C-peptide and metabolic outcomes in trials of disease modifying therapy in new-onset type 1 diabetes: an individual participant meta-analysis

Peter N Taylor, Kimberly S Collins*, Anna Lam*, Stephen R Karpen, Brianna Greeno, Frank Walker, Alejandro Lozano, Elnaz Atabakhsh, Simi T Ahmed, Marjana Marinac, Esther Latres, Peter A Senior, Mark Rigby, Peter A Gottlieb, Colin M Dayan on behalf of the Trial Outcome Markers Initiative collaboration†*

Summary

Background **Metabolic outcomes in type 1 diabetes remain suboptimal. Disease modifying therapy to prevent β-cell loss presents an alternative treatment framework but the effect on metabolic outcomes is unclear. We, therefore, aimed to define the relationship between insulin C-peptide as a marker of β-cell function and metabolic outcomes in new-onset type 1 diabetes.**

Methods **21 trials of disease-modifying interventions within 100 days of type 1 diabetes diagnosis comprising 1315 adults (ie, those 18 years and older) and 1396 children (ie, those younger than 18 years) were combined. Endpoints assessed were stimulated area under the curve C-peptide, HbA_{1c}, insulin use, hypoglycaemic events, and composite scores (such as insulin dose adjusted A_{1c}, total daily insulin, U/kg per day, and BETA-2 score). Positive studies were defined as those meeting their primary endpoint. Differences in outcomes between active and control groups were assessed using the Wilcoxon rank test.**

Findings **6 months after treatment, a 24·8% greater C-peptide preservation in positive studies was associated with a 0·55% lower HbA_{1c} ($p<0·0001$), with differences being detectable as early as 3 months. Cross-sectional analysis, combining positive and negative studies, was consistent with this proportionality: a 55% improvement in C-peptide preservation was associated with 0·64% lower HbA_{1c} ($p<0·0001$). Higher initial C-peptide levels and greater preservation were associated with greater improvement in HbA_{1c}. For HbA_{1c}, IDAAC, and BETA-2 score, sample size predictions indicated that 2–3 times as many participants per group would be required to show a difference at 6 months as compared with C-peptide. Detecting a reduction in hypoglycaemia was affected by reporting methods.**

Interpretation **Interventions that preserve β-cell function are effective at improving metabolic outcomes in new-onset type 1 diabetes, confirming their potential as adjuncts to insulin. We have shown that improvements in HbA_{1c} are directly proportional to the degree of C-peptide preservation, quantifying this relationship, and supporting the use of C-peptides as a surrogate endpoint in clinical trials.**

Funding **JDRF and Diabetes UK.**

Introduction

Type 1 diabetes results from the autoimmune destruction of insulin producing β cells.[1] The subsequent inability to produce insulin has profound adverse metabolic and clinical consequences.[1] Three stages of type 1 diabetes are defined: stage 1 is the presence of β-cell autoimmunity (ie, two or more islet autoantibodies), stage 2 is the presence of β-cell autoimmunity and dysglycaemia, and stage 3 is the onset of symptomatic disease.[2] For the past century, insulin has been the mainstay of treatment in stage 3 type 1 diabetes, but despite substantial improvements in glucose monitoring and insulin delivery the majority of people with diabetes do not meet glycaemic targets.[3] As a result, substantial morbidity and excess premature death by more than a decade persist in people with type 1 diabetes.[4,5]

Disease-modifying therapy to preserve β-cell function represents an alternative and adjunctive treatment framework to insulin therapy in type 1 diabetes that could enable more people with diabetes to meet glycaemic targets. C-peptide is co-secreted in a 1:1 ratio with insulin and is a measure of endogenous insulin secretion and β-cell function.[6] Randomised prospective trials in early stage 3 type 1 diabetes have identified nine disease-modifying interventions that have shown evidence of β-cell preservation as shown by improved meal-stimulated area under the curve (AUC) C-peptide[7–18] at 12 months post-diagnosis. Large population cohort-based studies show that persistence of even modest concentrations of C-peptide in type 1 diabetes are associated with better clinical outcomes including reductions in hypoglycaemia, neuropathy, and

Lancet Diabetes Endocrinol 2023

Published Online November 3, 2023 https://doi.org/10.1016/S2213-8587(23)00267-X

See Online/Comment https://doi.org/10.1016/S2213-8587(23)00299-1

*Contributed equally

†The Trial Outcome Markers Initiative collaborators are listed in the appendix (p 20)

Department of Infection and Immunity, Cardiff University School of Medicine, Cardiff University, Cardiff, UK (P N Taylor PhD, Prof C M Dayan PhD); **Critical Path Institute, Tucson, AZ, USA** (K S Collins PhD, S R Karpen PharmD, B Greeno PMP, F Walker BSc, A Lozano BSc, E Atabakhsh PhD, M Rigby PhD); **Alberta Diabetes Institute, University of Alberta, Edmonton, AB, Canada** (A Lam MD, Prof P A Senior PhD); **The New York Stem Cell Foundation Research Institute, New York, NY, USA** (S T Ahmed PhD); **JDRF, New York, NY, USA** (M Marinac PharmD, E Latres PhD); **University of Colorado School of Medicine, Aurora, CO, USA** (Prof P A Gottlieb MD)

Correspondence to: Prof Colin M Dayan, Department of Infection and Immunity, Cardiff University School of Medicine, Cardiff University, Cardiff CF14 4XN, UK **dayancm@cardiff.ac.uk**

See Online for appendix

Research in Context

Evidence before this study

We searched PubMed and Embase on Aug 1, 2021, for reports published in English between Jan 1, 2000, and July 30, 2020, related to "C-peptide" and "metabolic outcomes" and also "trials" of "immunotherapy" in "stage 3 type 1 diabetes". We also consulted with experts for additional relevant studies and reviewed the epidemiology of type 1 diabetes. 8·75 million people are living with type 1 diabetes worldwide of whom 1·52 million are younger than 20 years with a median age of diagnosis of 12 years. Despite recent advances in insulin therapy, only 20% of children and 30% of adults meet glycaemic targets even in high-income countries, resulting in a 16-year reduction in lifespan in those diagnosed younger than 10 years. Despite this huge unmet need, no new drugs for type 1 diabetes other than insulin have been developed within the past 100 years. Type 1 diabetes is an autoimmune disease and at least seven immunotherapies have been shown to slow the loss of pancreatic β-cell function as measured by insulin C-peptide concentrations. However, trials of immunotherapy in new-onset type 1 diabetes to date have not shown a significant effect on clinical endpoints such as HbA_{1c} or hypoglycaemia rates. As a result, almost all major drug companies have closed their type 1 diabetes immunotherapy programmes within the past 5 years.

Added value of this study

In this Article, we provide prospective trial evidence that earlier studies not showing clinical benefit from immunotherapy was predominantly an issue of insufficient statistical power, not a lack of effect. To show this, individual participant data were combined from 21 randomised controlled trials of immunotherapy in new-onset type 1 diabetes, comprising 1315 adults (ie, those 18 years and older) and 1396 children (ie, those younger than 18 years)–6 times larger than any previous study. In addition, we have quantified the relationship between the degree of C-peptide preservation and metabolic benefit: a 24·8% greater C-peptide preservation was associated with a 0·55% lower HbA_{1c} ($p<0·0001$) level. These differences were detectable as early as 3 months after trial commencement. Overall, our data suggest that preservation of >76% of initial C-peptide levels should allow almost all individuals to achieve optimal glycaemic targets. This study represents the work of a global collaboration between large pharmaceutical companies, smaller biopharmaceutical companies, publicly funded networks, health charities, and academic researchers coordinated by a not-for-profit company created under the auspices of the Food and Drug Administration's Critical Path Initiative programme to address this problem.

Implications of all the available evidence

Our prospective randomised controlled trial data are consistent with earlier observational data on the clinical benefits of C-peptide preservation from studies such as DCCT and SDRNT1Bio. The demonstration that β-cell (C-peptide) preservation via immunotherapy can substantially improve metabolic outcomes and the quantification of this relationship breaks the deadlock preventing progress in developing immunotherapies. Furthermore, the evidence supports the use of C-peptide as a surrogate outcome measure in clinical trials and regulatory submissions allowing new drugs to be assessed and clinical trials to be designed with confidence. Taken together these data now provide a roadmap for how, 100 years after the first clinical use of insulin, immunotherapy and β-cell preservation can begin to replace insulin as the first line therapy for type 1 diabetes. This will allow many more individuals to achieve glycaemic targets than at present, lessening the burden of insulin therapy while improving clinical outcomes.

retinopathy.[19–21] However, in contrast to the cohort-based observational studies, a significant improvement in metabolic outcomes, such as lower HbA_{1c} or a reduction in hypoglycaemia rates was rarely seen in trials of disease-modifying therapies.[11,18] The reasons why significant metabolic improvement was not observed in prospective studies are unclear. Explanations include too small a sample size, features of the populations studied (eg, adults *vs* children), failure of C-peptide to accurately reflect β-cell function, confounding effects of insulin therapy, or failure to reach a threshold amount of β-cell function to effect metabolic control.

Prospective studies in new-onset type 1 diabetes not meeting clinical endpoints[11,18,22] despite preserving C-peptide has raised concerns among drug developers and has challenged the role of C-peptide as a surrogate endpoint. There is therefore an urgent need to define the relationship between β-cell preservation and metabolic outcomes in prospective studies, and to confirm the role of C-peptide as a reliable surrogate outcome marker. Here we aimed to address this issue by combining individual participant data from multiple disease-modifying intervention trials conducted over the past 15 years in early stage 3 type 1 diabetes, comprising more than 2500 participants as part of the Trial Outcome Markers Initiative. We also aimed to explore the use of composite clinical endpoints and define the likely size of clinical trials required to show a clinically significant metabolic benefit.

Methods

Studies used

We identified 31 studies comprising 3156 participants from published clinical trials of immunomodulating

therapies in new-onset type 1 diabetes. The main barrier for study inclusion was establishing data sharing agreements and transfers in a timely manner. We therefore prioritised the inclusion of larger phase 2 and phase 3 trials, as well as TrialNet and Immune Tolerance Network studies, over smaller studies. Participant-level data from 21 disease modifying intervention trials in children (ie, those younger than 18 years) and adults (ie, those 18 years and older) with new-onset type 1 diabetes were used[10–18,23–34] (appendix pp 2–6, 17–19). New-onset type 1 diabetes was defined as recruitment to a clinical trial within 100 days of type 1 diabetes diagnosis. Studies were defined as positive if they had met their primary outcome, which was generally a statistically significant difference ($p<0·05$) in AUC C-peptide after a mixed-meal tolerance test. This analysis involved the secondary use of de-identified and anonymised data for which no direct linkage with an individual study participant is possible and therefore research ethics board approval was not required. The Western Institutional Review Board approved this approach in 2020.

Endpoints

The endpoints assessed were AUC C-peptide (nmol/L), HbA_{1c} (%), total daily insulin (U/kg per day), insulin dose adjusted A_{1c} (%),[35] BETA-2 score,[36] the Secretory Unit of Islet Transplant Objects index,[37] and hypoglycaemia (mmol/L). AUC C-peptide was time-normalised and calculated using the trapezoidal rule. AUCs missing baseline and derived from fewer than three timepoints were excluded.

Composite scores

Insulin dose adjusted A_{1c} combines HbA_{1c} with insulin dose; a lower score indicates greater β-cell function.[35] The BETA-2 score uses fasting plasma glucose, fasting C-peptide, HbA_{1c}, and insulin use. It has been shown to predict insulin independence, abnormal glucose tolerance,[36] as well as early and long-term islet cell graft function.[38] The Secretory Unit of Islet Transplant Objects index,[37] another measure of β-cell function, uses fasting C-peptide and glucose concentrations. The calculation of these scores is shown in the appendix (p 7).

Assessment of hypoglycaemia

There was substantial variation between studies in how hypoglycaemia was recorded. Some trials captured home capillary blood glucose readings in diaries, whereas others used participants recall of hypoglycaemic events. Because of this, the number of hypoglycaemic events ranged per participant ranged between 0 and 253. We therefore examined hypoglycaemia separately for studies using methods capturing large numbers of hypoglycaemic events and those using methods capturing smaller number of events.

Hypoglycaemic episodes were captured from adverse event reports, participant recall, and diaries that included capillary blood glucose records. A level 2 hypoglycaemic event was defined as a blood glucose measurement of less than 54 mg/dL (<3·0 mmol/L) regardless of clinical symptoms, and level 3 was defined as having a severe cognitive impairment that required external assistance, according to international consensus definitions.[39] As continuous glucose monitoring data were only available in 265 participants, continuous-glucose-monitoring-detected hypoglycaemia was not included in this analysis. Further details of hypoglycaemic modelling are described in the appendix (p 8).

	All studies (n=2712)	Negative studies (n=1932)	Positive studies (n=780)	p values
Placebo	812 (29·9%)	566 (29·3%)	246 (31·5%)	..
Intervention	1900 (70·1%)	1366 (70·7%)	534 (68·5%)	..
Age, years*	17 (13–25)	17 (12–24)	18 (13–26)	p=0·0001
Children (ie, those younger than 18 years)*	1396 (51·5%)	1039 (53·8%)	357 (45·8%)	p=0·0002
Adults (ie, those 18 years and older)*	1315 (48·5%)	893 (46·2%)	422 (54·1%)	..
Sex				
Female	1085 (40·0%)	776 (40·2%)	309 (39·6%)	p=0·82
Male	1627 (60·0%)	1156 (59·8%)	471 (60·4%)	..
Time normalised C-peptide, nmol/L	0·64 (0·44–0·92)	0·64 (0·43–0·92)	0·65 (0·46–0·92)	p=0·42
Time since diagnosis, days*	69 (49–85)	67 (47–83)	75 (53–90)	p<0·0001
BMI and weight				
BMI for children, kg/m²*	19·4 (17·4–22·0)	19·4 (17·4–21·7)	19·6 (17·5–23·0)	p=0·060
BMI for children, Z-score*	–0·2 (–0·7 to 0·5)	–0·2 (–0·7 to 0·4)	–0·1 (–0·6 to 0·8)	..
BMI for adults, kg/m²*	23·0 (20·7–25·4)	23·0 (20·6–25·4)	23·0 (20·8–25·4)	p=0·54
BMI for adults, Z-score*	–0·1 (–0·7 to 0·5)	–0·1 (–0·7 to 0·5)	–0·1 (–0·7 to 0·5)	..
Weight for children, kg*	49·6 (39·0–61·3)	49·7 (39·0–60·0)	49·0 (39·0–65·0)	p=0·43
Weight for adults, kg*	69·6 (60·0–79·8)	69·6 (59·5–79·7)	69·7 (61·0–80·3)	p=0·33
Race				
American Indian or Alaska Native*	10 (0·4%)	8 (0·4%)	2 (0·3%)	p<0·0001
Asian*	171 (6·3%)	168 (8·7%)	3 (0·4%)	..
Black*	50 (1·8%)	33 (1·7%)	17 (2·2%)	..
White*	2154 (79·4%)	1411 (73·0%)	743 (95·3%)	..
More than one race*	23 (0·8%)	18 (0·9%)	5 (0·6%)	..
Other*	17 (0·6%)	9 (0·5%)	8 (1·0%)	..
Unknown	287 (10·6%)	285 (14·8%)	2 (0·3%)	
Ethnicity				
Hispanic or Latino	160 (5·9%)	117 (6·1%)	43 (5·5%)	p=0·18
Not Hispanic or Latino	2271 (83·7%)	1536 (79·5%)	735 (94·2%)	..
Unknown	281(10·4%)	279 (14·4%)	2 (0·3%)	

Data are n (%) or median (IQR). There was one individual with missing data on age, 287 individuals with missing data on race, and 281 individuals with missing ethnicity data. *Significant difference between negative and positive studies.

Table 1: **Summary of participants in the included studies**

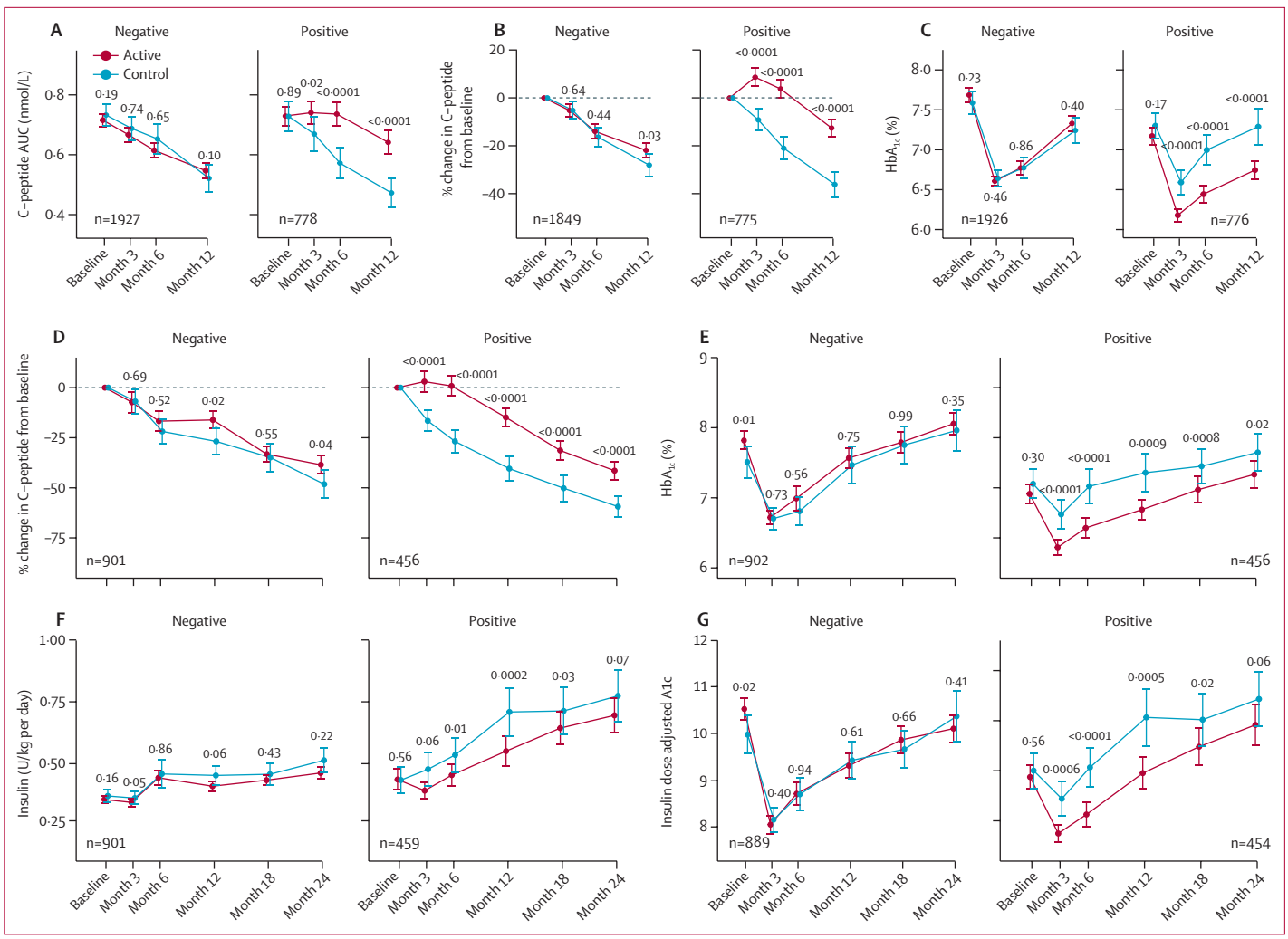

Figure 1: **Time normalised C-peptide AUC, change in C-peptide from baseline and HbA_{1c} means across 12 months, and change in C-peptide from baseline, HbA_{1c}, insulin dose, and insulin dose adjusted A_{1c} across 24 months**

Mean value of time-normalised C-peptide AUC across 12 months (A), percent change in C-peptide AUC from baseline across 12months (B), HbA_{1c} across 12 months (C), change in C-peptide AUC from baseline across 24 months (D), HbA_{1c} across 24 months (E), total daily insulin across 24 months (F), and insulin-dose adjusted A1c across 24 months (G). Error bars represent 95% CIs. Active groups are in red and control groups are in blue. AUC=area under the curve. p values are reported above each timepoint. Only studies with two years or more of follow-up were included in panels D–G.

Statistical analyses

Data were binned into baseline, 3, 6, 12, 18, and 24 months. Screening measurements were used for baseline when an endpoint was not captured at the study start. The binned time intervals included a range of 30 days SD. For C-peptide preservation at 2 years, a range of 90 days SD was used. At each timepoint, the mean and 95% CIs are shown. Locally estimated scatterplot smoothing curves were generated to depict the cumulative frequency of hypoglycaemic events and HbA_{1c} trajectories stratified by C-peptide quartiles. Statistical significance for each endpoint between active and control groups at each time-point was determined by the Wilcoxon rank sum test, for which a p-value of less than 0·05 was considered statistically significant given our hypothesis generation of exploring relationships with repeated measures across time for AUC C-peptide and the similar nature of several outcomes (eg, HbA_{1c} and insulin dose adjusted A_{1c}). The Kruskal-Wallis rank sum test was performed for the cumulative frequency of hypoglycaemic events across C-peptide quartiles. No correction was made for multiple testing. Exemplar sample size calculations were performed using the pwr R package. A two means, two-sided t-test with an alpha level of 0·05, power set to 80%, and Cohen's D effect size approximation, were calculated using the observed mean differences and SDs from the positive studies only within each age group (ie, children and adults) and time frame specified. Data were analysed using R (version 4.0.5) and R Studio (version 353).

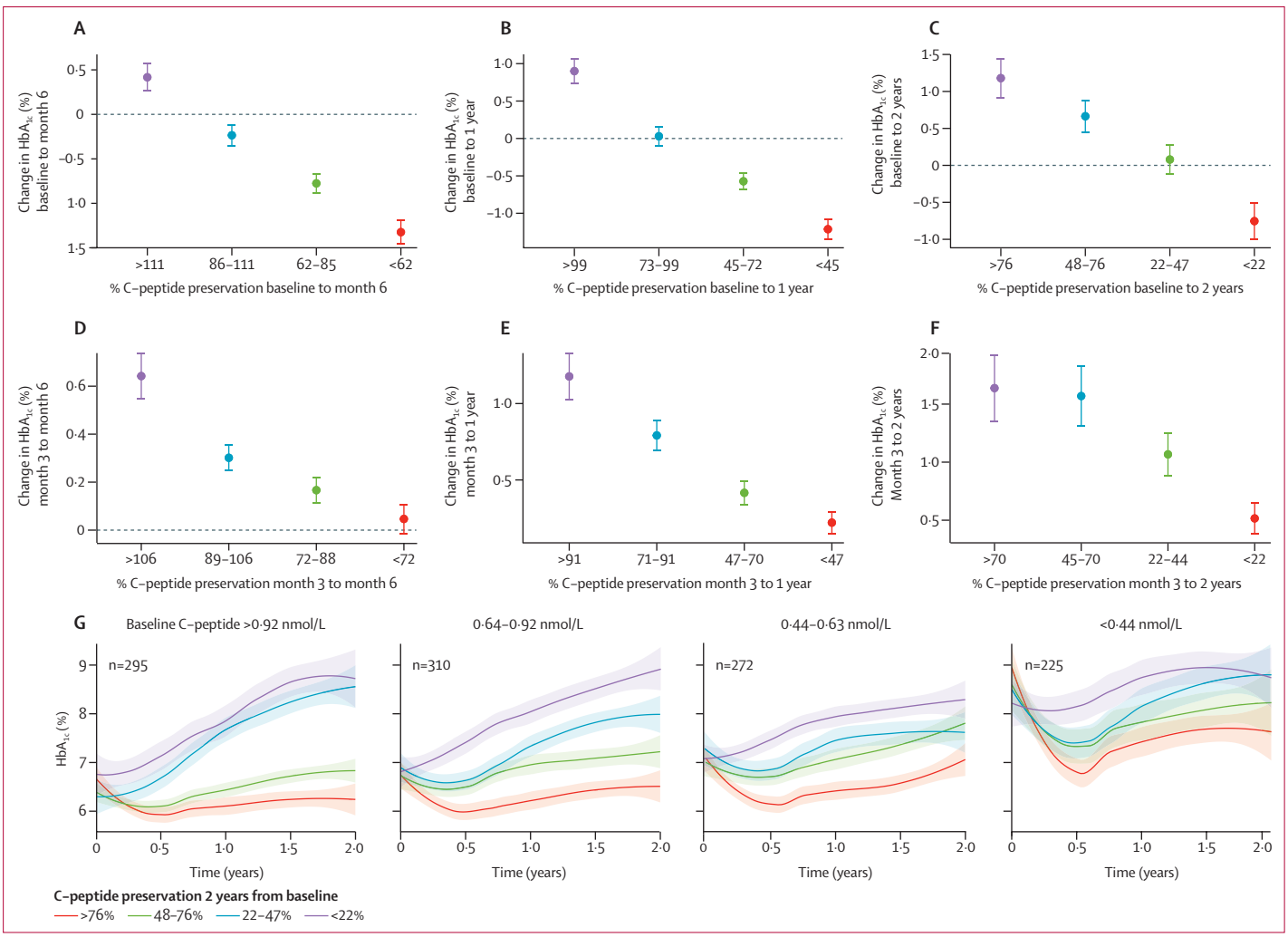

Figure 2: **C-peptide preservation and change in HbA_{1c} across time**

Mean change in HbA_{1c} (%) stratified by quartiles of percentage C-peptide preservation at 6 months (A), 1 year (B), and 2 years (C) from baseline, and at 6 months (D), 1 year (E), and 2 years (F) from 3 months. Error bars in panels A–F represent 95% CIs as do the shaded regions in panel G. Loess HbA_{1c} trajectories stratified by quantiles of baseline C-peptide and preservation at 2 years (G). Each panel represents a baseline C-peptide quartile >0·92, 0·64–0·92, 0·44–0·63, and <0·44 nmol/L. Loess curves within each panel are stratified by preservation of C-peptide from baseline at 2-year quartiles: >76%, 48-76%, 22-47%, and <22%. N represents the total number of individuals in each panel. Only individuals with 2-year C-peptide had data included.

Role of the funding source

As a patient-focused organisation and funder of the study, JDRF had a role in the initial concept and scope of the project, but no role in the study design, data collection, data analysis, or decision to submit for publication. However, EL and MM are employed by the JDRF and were involved in the interpretation of research outputs and drafting of the manuscript.

Results

Our dataset comprised 1315 adults and 1396 children studied within 100 days of diagnosis of type 1 diabetes from 21 studies (table 1). This represents 85·9% of study participants from the 31 eligible trials identified in our literature search. 780 participants were derived from eight positive studies (appendix pp 17–18). Across all studies, data were available beyond baseline in 2705 participants up to 12 months and 1358 participants up to 24 months.

AUC C-peptide levels declined linearly from diagnosis in the negative studies and the control groups of the positive studies. The control groups of positive studies fell to around 65% of baseline at 1 year and 40% at 2 years (figure 1). By contrast, the change in HbA_{1c} over time from diagnosis was U-shaped with an improvement in HbA_{1c} of up to 1% in the first 3 months followed by a linear rise after this time (figure 1).

In positive studies, disease modifying therapy was associated with an initial rise in C-peptide relative to baseline in the first 3 months, followed by a decline. C-peptide levels remained higher than controls up to 24 months despite no further treatment being given

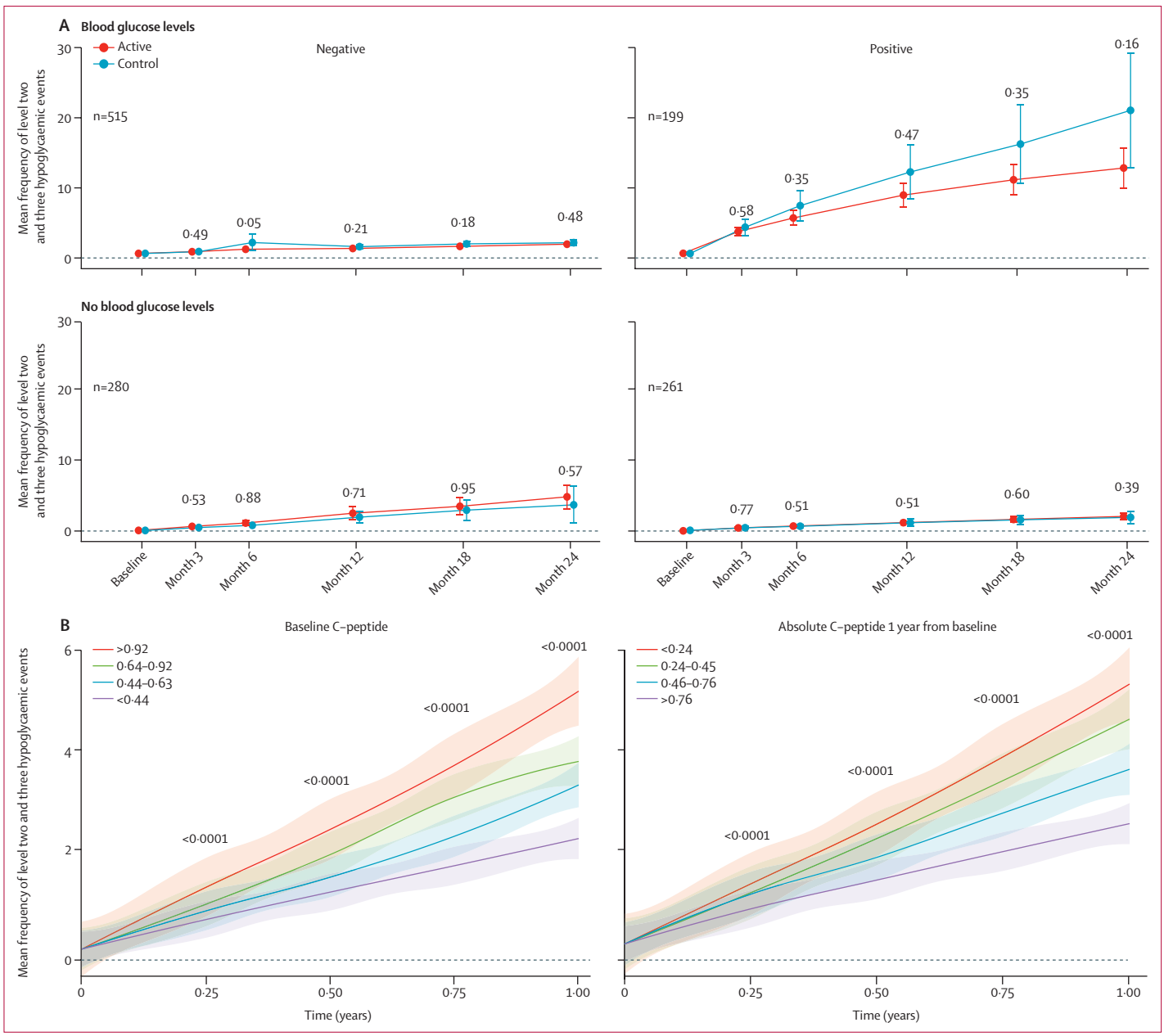

Figure 3: **Mean frequency of level two and level three hypoglycaemic events**

Mean frequency of the cumulative level two and level three hypoglycaemic events grouped by negative and positive study outcomes and whether blood glucose levels were provided (A). Error bars represent 95% CIs. Active groups are in red lines and control groups are in blue. N represents the total number of individuals in each panel. Only studies with 2 years or more of follow-up reported the occurrence of at least level two hypoglycaemic events. No statistical differences were found between active and control groups. Loess curves with 95% CIs of the mean frequency of combined level two and level three hypoglycaemic events across 1 year stratified by quartiles of C-peptide at baseline (left figure; B) and at 1 year (right figure; B). Significance reported at each time interval (0·00, 0·25, 0·5, 0·75, and 1 year). p values are reported above each timepoint.

beyond 12 months in all but one study (appendix pp 2–6). This preservation of C-peptide was associated with significantly lower HbA_{1c} concentrations compared with the controls as early as 3 months after the beginning of the intervention with the maximal difference being achieved at 6 months (figure 1). At 3 months there was a 17·8% difference in C-peptide preservation between active treatment and controls within positive studies and a 0·41% difference in HbA_{1c}; at 6 months a 24·8% difference in C-peptide preservation was associated with 0·55% difference in HbA_{1c} (figure 1). HbA_{1c} remained lower in the intervention group up to 24 months, although the difference between the groups appeared to reduce (figure 1).

The association between C-peptide preservation and HbA_{1c} seen between active groups and control groups in

the positive intervention studies was further supported in longitudinal analysis of the whole dataset (figure 2). Change in HbA_{1c} was linearly related to the degree of C-peptide preservation when studied from baseline or from the nadir of HbA_{1c} at 3 months (figure 2). A 32%, 27%, or 55% greater preservation of C-peptide from baseline to 1 year between each quartile was associated with a clinically meaningful improvement in HbA_{1c} of 0·87% (p<0·0001), 0·60% (p<0·0001), and 0·64% (p<0·0001) respectively. The relationship continued for 2 years, except for the analysis from 3 months to 2 years, which appeared to plateau at less than 56% C-peptide preservation (figure 2). A similar relationship was seen with the percentage of individuals achieving a target HbA_{1c} of less than 6·5% or an insulin dose adjusted A_{1c} of less than 9% (appendix pp 10–13) with some suggestion of a plateau with C-peptide levels of less than 0·85 nmol/l (appendix p 11).

Additional analysis of the whole dataset suggested that a higher initial C-peptide level in addition to higher degrees of β-cell preservation was associated with further improved HbA_{1c} levels at 2 years. An initial C-peptide level in the highest quartile (>0·92nmol/L) combined with preservation of more than 76% of C-peptide (N=81) was associated with a mean HbA_{1c} of 6·2% at 2 years as compared with around 8·6% in participants that maintained less than 22% of their initial C-peptide level of (>0·92nmol/l; N=47; figure 2).

Insulin doses increased steadily in the positive studies, but less so in the negative studies. Doses were lower for the first 6–18 months in the treatment group than the control groups in positive studies (figure 1). Although insulin doses increased, HbA_{1c} remained lower in the intervention group even after adjusting for insulin dose (insulin dose adjusted A_{1c}; figure 1). Higher insulin doses were given in positive studies than negative studies potentially reflecting differences in study populations. Participants in positive studies were older and had later recruitment after diagnosis (table 1). There might have been differences in glucose management, as HbA_{1c} concentrations tended to be lower in the control groups of positive studies than negative studies beyond 12 months.

Despite greater C-peptide preservation, no statistically significant differences in cumulative hypoglycaemic rates in the positive studies were shown. However, the cumulative number of hypoglycaemic events was numerically higher at 2 years in those receiving placebo in positive studies in which there was frequent recording of blood glucose levels (figure 3) in keeping with higher insulin doses needed in the placebo groups. There was no suggestion of any differences in studies that did not have frequent blood glucose measurements (figure 3). Longitudinal analysis of the whole dataset showed an important and significant difference in hypoglycaemic events as early as 3 months beyond baseline when those in the highest and lowest quartiles of baseline or C-peptide preservation at 1 year were compared (figure 3).

	Age group	Observed mean difference*	Active group (SD)	Control group (SD)	Sample size for each group (1:1), one-sided	Sample size for each group (1:1), two-sided
C-peptide preservation						
6 months	Children	29·40	41·36	31·01	20	25
6 months	Adult	18·18	46·41	40·74	72	92
6 months	Both	24·79	44·76	37·96	35	45
12 months	Children	29·38	41·35	30·79	20	25
12 months	Adult	12·06	46·06	44·82	176	224
12 months	Both	21·81	44·56	41·31	49	62
HbA_{1c}						
3 months	Children	−0·38	0·98	1·10	94	119
3 months	Adult	−0·38	1·02	1·29	116	148
3 months	Both	−0·41	1·03	1·22	94	120
6 months	Children	−0·57	1·18	1·35	62	79
6 months	Adult	−0·44	1·27	1·50	124	158
6 months	Both	−0·56	1·27	1·47	75	95
12 months	Children	−0·68	1·31	1·67	61	77
12 months	Adult	−0·31	1·32	1·70	299	379
12 months	Both	−0·54	1·35	1·75	104	132
BETA-2 score						
3 months	Children	1·98	6·84	6·68	145	184
3 months	Adult	1·86	6·28	5·70	129	164
3 months	Both	1·98	6·70	6·43	137	174
6 months	Children	2·65	8·25	6·61	99	126
6 months	Adult	2·78	6·57	6·20	66	84
6 months	Both	2·85	7·88	6·69	82	104
12 months	Children	2·88	8·37	6·57	85	108
12 months	Adult	2·08	5·79	4·70	80	102
12 months	Both	2·70	7·22	6·06	76	97
Insulin dose adjusted A_{1c}						
3 months	Children	−0·69	1·79	2·26	109	138
3 months	Adult	−0·72	1·40	2·09	76	97
3 months	Both	−0·74	1·77	2·28	95	120
6 months	Children	−0·94	2·22	2·49	79	100
6 months	Adult	−0·91	1·81	2·50	72	91
6 months	Both	−1·01	2·21	2·60	71	91
12 months	Children	−1·13	3·03	3·01	89	113
12 months	Adult	−0·83	1·70	2·93	104	132
12 months	Both	−1·14	2·80	3·18	86	109

(Table 2 continues on next page)

Exemplar power calculations using the observed differences between the active and control groups in the dataset indicated that 1·5–4·5-fold smaller samples are required for an AUC C-peptide versus a HbA_{1c} endpoint (table 2). Smaller sample sizes are required for studies in children compared with adults, although this is less marked with HbA_{1c} than a C-peptide endpoint. In keeping with this there is a faster decline in C-peptide in younger individuals (appendix pp 14–15). A sample size of 158 in each group in adults or 79 in children was adequate to see a difference in HbA_{1c} at 6 months at 80% power and a significance

	Age group	Observed mean difference*	Active group (SD)	Control group (SD)	Sample size for each group (1:1), one-sided	Sample size for each group (1:1), two-sided
(Continued from previous page)						
Level 2 or 3 hypoglycaemia						
6 months	Children	−0·50	5·06	6·28	1609	2043
6 months	Adult	−0·62	4·57	6·37	989	1256
6 months	Both	−0·52	4·79	6·31	1436	1823
12 months	Children	−1·16	7·80	10·95	831	1055
12 months	Adult	−1·23	7·79	11·66	804	1021
12 months	Both	−1·13	7·79	11·28	911	1156
Level 3 hypoglycaemia						
6 months	Children	−0·02	0·26	0·35	2939	3731
6 months	Adult	−0·01	0·14	0·16	2795	3549
6 months	Both	−0·01	0·20	0·27	6981	8862
12 months	Children	0·004	0·44	0·38	130 607	165 808
12 months	Adult	−0·01	0·19	0·19	4464	5668
12 months	Both	−0·01	0·33	0·30	12 298	15 612

Children are those younger than 18 years and adults are those 18 years and older. *C-peptide preservation (% preserved from baseline), HbA_{1c}, and insulin dose adjusted A_{1c} difference, hypoglycaemic events (total frequency at 6 or 12 months). Power was set to 80% and alpha at 0·05 for all sample size calculations.

Table 2: **Exemplar sample size calculations for different endpoints and follow-up for children, and both populations**

level of $p<0·05$ for two-sided tests. Extending the study to a 12-month endpoint did not reduce the sample size required. The use of insulin dose adjusted A_{1c} rather than HbA_{1c} as an endpoint reduced the sample size required in adults but not children (table 2). The BETA-2 score which obviates the requirement for mixed meal tolerance challenge testing required similar sample sizes to HbA_{1c} (appendix pp 16).

Discussion

We have shown in this individual participant data meta-analysis that successful disease-modifying therapy resulting in C-peptide preservation improves metabolic outcomes in type 1 diabetes, notably glycaemic control as measured by HbA_{1c}. Preservation of approximately 20% greater stimulated C-peptide levels compared with controls results in a clinically important improvement in HbA_{1c} (0·5%). Furthermore, this effect shows linear proportionality, with higher initial C-peptide and greater degrees of C-peptide preservation being associated with a greater effect on HbA_{1c}. For example, initial AUC C-peptide levels of greater than 0·92 nmol/L and preservation of greater than 76% of initial values was associated with maintenance of mean HbA_{1c} levels at around 6%. The onset of the effect is rapid, with differences being apparent within 3 months of commencing treatment. This effect was seen in aggregated prospective intervention trials and replicated in the cohort analysis of the association between decline in C-peptide and HbA_{1c} in the larger dataset of all study participants (including positive and negative studies). These data therefore provide strong support for the paradigm that treatments to preserve β-cell function will allow more individuals to reach glycaemic targets than insulin therapy alone. Quantification of the duration of metabolic benefits associated with C-peptide preservation are still required as our findings at 2 years regarding metabolic benefits were less robust. However, almost all the trials were not designed to address this issue. The less robust conclusions at 2 years are therefore likely due to a combination of treatment only being given in the first year in all but one study and reduced statistical power due to lower numbers with 2-year data.

Our data also indicate that the not observing significant metabolic improvement in previous studies despite C-peptide preservation was largely due to inadequate sample size rather than a failure of C-peptide to accurately reflect β-cell function. The spectrum of immunotherapies targeting diverse pathways used in the positive studies in our dataset achieved a mean of 29% greater C-peptide preservation compared with controls in children at 6 months and 18% greater C-peptide preservation in adults also at 6 months. This translates to a sample size requirement of around 62 children or 114 adults per study group to detect a difference of 0·57% and 0·44% in HbA_{1c}, respectively. By comparison, the largest of the individual positive studies had 63 adults in the control group,[11] whereas the remainder had between 16 and 34 participants per group.

The status of AUC C-peptide as a surrogate endpoint for regulatory approval of disease-modifying interventions has been widely debated in recent years.[19] Although C-peptide qualifies in terms of biological plausibility and likely causality, it has previously been difficult to show proportionality of clinical benefit to C-peptide. Our data provide clarification, suggesting an essentially linear, proportional relationship between C-peptide preservation and HbA_{1c} at least down to C-peptide levels less than 22% of the level at month 3 after study entry. Our data showing a direct effect of C-peptide preservation on HbA_{1c} levels in prospective studies of disease-modifying therapies, substantially adds to the evidence cited by Palmer and colleagues,[19] which used the DCCT study to show an association between C-peptide and metabolic outcomes. This provides robust supportive evidence that AUC C-peptide can be used as a reasonably likely surrogate endpoint, which could be a key factor in regulatory decisions. AUC C-peptide is also more efficient than metabolic markers in showing the efficacy of a disease-modifying intervention in type 1 diabetes. Our data indicate that 6 months could be long enough for a primary outcome in proof-of-concept studies (phase 2), although later timepoints will probably be required in phase 3 trials to show durability.

Composite outcomes adjusting for insulin use might have an advantage over HbA_{1c}. Insulin dose adjusted A_{1c}

appears to be an attractive choice in adults, requiring similar sample sizes between adults and children. The BETA-2 composite score is convenient as it can be used with fasting samples only and no requirement for meal stimulated measurements: it requires similar sample sizes to HbA_{1c}.

The relationship between C-peptide and hypoglycaemia was more difficult to define in the current dataset. The hypoglycaemic data were non-standardised and many events were most likely missed given the scarcity of continuous glucose monitoring data. Analysis of the whole dataset showed that higher levels of C-peptide at baseline or 1 year after study entry were associated with less frequent hypoglycaemic events as early as 3 months beyond baseline. However, there were too few hypoglycaemic events and too wide variation in numbers of recorded events to confirm a causative role for C-peptide in the positive studies. In the absence of a more systematic capture of hypoglycaemic events, our data suggest that large numbers of participants are required to power a trial based on this clinical outcome.

Our results on ethnicity need careful interpretation, White individuals were substantially more likely to be in positive studies. This finding was mainly driven by the largest negative study (Protégé),[18] which recruited a large number of participants from the Indian sub-continent who had lower C-peptide concentrations, higher insulin use, and higher HbA_{1c} than those recruited from North America and Europe.[18] Our finding is most likely due to these baseline differences rather than a genuine interaction in immunotherapy effect by ethnicity.

We observed higher insulin use in positive studies. This finding is most likely a reflection of the scarcity of standardised insulin titration across the studies rather than being related to their outcome: if anything, higher insulin use will underestimate the benefits of immunotherapy, as controls also received more insulin. This conclusion is supported by the finding that the results were similar when adjusting HbA_{1c} for insulin dose as seen with the metric insulin dose adjusted A_{1c}.

The strengths of our study are the large sample size, inclusion of multiple and diverse successful interventions, and the availability of substantial amounts of data at 2 years. Weaknesses include the heterogeneity in study populations between trials and particularly the heterogeneity and under-reporting of hypoglycaemia. Differences in age (children *vs* adults), BMI, ethnic origin, and baseline C-peptide levels all effect metabolic outcomes in addition to C-peptide preservation. A clinical trial simulation tool is under development based on our dataset. This tool will enable users to model different trial design scenarios, endpoints, and treatment effect sizes starting with different populations.

In summary, we have provided strong evidence that interventions that preserve β-cell function are effective at improving metabolic outcomes in new-onset type 1 diabetes. Furthermore, we have quantified this relationship by showing that improvements in HbA_{1c} are directly proportional to the degree of C-peptide preservation, supporting the use of C-peptide as a surrogate endpoint in clinical trials. Optimal metabolic control remains an unmet need in type 1 diabetes. The evidence presented here supports consideration of an alternative approach to address this, focussing on treatment of the disease (autoimmune destruction of β cells), rather than relying on insulin to treat the resulting disability (insulin deficiency).

Contributors

CMD, PAG, PAS, MR, and SRK conceived and designed the study. KSC, PNT, ALa, CMD, and PAS designed the statistical analysis plan and KSC performed the statistical analysis. All authors contributed to interpreting the results, drafting the manuscript, (initial draft done by PNT), and editing subsequent revisions. KSC, EA, FW, and ALo had full access to the data in the study and had final responsibility for the decision to submit for publication and KSC and EA verified the data. All authors gave final approval of the version to be published and accept responsibility to submit the manuscript for publication.

Declaration of interests

PNT declares personal consulting fees from Immunovant and leadership roles in the Society for Endocrinology and British Thyroid Association. CMD declares lecturing or has been involved as an advisor for: Novo Nordisk, Sanofi-Genzyme, Janssen, Servier, Lilly, AstraZeneca, Provention Bio, UCB, MSD, Vielo Bio, Avotres, Worg, and Novartis. He holds patents jointly with Midatech, and Provention Bio and Sanofi. PAG declares consulting fees from Provention Bio and Viacyte and co-founded ImmunoMolecular Therapeutics for which he holds shares and serves as Chief Medical Officer and is a board member. He has received research support from the National Institutes of Health, Helmsley Charitable Trust, the Juvenile Diabetes Research Foundation, Nova Pharmaceuticals, Intrexon T1D Partners, Novartis, Imcyse, and Provention Bio. ALa received salary funding from JDRF in partnership with Diabetes UK. EL is a former Regeneron Pharmaceuticals employee and owns stock in the company. PAS holds the Charles A Allard Chair in Diabetes Research. He is a co-investigator in Diabetes Action Canada, and the board chair of Diabetes Canada. He has received consulting fees or honoraria from Abbott, Bayer, Eli Lilly, Insulet, Novo Nordisk, and Vertex. ALo, BG, EA, FW, and KSC are employees of the Critical Path Institute, a non-profit organisation supported by the US Food and Drug Administration of the Department of Health and Human Services. The Critical Path Institute is 55% funded by the Food and Drug Administration of the Department of Health and Human Services, totalling US $17 612 250, and 45% funded by non-government sources, totalling $14 203 111. The contents are those of the authors and do not necessarily represent the official views of, nor are an endorsement by, the Food and Drug Administration of the Department of Health and Human Services or the US Government. The Type 1 Diabetes Consortium at the Critical Path Institute receives funding from the following organisations: JDRF, Helmsley Charitable Trust, Novo Nordisk, Provention Bio, Sanofi, and Diamyd Medical. EA is a former employee of Abcam and owns stock in the company. All other authors declare no competing interests.

Data sharing

The Trial Outcome Markers Initiative on type 1 diabetes has generated an aggregated database of deidentified, participant-level data from 21 historical clinical trials and observational studies in new-onset type 1 diabetes. This database will be used to develop a publicly available clinical trial simulation platform and accompanying graphical user interface, which will be readily accessible via the Critical Path Institute's website.

For the **Critical Path Institute website** see https://c-path.org

For more on the **clinical trial simulation tool** see https://c-path.org/programs/tomi-t1d/

Acknowledgments

We thank the participants who participated in these clinical trials and the researchers from the clinical trials who enabled the data to be used in the Trial Outcome Markers Initiative project. We would like to acknowledge the data provided by the National Institute of Diabetes and Digestive and Kidney Diseases Central Repository. Data were used through the Type 1 Diabetes TrialNet Study Group, a clinical trials network funded through a cooperative agreement by the National Institutes of Health through the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Allergy and Infectious Diseases, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, and JDRF. In addition, we recognise the following data contributors: Diamyd Medical, Stockholm, Sweden; George Washington University, Washington DC, USA; GlaxoSmithKline, Brentford, UK; Harvard University, Cambridge, MA, USA; Immune Tolerance Network, Seattle, WA, USA; Jaeb Center for Health Research, Tampa, FL, USA; Janssen Pharmaceutical Companies of Johnson and Johnson, Titusville, NJ, USA; Linköping University, Linköping, Sweden; Novo Nordisk, Crawley, UK; Provention Bio, a Sanofi Company, Red Bank, NJ, USA; University of Birmingham, Birmingham, UK; University of California San Francisco, San Francisco, CA, USA; University of Colorado, Boulder, CO, USA; University of Exeter, Exeter, UK; University of Florida, Gainesville, FL, USA; and Wake Forest School of Medicine, Winston-Salem, NC, USA.

References

1 Quattrin T, Mastrandrea LD, Walker LSK. Type 1 diabetes. *Lancet* 2023; **401:** 2149–62.

2 Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care* 2015; **38:** 1964–74.

3 Miller KM, Beck RW, Foster NC, Maahs DM. HbA1c levels in type 1 diabetes from early childhood to older adults: a deeper dive into the influence of technology and socioeconomic status on HbA1c in the T1D exchange clinic registry findings. *Diabetes Technol Ther* 2020; **22:** 645–50.

4 Rawshani A, Sattar N, Franzén S, et al. Excess mortality and cardiovascular disease in young adults with type 1 diabetes in relation to age at onset: a nationwide, register-based cohort study. *Lancet* 2018; **392:** 477–86.

5 Livingstone SJ, Levin D, Looker HC, et al. Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008–2010. *JAMA* 2015; **313:** 37–44.

6 Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. *Diabet Med* 2013; **30:** 803–17.

7 Allen LA, Dayan CM. Immunotherapy for type 1 diabetes. *Br Med Bull* 2021; **140:** 76–90.

8 Pearson JA, McKinney EF, Walker LSK. 100 years post-insulin: immunotherapy as the next frontier in type 1 diabetes. *Immunother Adv* 2021; **1:** ltab024.

9 Forlenza GP, McVean J, Beck RW, et al. Effect of verapamil on pancreatic beta cell function in newly diagnosed pediatric type 1 diabetes: a randomized clinical trial. *JAMA* 2023; **329:** 990–99.

10 Herold KC, Gitelman SE, Ehlers MR, et al. Teplizumab (anti-CD3 mAb) treatment preserves C-peptide responses in patients with new-onset type 1 diabetes in a randomized controlled trial: metabolic and immunologic features at baseline identify a subgroup of responders. *Diabetes* 2013; **62:** 3766–74.

11 von Herrath M, Bain SC, Bode B, et al. Anti-interleukin-21 antibody and liraglutide for the preservation of β-cell function in adults with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol* 2021; **9:** 212–24.

12 Rigby MR, DiMeglio LA, Rendell MS, et al. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol* 2013; **1:** 284–94.

13 Quattrin T, Haller MJ, Steck AK, et al. Golimumab and beta-cell function in youth with new-onset type 1 diabetes. *N Engl J Med* 2020; **383:** 2007–17.

14 Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N Engl J Med* 2009; **361:** 2143–52.

15 Orban T, Bundy B, Becker DJ, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011; **378:** 412–19.

16 Haller MJ, Schatz DA, Skyler JS, et al. Low-dose anti-thymocyte globulin (ATG) preserves β-cell function and improves HbA_{1c} in new-onset type 1 diabetes. *Diabetes Care* 2018; **41:** 1917–25.

17 Gitelman SE, Bundy BN, Ferrannini E, et al. Imatinib therapy for patients with recent-onset type 1 diabetes: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol* 2021; **9:** 502–14.

18 Sherry N, Hagopian W, Ludvigsson J, et al. Teplizumab for treatment of type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial. *Lancet* 2011; **378:** 487–97.

19 Palmer JP, Fleming GA, Greenbaum CJ, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21–22 October 2001. *Diabetes* 2004; **53:** 250–64.

20 Jeyam A, Colhoun H, McGurnaghan S, et al. Clinical impact of residual C-peptide secretion in type 1 diabetes on glycemia and microvascular complications. *Diabetes Care* 2021; **44:** 390–98.

21 Gubitosi-Klug RA, Braffett BH, Hitt S, et al. Residual β cell function in long-term type 1 diabetes associates with reduced incidence of hypoglycemia. *J Clin Invest* 2021; **131:** e143011.

22 Hagopian W, Ferry RJ Jr, Sherry N, et al. Teplizumab preserves C-peptide in recent-onset type 1 diabetes: two-year results from the randomized, placebo-controlled Protégé trial. *Diabetes* 2013; **62:** 3901–08.

23 Aronson R, Gottlieb PA, Christiansen JS, et al. Low-dose otelixizumab anti-CD3 monoclonal antibody DEFEND-1 study: results of the randomized phase III study in recent-onset human type 1 diabetes. *Diabetes Care* 2014; **37:** 2746–54.

24 Ambery P, Donner TW, Biswas N, Donaldson J, Parkin J, Dayan CM. Efficacy and safety of low-dose otelixizumab anti-CD3 monoclonal antibody in preserving C-peptide secretion in adolescent type 1 diabetes: DEFEND-2, a randomized, placebo-controlled, double-blind, multi-centre study. *Diabet Med* 2014; **31:** 399–402.

25 Ludvigsson J, Eriksson L, Nowak C, et al. Phase III, randomised, double-blind, placebo-controlled, multicentre trial to evaluate the efficacy and safety of rhGAD65 to preserve endogenous beta cell function in adolescents and adults with recently diagnosed type 1 diabetes, carrying the genetic HLA DR3-DQ2 haplotype: the DIAGNODE-3 study protocol. *BMJ Open* 2022; **12:** e061776.

26 Greenbaum CJ, Serti E, Lambert K, et al. IL-6 receptor blockade does not slow β cell loss in new-onset type 1 diabetes. *JCI Insight* 2021; **6:** e150074.

27 Narendran P, Jackson N, Daley A, et al. Exercise to preserve β-cell function in recent-onset Type 1 diabetes mellitus (EXTOD)—a randomized controlled pilot trial. *Diabet Med* 2017; **34:** 1521–31.

28 Pozzilli P, Bosi E, Cirkel D, et al. Randomized 52-week phase 2 trial of albiglutide versus placebo in adult patients with newly diagnosed type 1 diabetes. *J Clin Endocrinol Metab* 2020; **105:** dgaa149.

29 Buckingham BA, Beck RW, Ruedy KJ, et al. The effects of inpatient hybrid closed-loop therapy initiated within 1 week of type 1 diabetes diagnosis. *Diabetes Technol Ther* 2013; **15:** 401–08.

30 Lagarde WH, Courtney KL, Reiner B, Steinmann K, Tsalikian E, Willi SM. Human plasma-derived $alpha_1$-proteinase inhibitor in patients with new-onset type 1 diabetes mellitus: a randomized, placebo-controlled proof-of-concept study. *Pediatr Diabetes* 2021; **22:** 192–201.

31 Gitelman SE, Gottlieb PA, Rigby MR, et al. Antithymocyte globulin treatment for patients with recent-onset type 1 diabetes: 12-month results of a randomised, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol* 2013; **1:** 306–16.

32 Gottlieb PA, Quinlan S, Krause-Steinrauf H, et al. Failure to preserve beta-cell function with mycophenolate mofetil and daclizumab combined therapy in patients with new-onset type 1 diabetes. *Diabetes Care* 2010; **33:** 826–32.

33 Wherrett DK, Bundy B, Becker DJ, et al. Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial. *Lancet* 2011; **378:** 319–27.

34 Moran A, Bundy B, Becker DJ, et al. Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. *Lancet* 2013; **381:** 1905–15.

35 Mortensen HB, Hougaard P, Swift P, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. *Diabetes Care* 2009; **32:** 1384–90.

36 Forbes S, Oram RA, Smith A, et al. Validation of the BETA-2 score: an improved tool to estimate beta cell function after clinical islet transplantation using a single fasting blood sample. *Am J Transplant* 2016; **16:** 2704–13.

37 Takita M, Matusmoto S. SUITO index for evaluation of clinical islet transplantation. *Cell Transplant* 2012; **21:** 1341–47.

38 Lam A, Oram RA, Forbes S, et al. Estimation of early graft function using the BETA-2 score following clinical islet transplantation. *Transpl Int* 2022; **35:** 10335.

39 Amiel SA, Aschner P, Childs B, et al. Glucose concentrations of less than 3·0 mmol/L (54 mg/dL) should be reported in clinical trials: a joint position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2017; **40:** 155–57.