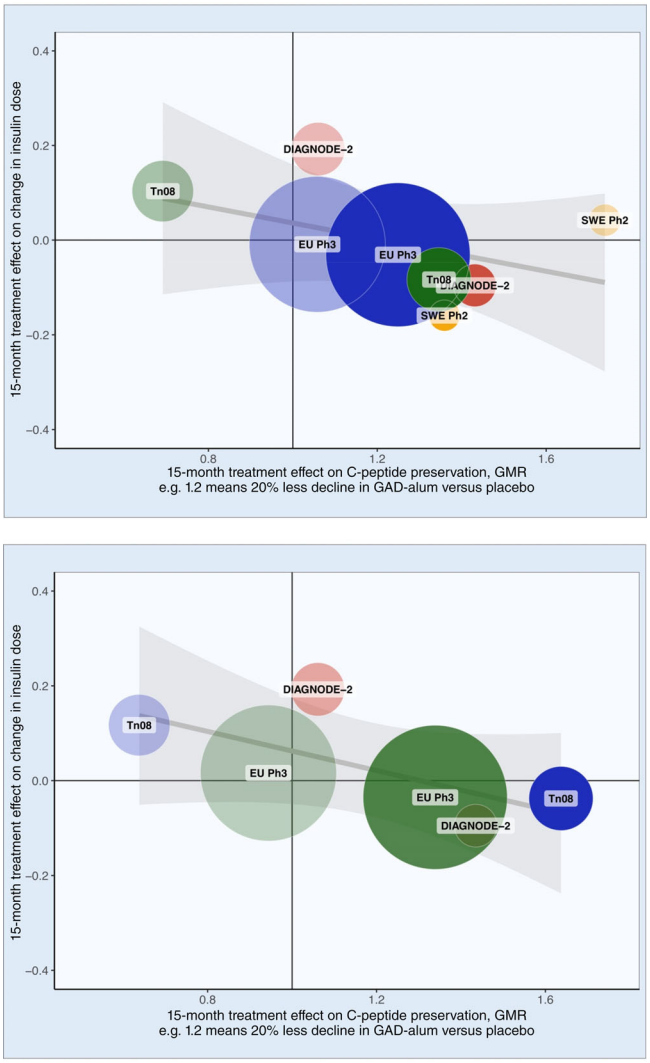


FIGURE A7 Scatter plots illustrating the association between treatment effect (change from baseline to Month 15 compared to placebo) on C-peptide on the x-axis and insulin dose on the y-axis. Upper panel: 2–4 injections. Lower panel: 3 or 4 injections

18 months. A covariate term for which study the patient took part in was included in the analysis to account for these differences in design. Common and important inclusion criteria included written informed consent given by patients and/or their caregiver, a confirmed diagnosis if clinical T1D, detectable GAD65 antibodies, and a fasting C-peptide ≥0.12 nmol/L (0.36 ng/mL).

Meal stimulated C-peptide was assessed using the mixed meal tolerance test (MMTT). After ingestion of a standardized liquid meal, C-peptide in serum was measured at 0, 30, 60, 90 and 120 min following ingestion. The mean C-peptide Area Under Curve was calculated by the trapezoid rule for the five measurements and divided by 120 min. For analysis, C-peptide AUCmean 0–120 min values were natural log-transformed. Circulating HbA1c levels were analysed at a central laboratory and not transformed.

Mean changes from baseline were analysed using a Restricted Maximum Likelihood-based repeated measures approach (Mixed Model for Repeated Measures [MMRM]). The model was adjusted for the fixed effects of baseline C-peptide (or baseline HbA1c), study, treatment, HLA subgroup, visit, country, sex and age, as well as the interaction of baseline C-peptide (or HbA1c) by visit and treatment by HLA subgroup by visit. Baseline value, age and visit were treated as continuous variables. Study, treatment, HLA subgroup, country and sex were treated as categorical variables. Patient identification number and country were included as categorical random effects to yield a variance components structure. An unstructured (co)variance structure was used to model the within-patient errors. If this analysis failed to converge, compound symmetry and autoregressive were tested (the [co]variance structure converging to the best fit of the two models, as determined by Akaike's information criterion, was used as the primary analysis). The Kenward-Roger approximation was used to estimate denominator degrees of freedom. The primary treatment comparison was the contrast between treatments at Month 15 for active treatments versus placebo. Back-transformed least square (LS) mean estimate for change from baseline to 15 months in C-peptide AUCmean 0-120 min during MMTT were used to quantify the model-based estimated treatment effect. The back-transformed estimates of the treatment difference provides an estimate for the Diamyd/placebo-ratio in relative change from baseline AUC. Here a ratio of e.g. 1.40 meant that the change from baseline to Month 15 in C-peptide level was 40% smaller for Diamyd than for placebo at Month 15, i.e. the retention on insulin secretive function was 40% larger.

The Table below shows the number of participants by HLA type.

HLA subgroup	NCT00435981	NCT00529399	NCT00723411	NCT03345004	Total
DR3-DQ2 *N* (% study, % total)	34 (49%, 11%)	71 (51%, 23%)	161 (51%, 51%)	47 (44%, 15%)	313
Not DR3-DQ2 *N* (% study, % total)	35 (51%, 11%)	68 (49%, 22%)	152 (49%, 48%)	59 (53%, 9.4%)	314