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Immunomodulatory interventions in type 1 diabetes: a systematic review and meta-analysis revealing paradoxical dissociation between beta-cell preservation and glycemic control

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Abstract

Background Immunomodulatory therapies aim to preserve beta-cell function in type 1diabetes (T1D), yet their clinical translation remains inconsistent. Weconducted a systematic review and meta-analysis to quantify treatment effectson beta-cell preservation, glycemic control, and insulin requirements whileexamining time-dependent efficacy and safety profiles.

Methods We searched PubMed, Embase, and Cochrane CENTRAL for randomizedcontrolled trials evaluating immunomodulatory interventions (teplizumab,otelixizumab, low-dose IL-2) in T1D patients. Primary outcomes includedC-peptide area under curve, HbA1c, and insulin requirements analyzed asstandardized mean differences (SMD) using random-effects models.Meta-regression examined time-dependent treatment attenuation.

Results Nineteen studies ($n = 1,852$) were analyzed. Immunotherapy significantlypreserved C-peptide (SMD = 0.221, 95%CI: 0.069–0.373, $p = 0.007$) without improvingHbA1c (SMD = 0.033, 95%CI: −0.070–0.136, $p = 0.488$), demonstratingbiomarker-clinical outcome disconnect. Paradoxically, low-dose IL-2 producedmassive regulatory T-cell expansion (SMD = 2.23, $p = 0.003$) and improved HbA1c(SMD = −0.514, $p = 0.025$) without preserving C-peptide (SMD = 0.020, $p = 0.967$),suggesting non-immunological metabolic pathways. Insulin requirements showedmodest reduction in sensitivity analysis (SMD = −0.453, $p = 0.021$) with significanttime-dependent attenuation ($R^2 = 49.06\%$, $p = 0.009$), indicating benefits fadeprogressively. WBCs Clearance agents demonstrated 3.2-fold higher seriousadverse

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event rates than Treg Enhancement agents (34.5% vs 10.8%), with all five reported deaths occurring with WBCs Clearance.

Conclusion Immunomodulation provides modest, temporary beta-cell preservationwithout consistent glycemic improvement. The C-peptide-HbA1c disconnect likelyreflects intensive insulin management masking treatment benefits. IL-2'sparadoxical HbA1c improvement without beta-cell preservation warrants mechanistic investigation. Time-dependent benefit attenuation suggests maintenance therapy may be necessary. Safety profiles favor Treg Enhancementover WBCs Clearance approaches. Future trials should employ continuous glucosemonitoring endpoints, longer follow-up, and combination strategies to optimizedisease-modifying therapy development.

Clinical trial number Not applicable.

Language model assistance

Large language models (specifically Claude 4, Anthropic) were used to assist with grammar checking, manuscript formatting, and language editing to improve clarity and readability. All scientific content, data analysis, interpretation, and conclusions were developed by the authors.

Keywords Type 1 diabetes, Immunotherapy, Teplizumab, C-peptide preservation, Meta-analysis, Regulatory t cells, Disease-modifying therapy

Introduction

Type 1 diabetes (T1D) affects approximately 9.5 million individuals globally, with incidence increasing 2.4% annually [1]. The disease results from autoimmune destruction of pancreatic beta-cells, leading to absolute insulin deficiency and lifelong dependence on exogenous insulin therapy. Despite remarkable advances in insulin delivery technologies and continuous glucose monitoring, patients continue to face substantial burdens including hypoglycemia, glycemic variability, and long-term microvascular and macrovascular complications. Current management remains symptomatic, addressing insulin deficiency without halting the underlying autoimmune process. This reality underscores the critical unmet need for disease-modifying therapies that preserve endogenous beta-cell function and alter the natural history of T1D.

Preservation of residual beta-cell function, measured by C-peptide secretion, offers substantial clinical benefits even at remarkably low levels. Long-term observational studies from the Diabetes Control and Complications Trial (DCCT) and its follow-up study (EDIC) demonstrate that even modest C-peptide preservation is associated with reduced hypoglycemia risk, lower insulin requirements, and improved glycemic stability [2]. Critically, these benefits occur across the entire measurable range of C-peptide with no apparent threshold, even increases from 0.10 to 0.15 nmol/L are associated with clinically meaningful reductions in severe hypoglycemia (8.2% risk reduction) and sustained retinopathy (25% risk reduction) [2]. The long-term protective effects persist for decades after diagnosis, with patients maintaining C-peptide levels as low as 0.03 nmol/L demonstrating significantly reduced severe hypoglycemia rates compared to those with undetectable C-peptide [3]. These findings establish beta-cell preservation as a rational therapeutic target, with C-peptide serving as the primary efficacy biomarker in clinical trials and recently gaining acceptance as a validated surrogate endpoint for demonstrating clinical benefit in disease-modifying therapy development [4].

Recognizing T1D's autoimmune etiology, numerous immunomodulatory strategies have been investigated over the past two decades. Early landmark trials with anti-CD3 monoclonal antibodies demonstrated proof-of-concept that short-course immunotherapy could preserve insulin production in new-onset patients [5]. These T-cell directed therapies aim to deplete or modulate autoreactive T cells through partial agonist signaling on CD3, potentially enhancing regulatory T-cell function while reducing effector responses. In parallel, alternative approaches have emerged, including regulatory T-cell (Treg) enhancement strategies using low-dose interleukin-2, which selectively expand immunosuppressive CD4+CD25+FOXP3+ populations to restore immune tolerance. The landscape expanded further with antigen-specific therapies, co-stimulation blockade, B-cell depletion, and various anti-inflammatory agents, each targeting distinct nodes in the autoimmune cascade. Recently, teplizumab achieved a major regulatory milestone as the first FDA-approved disease-modifying therapy for T1D, demonstrating a median 32.5-month delay in disease onset when administered to high-risk relatives before clinical diagnosis [6]. This approval represents a paradigm shift from purely symptomatic management toward addressing underlying disease pathogenesis.

Despite two decades of clinical investigation and this recent regulatory success, critical questions remain regarding the translation of immunotherapy from biological efficacy to sustained clinical benefit. Meta-analyses have consistently documented that nonantigen-based immunotherapies preserve C-peptide and reduce insulin

dosage, yet fail to produce substantial improvements in HbA1c or fasting plasma glucose [7]. Individual trials exemplify this disconnect: the Protégé trial demonstrated statistically significant C-peptide preservation at 2 years yet showed no differences in HbA1c between teplizumab and placebo groups at any timepoint [8]. Whether this biomarker-clinical outcome discordance reflects fundamental limitations in the trial design particularly intensive insulin management protocols applied equally to both study arms with insufficient statistical power to detect glycemic differences, inadequate effect sizes to overcome normal glycemic variability, or genuine biological constraints on translating beta-cell preservation into measurable glycemic improvement remains debated [9]. The resolution of this paradox has profound implications for endpoint selection, trial design, and regulatory pathways for future therapies.

Mechanistic understanding of therapeutic success and failure is similarly incomplete. Regulatory T-cell expansion strategies, while immunologically compelling, have shown particularly variable clinical translation. Low-dose IL-2 therapy consistently produces robust, dose-dependent Treg expansion with increases reaching 9.6-fold above baseline at optimal doses yet metabolic benefits remain inconsistent or absent despite dramatic immunological responses [10]. The disconnect between impressive cellular immunology outcomes and clinical metabolic endpoints suggests either that numerical Treg expansion does not equal functional immunosuppressive capacity, that the duration of Treg elevation (typically days to weeks) is insufficient for diseases requiring months to years of sustained immune regulation, or that compensatory activation of other immune populations negates intended tolerogenic effects. Safety profiles vary substantially across intervention classes, with early T-cell depleting approaches experiencing significant adverse events including dose-limiting Epstein-Barr virus reactivation and cytokine release syndrome [11], while alternative strategies appear better tolerated. Furthermore, the durability of therapeutic benefits remains poorly characterized, with some studies suggesting systematic time-dependent attenuation of treatment effects even during continuous therapy [12]. Patient selection criteria and optimal intervention timing, whether at clinical onset, during the honeymoon period, or in the presymptomatic stage remain inadequately defined despite emerging evidence that baseline metabolic status, age, human leukocyte antigen genotype, and autoantibody profiles may predict differential treatment responses.

These inconsistencies and knowledge gaps motivated the current systematic review and meta-analysis. Our primary objectives were to: (1) quantify treatment effects of immunomodulatory interventions on beta-cell preservation (C-peptide), glycemic control (HbA1c), and insulin requirements across randomized controlled trials; (2) examine the relationship between biomarker improvements and clinical outcomes to address the C-peptide-HbA1c translation paradox; (3) assess durability of therapeutic benefits through meta-regression analysis of follow-up duration as a moderator of treatment effects; (4) compare efficacy and safety profiles across distinct intervention mechanisms, particularly T-cell depletion versus regulatory T-cell enhancement approaches; and (5) identify sources of heterogeneity in treatment responses to inform patient selection and trial design. By systematically synthesizing evidence across trials, this analysis aims to clarify contradictions in the literature, quantify the magnitude and consistency of treatment effects, and provide evidence-based guidance for optimizing future clinical trial design and therapeutic development in type 1 diabetes.

Methods

Search strategy and study selection

We included randomized controlled trials evaluating Treg analogs or immunomodulatory interventions such as immunity washout agents or Treg enhancer agents (teplizumab, otelixizumab, low-dose IL-2, etc) in type 1 diabetes patients that reported C-peptide, HbA1c, or daily insulin requirements with sufficient data for meta-analysis. Studies lacking standard deviations necessary for effect size calculation were excluded.

A comprehensive literature search was performed across PubMed, Embase by ovid, and the Cochrane Central Register of Controlled Trials (CENTRAL) to identify randomized controlled trials (RCTs). The detailed search strategy (Supplementary Table 1) combined Medical Subject Headings (MeSH) and free-text terms related to the population, intervention and outcomes.

Data extraction

Two reviewers independently extracted data from included studies using standardized electronic forms. Extracted information included: study characteristics (first author, publication year, trial name, country, design, follow-up duration), participant demographics (sample size, age, sex distribution, diabetes duration, time from diagnosis to enrollment), intervention details (drug name, dose, administration schedule, number of courses), comparator (placebo or standard care), and outcome data (means, standard deviations, sample sizes at each timepoint). For studies reporting outcomes at multiple timepoints, we extracted data from all available timepoints and selected the primary timepoint for main analyses based on study-specified primary endpoints or, when unspecified, the longest follow-up duration reported. Discrepancies in data extraction were resolved through discussion and verification against original publications.

Complete data extraction is provided in (Supplementary Table 5).

Outcomes

Primary outcomes

(1) change in stimulated C-peptide secretion from baseline, measured as area under the curve (AUC) during mixed-meal tolerance tests, representing beta-cell function preservation; (2) change in glycated hemoglobin (HbA1c) from baseline, representing glycemic control; and (3) daily insulin requirements analyzed as standardized mean differences, representing clinical insulin dependency.

Secondary outcomes

included: (1) serious adverse events (SAEs), defined as any adverse event resulting in death, life-threatening consequences, hospitalization, persistent disability, or other medically important conditions, analyzed using proportion meta-analysis; (2) regulatory T-cell (Treg) expansion, quantified as change in CD4+CD25+FOXP3+ cell frequency or absolute count, analyzed using standardized mean differences; and (3) additional immunological and safety outcomes including Epstein-Barr virus reactivation rates, anti-drug antibody development, CD4:CD8 ratios, CD8+ T-cell exhaustion markers, treatment discontinuation rates, mortality, and Grade 3/4 adverse events, which were synthesized descriptively due to heterogeneous reporting. Detailed safety outcomes are in (Supplementary Table 6), and immunological outcomes are summarized in (Supplementary Table 3).

Statistical analysis

Effect size metrics

For all primary outcomes including C-peptide, HbA1c, and insulin requirements, we calculated Hedges' g, a bias-corrected standardized mean difference that adjusts for small sample bias by applying a correction factor [13]. Hedges' g was chosen over Cohen's d due to the presence of studies with small sample sizes ($n < 20$ per group) in our dataset. This approach enables consistent cross-outcome comparisons while maintaining interpretability. For serious adverse events, we calculated event proportions with 95% confidence intervals using the Freeman-Tukey double arcsine transformation to stabilize variance, particularly important for proportions near boundaries (0% or 100%). Effect sizes were interpreted using conventional benchmarks: for standardized mean differences, $|g| = 0.2$ represents a small effect, $|g| = 0.5$ a medium effect, and $|g| = 0.8$ a large effect.

Meta-analytic models

All meta-analyses employed random-effects models using the restricted maximum-likelihood (REML) estimator for between-study variance (τ^2), which provides less biased estimates than the DerSimonian-Laird method, particularly when heterogeneity is present or the number of studies is small. We applied the Hartung-Knapp adjustment to confidence intervals and p-values, which uses a t-distribution rather than the standard normal distribution and provides more conservative inference when the number of studies is limited [14]. This adjustment is particularly important for meta-analyses with fewer than 10 studies, as it reduces Type I error rates when between-study heterogeneity is substantial.

Heterogeneity was quantified using multiple metrics: (1) the I^2 statistic [15], representing the percentage of total variation across studies attributable to heterogeneity rather than sampling error, with interpretation thresholds of 0–40% indicating heterogeneity that might not be important, 30–60% representing moderate heterogeneity, 50–90% substantial heterogeneity, and 75–100% considerable heterogeneity; (2) τ^2, the estimated between-study variance; and (3) Cochran's Q-test, with $p < 0.10$ indicating statistically significant heterogeneity. When interpreting I^2, we considered the magnitude and direction of effects alongside the I^2 value, as recommended in contemporary meta-analysis guidelines.

Primary versus sensitivity analysis strategy

Given substantial initial heterogeneity in preliminary analyses, we employed a two-tiered analytic strategy balancing interpretability with comprehensiveness. Primary analyses excluded studies identified as statistical outliers (standardized residuals > 3.0) or those contributing disproportionately to heterogeneity based on influence diagnostics, providing more homogeneous and interpretable effect estimates. Sensitivity analyses included all eligible studies to ensure comprehensive evaluation and assess robustness of findings. This approach allows transparent assessment of how outlier studies influence overall conclusions while maintaining statistical rigor.

For C-peptide, primary analysis included 17 arms from 11 studies ($n = 1{,}358$; $I^2 = 20.1\%$) while sensitivity analysis included 20 arms from 14 studies ($n = 1{,}852$; $I^2 = 61.0\%$), excluding 3 outliers (Herold 2013-Diabetes, Ambery 2014, Ramos 2023) plus 5 studies with missing variance data. For HbA1c, primary analysis included 11 arms from 7 studies ($n = 1{,}473$; $I^2 = 0.0\%$) while sensitivity analysis included 14 arms from 10 studies ($n = 1{,}603$; $I^2 = 57.6\%$), excluding 3 outliers (Keymeulen 2010, Herold 2005, Herold 2002). For insulin requirements, primary analysis included 12 arms from 8 studies ($n = 898$; $I^2 = 70.1\%$) while sensitivity analysis included 15 arms from 11 studies ($n = 1{,}394$; $I^2 = 77.3\%$), excluding 3 outliers (Keymeulen 2010, Ramos 2023, Aronson 2014). Serious adverse events analysis included all 4 studies with complete SAE reporting ($n = 277$; $I^2 = 79.1\%$).

Complete justification for outlier identification and exclusion criteria is provided in supplementary materials, including standardized residuals, Cook's distances, and leverage statistics for all studies.

Subgroup and meta-regression analyses

We conducted subgroup analyses stratified by intervention type (teplizumab, otelixizumab, low-dose IL-2) using random-effects models within each subgroup. Between-subgroup heterogeneity was assessed using the Q-test for subgroup differences, with $p < 0.05$ indicating statistically significant differences in treatment effects across intervention classes. For insulin requirements, we additionally stratified analyses by timing of assessment (≤12 months versus > 12 months follow-up) to examine potential time-dependent patterns in treatment effects. For serious adverse events, we compared proportions between mechanistic categories: Treg Enhancement agents (low-dose IL-2) versus WBCs (whole blood cells) Clearance agents (anti-CD3 antibodies), reflecting fundamental differences in mechanism of action while selective immunoregulatory enhancement versus broad T-cell depletion.

Random-effects meta-regression examined potential moderators of treatment effects using mixed-effects models. For continuous moderators, we analyzed follow-up duration (in months) to assess time-dependent attenuation of treatment benefits. For categorical moderators, we examined intervention type. Meta-regression results are reported as regression coefficients (β) with 95% confidence intervals, p-values testing the null hypothesis that $\beta = 0$, and R^2 values representing the proportion of between-study heterogeneity explained by the covariate. Statistical significance was set at $p < 0.05$ for all meta-regression analyses.

Regulatory T-cell expansion meta-analysis

For the Hartemann (2013) study, which compared three doses of low-dose IL-2 (0.33, 1.0, and 3.0 MIU/day × 5 days) versus placebo in evaluating Treg expansion, we treated each dose-placebo comparison as a separate study arm in the meta-analysis. This approach appropriately accounts for within-study correlation while maximizing information extraction from the dose-finding design. Effect sizes were pooled using random-effects models. Leave-one-out sensitivity analysis assessed the influence of individual dose comparisons on the overall pooled estimate by iteratively removing each comparison and recalculating the summary effect.

Publication bias and additional sensitivity analyses

Publication bias was assessed using funnel plots with visual inspection for asymmetry (Supplementary Figures 4–8) and Egger's regression test for funnel plot asymmetry where feasible (k ≥ 10 studies). For outcomes with fewer than 10 studies, formal statistical tests for publication bias were not conducted due to insufficient power. Leave-one-out influence analyses were performed for all primary outcomes to identify studies exerting disproportionate influence on pooled estimates. We compared results from random-effects versus common-effect (fixed-effect) models as an additional sensitivity check. Forest plots were generated for all meta-analyses showing individual study effects, pooled estimates, and heterogeneity statistics.

Statistical software

All statistical analyses were performed using R statistical software (version 4.5.1, R Foundation for Statistical Computing, Vienna, Austria) with the meta package (version 8.2.1) [16] for standard meta-analyses and the metafor package (version 4.8.0) [17] for meta-regression and advanced diagnostic analyses. Complete R code for all analyses is available upon request from the corresponding author.

Risk of bias assessment

Risk of bias assessment was conducted independently by two reviewers using the Cochrane Risk of Bias 2 (RoB 2) tool [18]. Each study was evaluated across five domains: (1) bias arising from the randomization process, (2) bias due to deviations from intended interventions, (3) bias due to missing outcome data, (4) bias in measurement of the outcome, and (5) bias in selection of the reported result. Overall risk of bias was classified as low, some concerns, moderate, or high according to RoB 2 criteria. Disagreements between reviewers were resolved through consensus discussion. Risk of bias assessments for individual studies are presented in (Supplementary Fig. 13) and summarized across studies in (Supplementary Table 7).

Reporting standards

This systematic review and meta-analysis adhered to the PRISMA 2020 reporting guidelines [19]. The completed PRISMA 2020 checklist is provided as Supplementary Material, with study selection detailed in (Fig. 1). The review protocol was not prospectively registered.

"The systematic review protocol was registered with the Open Science Framework (OSF) at https://osf.io/4y3sp."

Results

Study selection and characteristics

Our systematic search identified 19 studies across all outcomes [5, 8, 10–12, 20–33] (Fig. 1). The final analysis included:

- C-peptide: 20 study arms from 14 unique studies ($n = 1{,}852$ participants); primary analysis included

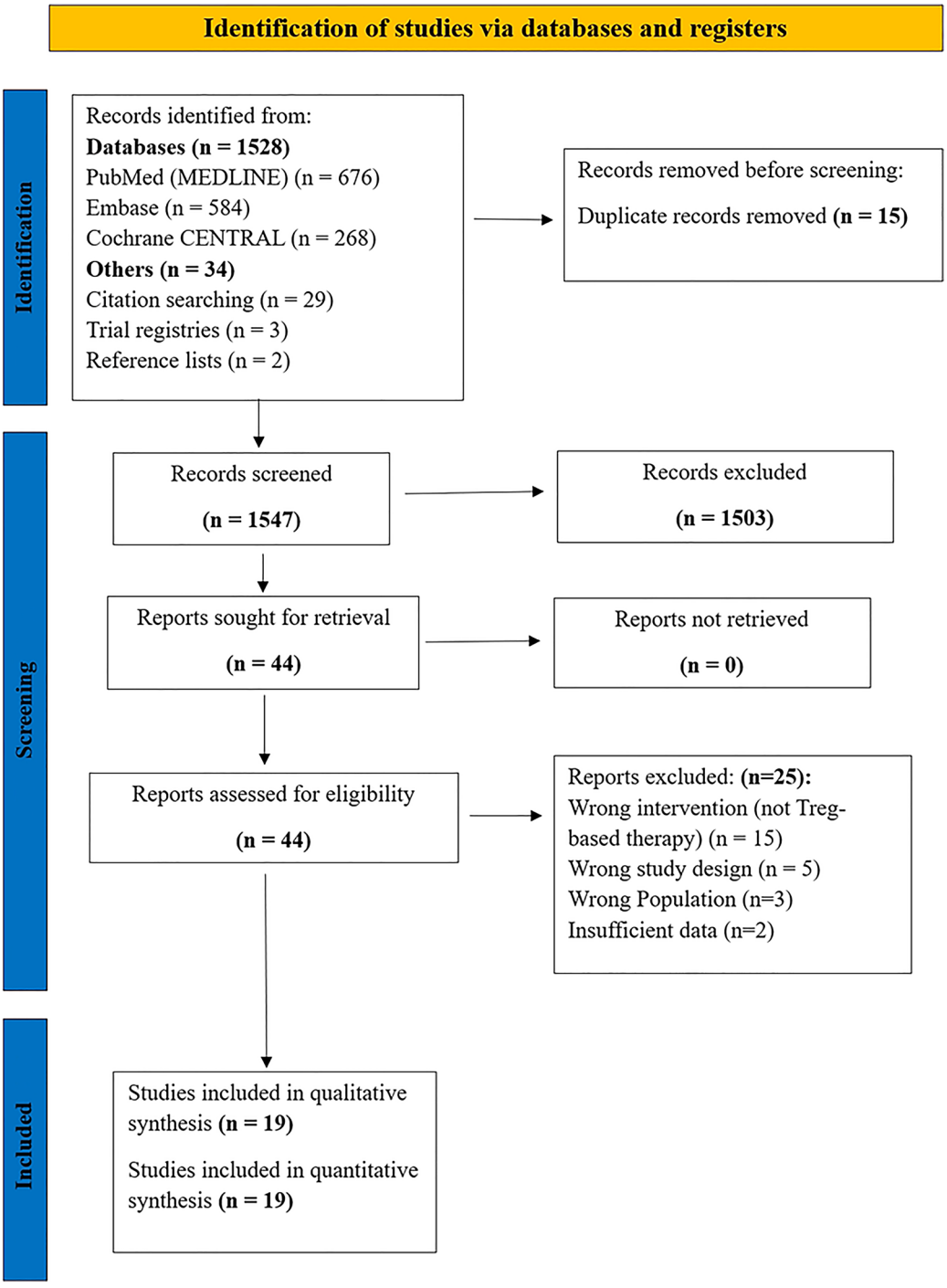

Fig. 1 Prisma flow diagram of study selection process. prisma flow diagram illustrating the systematic review and meta-analysis study selection process. A total of 1,528 records were identified from electronic databases including PubMed/MEDLINE (*n* = 676), Embase (*n* = 584), Cochrane central (*n* = 268), and other sources (*n* = 34), supplemented by citation searching (*n* = 29), trial registries (*n* = 3), and reference lists (*n* = 2). After removing 15 duplicates, 1,547 records were screened, with 1,503 excluded based on title and abstract. Forty-four full-text reports were assessed for eligibility, of which 25 were excluded: wrong intervention not targeting Treg-based therapy (*n* = 15), wrong study design (*n* = 5), wrong population (*n* = 3), and insufficient data (*n* = 2). Ultimately, 19 studies were included in both qualitative and quantitative synthesis

17 arms from 11 studies ($n = 1,358$) after excluding 5 studies with missing variance data and 3 outlier studies (Figure 2, Supplementary Figure 1)

- HbA1c: 14 arms from 10 studies ($n = 1,603$); primary analysis included 11 arms from 7 studies ($n = 1,473$) after excluding 3 outliers (Figure 3, Supplementary Figure 2)
- Insulin requirements: 15 study arms from 11 unique studies ($n = 1,394$); primary analysis included 12 arms from 8 studies ($n = 898$) after excluding 3 outliers (Figure 4, Supplementary Figure 3)

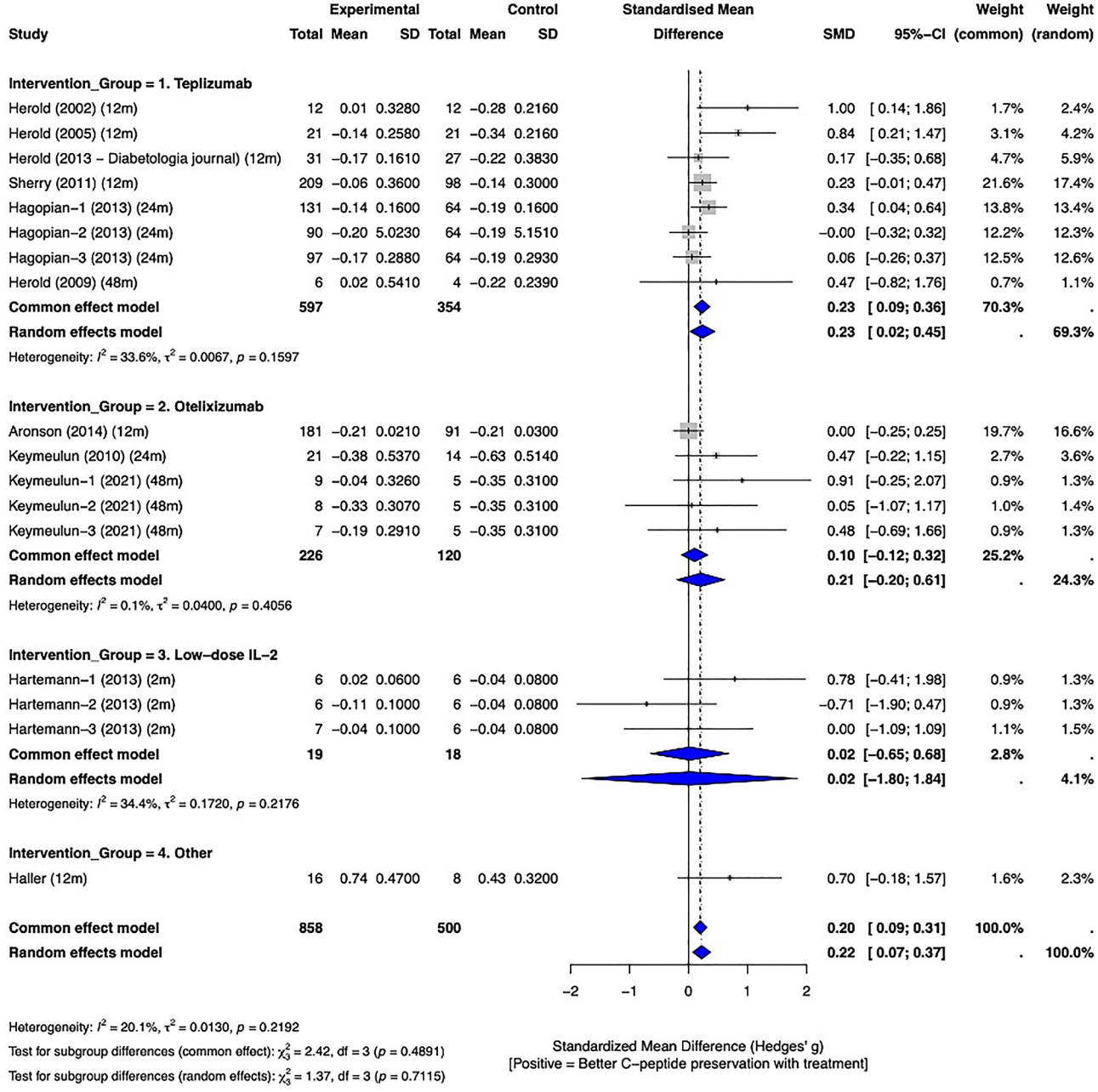

Fig. 2 Forest plot of C-peptide preservation outcomes by intervention type in patients with type 1 diabetes. Forest plot showing standardized mean differences (Hedges' g) for C-peptide levels comparing immunotherapy interventions to control across 11 studies with 17 treatment arms ($n = 858$ experimental, $n = 500$ control). Studies are stratified by intervention group: teplizumab (8 arms; $n = 597$ experimental, $n = 354$ control), Otelixizumab (5 arms; $n = 226$ experimental, $n = 120$ control), low-dose IL-2 (3 arms; $n = 19$ experimental, $n = 18$ control), and other interventions (1 arm; $n = 16$ experimental, $n = 8$ control). Positive values indicate better C-peptide preservation with treatment. Overall pooled effect: SMD = 0.22 (95% ci: 0.07–0.37, random effects model). Numbers in parentheses indicate follow-up duration in months. Diamond symbols represent pooled effects; horizontal lines indicate 95% confidence intervals. Square sizes are proportional to study weights. Overall heterogeneity: $I^2 = 20.1\%$, $p = 0.22$

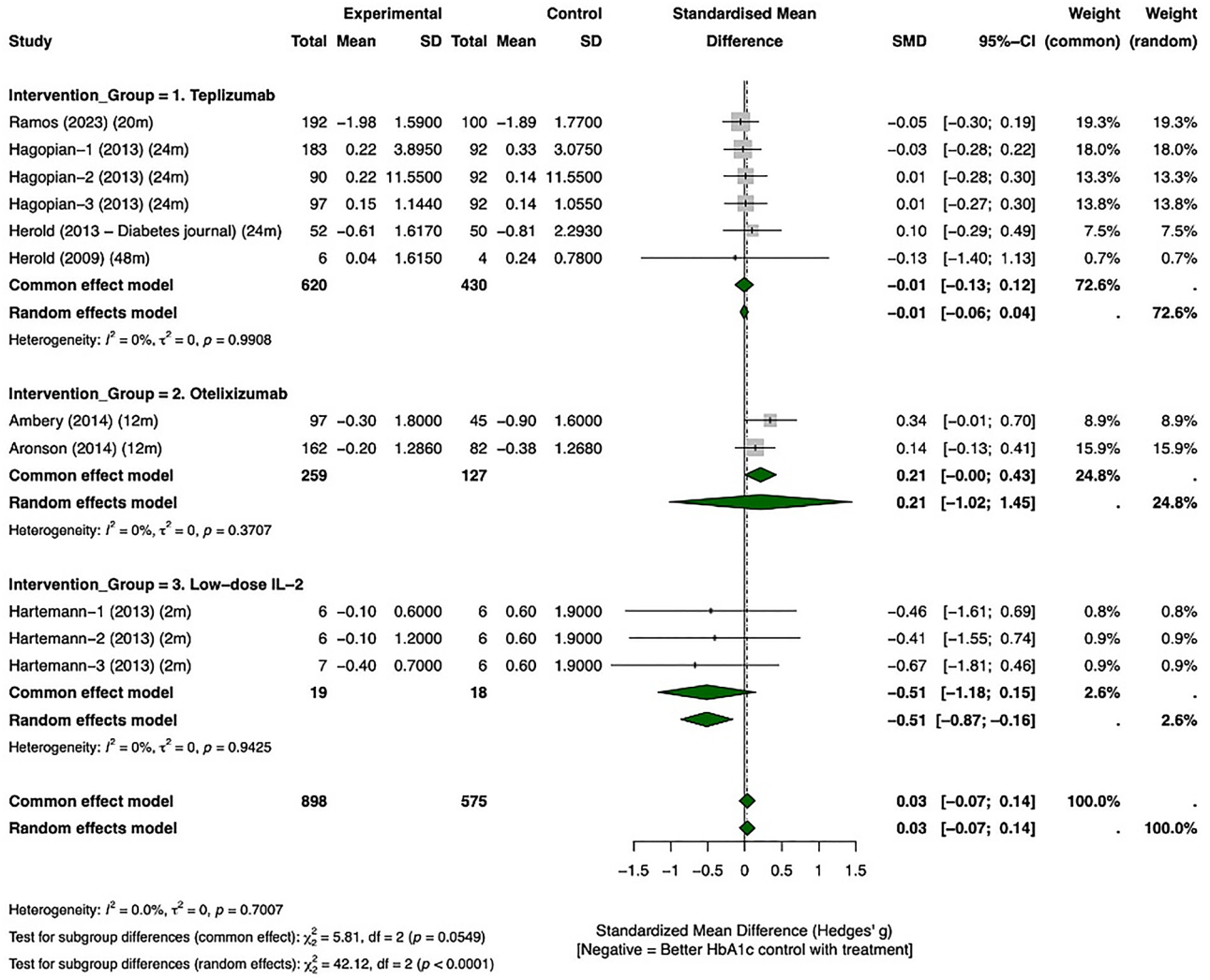

Fig. 3 Forest plot of HbA1c outcomes by intervention type in patients with type 1 diabetes. Forest plot showing standardized mean differences (Hedges' g) for HbA1c levels comparing immunotherapy interventions to control across 8 studies with 12 treatment arms (n = 898 experimental, n = 575 control). Studies are stratified by intervention group: teplizumab (5 studies, 7 arms; n = 620 experimental, n = 430 control), Otelixizumab (2 studies, 2 arms; n = 259 experimental, n = 127 control), and Low-dose IL-2 (1 study, 3 arms; n = 19 experimental, n = 18 control). Negative values indicate better HbA1c control with treatment. Overall pooled effect: SMD = 0.03 (95% ci: −0.07 to 0.14, random effects model), indicating no significant difference between treatment and control. Numbers in parentheses indicate follow-up duration in months. Diamond symbols represent pooled effects; horizontal lines indicate 95% confidence intervals. Square sizes are proportional to study weights. Overall heterogeneity: I^2 = 0.0%, p = 0.70

- Serious adverse events: 4 studies with complete SAE reporting (n = 277 participants, 39 events) (Supplementary Figure 9)

Interventions evaluated included Teplizumab (6–10 arms depending on outcome), Otelixizumab (2–6 arms), and low-dose IL-2 (3 arms). Studies predominantly enrolled pediatric and young adult patients with recent-onset type 1 diabetes (within first year of diagnosis). Follow-up ranged from 2 to 24 months.

Primary outcome 1: C-peptide auc (beta-cell function preservation)

Primary Analysis (17 studies, n = 1,358) Immunomodulatory interventions significantly preserved C-peptide production compared to placebo/control (Fig. 2):

- Hedges' g = 0.221 (95% CI: 0.069 to 0.373)
- p = 0.0072 (statistically significant)
- I^2 = 20.1% (low heterogeneity)

This small but statistically significant effect indicates better preservation of beta-cell function in treated patients. The low heterogeneity suggests consistency across studies.

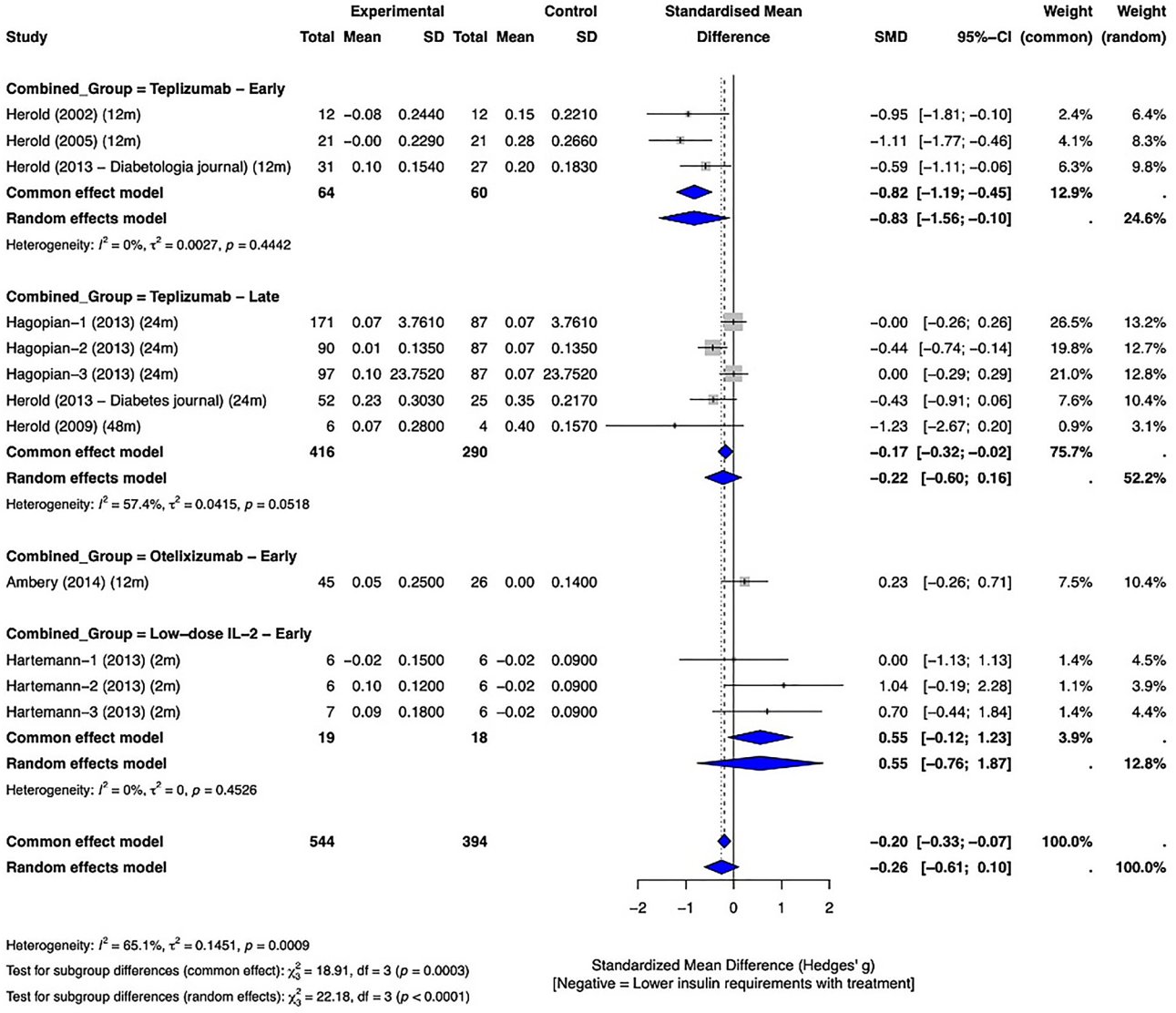

Fig. 4 Forest plot of insulin dose requirements by intervention type and timing in patients with type 1 diabetes. Forest plot showing standardized mean differences (Hedges' g) for insulin requirements comparing immunotherapy interventions to control across 8 studies with 12 treatment arms (n = 544 experimental, n = 394 control). Studies are stratified by intervention and timing: teplizumab-early (3 studies, 3 arms; n = 64 experimental, n = 60 control), teplizumab-late (3 studies, 5 arms; n = 416 experimental, n = 290 control), otelixizumab-early (1 study, 1 arm; n = 19 experimental, n = 18 control), and low-dose IL-2-Early (1 study, 3 arms; n = 45 experimental, n = 26 control). Negative values indicate lower insulin requirements with treatment. Overall pooled effect: SMD = −0.26 (95% ci: −0.61 to 0.10, random effects model). Numbers in parentheses indicate follow-up duration in months. Diamond symbols represent pooled effects; horizontal lines indicate 95% confidence intervals. Square sizes are proportional to study weights. Overall heterogeneity: I^2 = 65.1%, p = 0.0009

Subgroup analysis by intervention type (Supplementary Table 4) revealed that only Teplizumab achieved statistical significance (SMD = 0.235, 95% CI: 0.023 to 0.446, p = 0.034), while Otelixizumab (SMD = 0.207, p = 0.229) and low-dose IL-2 (SMD = 0.020, p = 0.967) did not reach significance. However, the test for subgroup differences was not significant (Q = 1.37, p = 0.711), suggesting insufficient power to detect true differences between interventions.

Meta-regression found no significant relationship between follow-up duration and C-peptide effect size (β = 0.0084 per month, p = 0.354, R^2 = 3.56%), nor did intervention type significantly explain heterogeneity (p = 0.372, R^2 = 15.06%).

Sensitivity analysis including all 20 eligible studies showed a larger effect (g = 0.312, 95% CI: 0.111 to 0.512, p = 0.004) but with substantially higher heterogeneity (I^2 = 61.0%), supporting the primary analysis approach of excluding outliers (Supplementary Figure 1).

Primary outcome 2: HbA1c

Primary Analysis (11 studies, $n = 1,473$) Overall, immunomodulatory interventions did NOT significantly improve HbA1c (Fig. 3):

- Hedges' g = 0.033 (95% CI: −0.070 to 0.136)
- $p = 0.488$ (not statistically significant)
- $I^2 = 0.0\%$ (no heterogeneity)

The narrow confidence interval and complete absence of heterogeneity indicate high precision around a null effect. Despite preserving C-peptide, immunotherapy did not translate to improved glycemic control overall.

The IL-2 paradox - critical finding

Subgroup analysis revealed a striking and unexpected pattern (Supplementary Table 4). While Teplizumab (SMD = −0.009, 95% CI: −0.062 to 0.045, $p = 0.692$) and Otelixizumab (SMD = 0.213, 95% CI: −1.023 to 1.449, $p = 0.273$) showed no HbA1c benefit, low-dose IL-2 was the only intervention demonstrating significant improvement:

- Low-dose IL-2: Hedges' g = −0.514 (95% CI: −0.867 to −0.161)
- $p = 0.025$ (statistically significant)
- Medium-sized effect by Cohen's criteria
- Test for subgroup differences: Q = 42.12, $p < 0.0001$ (highly significant)

This finding is paradoxical because IL-2 showed essentially no C-peptide preservation effect (g = 0.020, $p = 0.967$ from Primary Outcome 1), yet significantly improved HbA1c. This breaks the expected biological pathway: preserved beta-cells → reduced insulin needs → improved HbA1c. The mechanism underlying IL-2's HbA1c benefit without beta-cell preservation remains unclear and warrants further investigation.

Meta-regression did not identify significant moderators (time: $p = 0.380$; intervention type:$p = 0.526$), and sensitivity analysis including all 14 studies showed similar non-significance (g = −0.154, $p = 0.200$) with substantial heterogeneity ($I^2 = 57.6\%$; (Supplementary Figure 2)).

Primary outcome 3: insulin requirements

The primary analysis (12 arms from 8 studies, $n = 898$) showed a trend toward reduced insulin requirements that did not reach statistical significance (Fig. 4, Tables 1 and 2, Supplementary Table 4):

- Hedges' g = −0.255 (95% CI: −0.614 to 0.104)
- $p = 0.146$ (not statistically significant)
- $I^2 = 65.1\%$ (substantial heterogeneity)

The sensitivity analysis including all 15 arms from 11 studies ($n = 1,394$) demonstrated a statistically significant reduction (Table 2, Supplementary Figure 3, Supplementary Table 4):

- Hedges' g = −0.453 (95% CI: −0.828 to −0.079)
- $p = 0.021$ (statistically significant)
- $I^2 = 86.8\%$ (considerable heterogeneity)

This represents a small-to-moderate effect size by Cohen's criteria, indicating meaningful reductions in insulin requirements with immunotherapy.

Time-dependent attenuation - major finding

To explore the substantial heterogeneity, we conducted meta-regression analyses (Supplementary Table 4). The most important finding was time-dependent attenuation of treatment benefits:

- Follow-up duration significantly predicted treatment effect ($p = 0.009$)
- $R^2 = 49.06\%$ (explains approximately half of heterogeneity)

Interpretation: Treatment benefits diminish progressively with longer follow-up periods, with the effect size decreasing as follow-up extends. Benefits are strongest immediately post-treatment but progressively attenuate over time, suggesting that single-course immunotherapy provides temporary rather than sustained disease modification.

Subgroup analysis by intervention and timing

Subgroup analysis revealed differential effects by intervention type and timing (Table 2, Supplementary Table 4). In the primary analysis, only Teplizumab at early timepoints achieved statistical significance (SMD = −0.827, 95% CI: −1.558 to −0.095, $p = 0.040$) (Table 2, MAIN - Teplizumab Early)**, r**epresenting a large effect size. Teplizumab at late timepoints showed a non-significant trend (SMD = −0.220, $p = 0.186$) (Table 2, MAIN - Teplizumab Late). Otelixizumab showed no effect (SMD = 0.228, $p = 0.356$ for early timepoint) (Table 2, MAIN - Otelixizumab Early), while low-dose IL-2 demonstrated numerically increased insulin requirements (SMD = 0.554, $p = 0.212$) (Table 2, MAIN - Low-dose IL-2 Early).

Secondary outcomes

Serious adverse events

Four studies with complete SAE reporting (Ludvigsson-1 2012, Ludvigsson-2 2012, Herold 2013-Diabetes, Herold 2009) contributed to the proportion meta-analysis ($n = 277$, 39 events) (Supplementary Figure 9). Individual study SAE rates ranged from 7.2% to 66.7%, with Herold

Table 1 Baseline characteristics of included studies

Study Information						Sample Size			Demographics					Baseline Clinical Parameters							Intervention Details						
Study ID	First Author	Year	Country	Study Design	Centers	Total N	Intervention N	Control N	Age (years)	Age Range	Male (%)	Disease Duration	Disease Category	HbA1c (%)	Insulin Dose (IU/kg/day)	Fasting C-peptide	C-peptide AUC	GAD+ (%)	IA2+ (%)	Multiple Autoantibodies	Intervention Type	Cell Source	Dose per Administration	Route	Total Doses	Treatment Duration	Follow-up Duration
1	Ambery	2014	Multi-country (11 countries)	RCT	127	179	118	61	Not reported	Dec-45	NR	≤90 days	Recent onset	7.4-7.9	Not reported	Not reported	0.70-0.77 (nmol/l×min)/min	≥1 required	≥1 required	≥1 required	Otelixizumab (anti-CD3 mAb)	N/A (antibody therapy)	3.1 mg total (escalating doses)	Intravenous	8	8 days	12 months
2	Aronson	2014	Multinational: U.S., Canada, Germany, Denmark, Spain, Finland, U.K., Italy, Sweden	RCT	Multicenter (9 countries)	272	181	91	Placebo: 25.3±7.2 Otelixizumab: 24.8±6.6	Dec-45	65.1	≤90 days	Recent onset	Placebo: 7.26±1.5%; Otelixizumab: 7.25±1.2%	0.36 IU/kg/day	Not separately reported	Placebo: 0.70±0.36; Otelixizumab: 0.75±0.42 (nmol/L×min)/min	≥1 required	≥1 required	≥1 required (GAD, IA2, ZnT8, or insulin)	Otelixizumab (anti-CD3ε mAb)	N/A (antibody therapy)	Day 1: 0.1 mg; Day 2: 0.2 mg; Day 3: 0.3 mg; Days 4-8: 0.5 mg/day (Total: 3.1 mg)	Intravenous	8	8 days	12 months
3	Hagopian	2013	Multi-country (USA, India, others)	RCT	Multicenter (not specified)	516	417	99	Not reported	Aug-35	NR	≤12 weeks	Recent onset	7.6-9.7 (regional variation)	0.47-0.98 (regional variation)	Not reported	0.65 nmol/L (0.53-0.77 by region)	Not reported	Variable by region	≥1 required	Teplizumab (anti-CD3 mAb)	N/A (antibody therapy)	~9,034 mg/m² (14-day full-dose)	Intravenous	14 per cycle × 2 cycles	14 days per cycle	24 months
4	Haller	2015	USA	RCT	3	25	17	8	24.6±9.9	Dec-45	71	1.04±0.55 years	Established	6.03-6.69	0.44-0.45	NR	0.71 nmol/L/min	Present	Present	≥1	ATG + GCSF	NR	ATG 2.5 mg/kg, G-CSF 6 mg	IV (ATG), SC (G-CSF)	ATG: 2, G-CSF: 6	12 weeks	12 months
5	Hartemann	2013	France	RCT	1	25	18	6	26.2-37.7	18-55	NR	12-28.5 months	Established	7.0-7.4	0.37-0.65	0.21-0.30 nmol/L	0.17-0.35 nmol/L/min	23/24	Jul-24	≥1	Low-dose IL-2	NR	0.33-3 MIU/day	Subcutaneous	5	5 days	60 days
6	Herold (2002)	2002	USA	RCT	4	24	12	12	13 median	7.5-30	75	<6 weeks	Newly diagnosed	8.27-9.27	0.44-0.57	0.20-0.21 nmol/L	111.5-133.2 nmol/L	17/24	Dec-24	≥1	Anti-CD3 mAb	NR	1.42-45.4 µg/kg	Intravenous	14	14 days	12-18 months
7	Herold (2005)	2005	USA	RCT	4	42	21	21	14.4 ± 1.24	7.5 - 30	62	>2 years	Established	8.38±0.32%	0.475±0.235	0.209±0.021	0.49±0.20	33/42	29/42	≥1	Teplizumab	NR	1.42 - 1818	Intravenous	NR	14 days or 12 days	24 months
8	Herold	2009	USA	RCT	4	10	6	4	13.1±4.5	Not specified	70	≤6 weeks (19-54 days)	Recent onset	6.68-7.65	0.28-0.38	Not reported	0.406-0.877 nmol/L	Required (% not reported)	Required (% not reported)	≥1 required	Teplizumab (anti-CD3 mAb)	N/A (antibody therapy)	Escalating: 460-1,818 mcg/m²/day	Intravenous	12	12 days	24 months (60 months extended)
9	Herold	2013	USA and Canada	RCT	6	83	52	25	12.7±4.7	Aug-30	56.2	<8 weeks (39±9 days)	Recent onset	7.43-7.70	0.39±0.22	Not reported	0.67-0.72 nmol/L (4-h)	76.5-95.7%	95.7-98.0%	≥1 required	Teplizumab (anti-CD3 mAb)	N/A (antibody therapy)	Escalating: 51-826 mg/m²/day	Intravenous	14 per cycle	14 days per cycle	24 months
10	Herold	2013	USA	RCT	4	63	34	29	12.4±4.7	Aug-26	57	4-12 months (7.1±2.4 months)	Established (4-12 months)	6.37-7.14	0.386-0.405	Not reported	0.6-0.618 nmol/L	55-74%	73-87%	≥1 required	Teplizumab (anti-CD3 mAb)	N/A (antibody therapy)	Escalating: 51-826 µg/m²/day	Intravenous	14	14 days	12 months
11	Keymeulen	2010	Belgium and Germany	RCT	5	80	40	40	27±6.5	Dec-39	66	21-25 days	Recent onset	8.1-8.8	0.39-0.46	Not reported	0.55-0.96 nmol/l×min	84-97%	Not separately reported	ICA: 71-79%	ChAglyCD3 (anti-CD3 mAb)	N/A (antibody therapy)	Not specified in follow-up paper	Intravenous	6	6 days	48 months
12	Keymeulen	2021	Belgium	Dose-escalation	5	30	24	6	22.2±3.3	16-27	65.5	<32 days	Recent onset	6.8-8.1	0.37-0.46	Not reported	0.55-0.80 nmol/l×min	26/29 (90%)	24/29 (83%)	28/29 (97%)	Otelixizumab (anti-CD3 mAb)	N/A (antibody therapy)	9mg, 18mg, or 27mg cumulative	Intravenous	6	6 days	24 months
13	Ludvigsson	2012	Multi-country (9 European)	RCT	63	334	219	115	13.0±2.3	Oct-20	51.8	<3 months (74±24 days)	Recent onset	7.1±1.2	0.57±0.30	0.286±0.162 nmol/L	0.66±0.32 nmol/L×h	Required (100%)	Not reported	≥1 required	GAD65 antigen therapy	N/A (protein antigen)	20 µg GAD-alum	Subcutaneous	4 or 2	270 days	15 months
14	Marek-Trzonkowska	2014	Poland	Non-RCT	Multiple	22	12	10	May-18	May-18	NR	<2 months	Newly diagnosed	NR	NR	>0.4 ng/mL	NR	Required	Required	≥1	Adoptive transfer	Autologous PBMC	10-30×10⁶/kg	Intravenous	01-Feb	1-2 infusions	12 months
15	Perdigoto	2019	USA (multi-center)	Follow-up study of RCT (AbATE trial)	6 medical centers	43	31	12	Returners: 12.03±0.47; Non-returners: 14.44±0.98	Aug-30	Control: 66.7%; Teplizumab: 58.1%	Control: 37.33±2.86 days; Teplizumab: 39.55±1.47 days	Recent onset	Control: 7.66±0.42%; Teplizumab: 7.42±0.19%	Control: 0.40±0.04; Teplizumab: 0.35±0.05	Not reported separately	Control: 0.50±0.05 nmol/l; Teplizumab: 0.52±0.03 nmol/l	All participants autoantibody positive	All participants autoantibody positive (anti-ICA512/IA-2)	≥1 required (anti-GAD65, anti-ICA512/IA-2, or ICA)	Teplizumab (Fc receptor non-binding anti-CD3 mAb)	N/A (antibody therapy)	Cycle 1: median 11.6 mg (IQR 5.7); Cycle 2: median 12.4 mg (IQR 5.08)	Intravenous	2	2 years (two treatment cycles)	Mean 7.02±0.15 years from baseline (median 6.70 years, range 5.32-9.22)
16	Ramos	2023	Multi-country (USA, Canada, Europe)	RCT	61	328	217	111	12.1±2.5	Aug-17	57.3	≤6 weeks (5.3±0.8 weeks)	Recent onset	9.00±1.80	0.426±0.293	Not reported	0.738±0.350 pmol/mL	≥1 required	≥1 required	≥1 required	Teplizumab (anti-CD3 mAb)	N/A (antibody therapy)	Escalating: 106-850 µg/m²/day	Intravenous	12 per course × 2 courses	12 days per course	78 weeks
17	Rosenzwajg	2020	France	RCT	4	24	17	7	9.3-10.6	Jul-14	50	<3 months	Newly diagnosed	6.8-8.1	0.3-0.6	0.23-0.33 nmol/L	0.96-1.23 nmol/L×h	18/24	17/24	≥1	Low-dose IL-2	NR	0.125-0.500 MIU/m²/day	Subcutaneous	29	1 year	436 days
18	Sherry	2011	Multi-national: USA, Canada, Mexico, India, Israel, Europe	RCT	83 clinical centers	516	417	99	14-day full: 18.9±7.6; 14-day low: 17.9±6.1; 6-day full: 18.1±6.9; Placebo: 18.2±7.3	Aug-35	14-day full: 63%; 14-day low: 61%; 6-day full: 68%; Placebo: 62%	14-day full: 8.4±2.6 wks; 14-day low: 8.4±2.6 wks; 6-day full: 9.0±4.5 wks; Placebo: 8.3±2.6 wks	Recent onset	14-day full: 8.3±2.0%; 14-day low: 8.4±2.1%; 6-day full: 8.1±1.8%; Placebo: 8.2±2.0%	14-day full: 0.63±0.42; 14-day low: 0.68±0.41; 6-day full: 0.63±0.39; Placebo: 0.65±0.32	Not separately reported	14-day full: 0.65±0.54; 14-day low: 0.69±0.45; 6-day full: 0.68±0.40; Placebo: 0.65±0.44 nmol/L per min	14-day full: 94%; 14-day low: 88%; 6-day full: 86%; Placebo: 91%	14-day full: 55%; 14-day low: 57%; 6-day full: 57%; Placebo: 54%	86-93% had 2-3 positive autoantibodies	Teplizumab (anti-CD3 mAb)	N/A (antibody therapy)	3 regimens: (1) 14-day full ~9034 µg/m²; (2) 14-day low ~2985 µg/m²; (3) 6-day full ~2426 µg/m²	Intravenous	2	2 cycles over 6 months	2 years (1-year data reported)
19	Wherrett	2011	USA and Canada	RCT	15	145	97	48	16.5±9.5	Mar-45	51	<3 months (87±15 days)	Recent onset	6.55±1.0	0.37±0.23	Not reported	0.685±0.34 pmol/mL	Required (100%)	Not reported	97.2% had ≥2	GAD65 antigen therapy	N/A (antigen therapy)	20 µg GAD-alum	Subcutaneous	3 (or 2+alum)	12 weeks	12 months

Table 2 Meta-analysis summary results

Outcome	Analysis	N_Studies	N_Arms	Effect_Size	CI_95_Lower	CI_95_Upper	P_value	I2_percent
Insulin	MAIN - Overall (Excluding key studies)	8	12	−0.255	−0.614	0.104	0.1459	65.1%
Insulin	MAIN - Teplizumab Early	3	3	−0.827	−1.558	−0.095	0.0398	0.0%
Insulin	MAIN - Teplizumab Late	3	5	−0.22	−0.603	0.163	0.1862	57.4%
Insulin	MAIN - Otelixizumab Early	1	1	0.228	−0.256	0.713	0.3557	NA%
Insulin	MAIN - Low-dose IL-2 Early	1	3	0.554	−0.761	1.869	0.2117	0.0%
Insulin	SENSITIVITY - Overall (All studies)	11	15	−0.453	−0.828	−0.079	0.0212	86.8%
Insulin	SENSITIVITY - Teplizumab Early	3	3	−0.827	−1.558	−0.095	0.0398	0.0%
Insulin	SENSITIVITY - Teplizumab Late	4	6	−0.328	−0.699	0.043	0.0723	70.4%
Insulin	SENSITIVITY - Otelixizumab Early	2	2	−0.705	−12.432	11.023	0.5849	97.5%
Insulin	SENSITIVITY - Otelixizumab Late	1	1	−0.93	−1.447	−0.412	0.0004	NA%
Insulin	SENSITIVITY - Low-dose IL-2 Early	1	3	0.554	−0.761	1.869	0.2117	0.0%
HbA1c	MAIN - Overall	8	12	0.033	−0.07	0.136	0.4875	0.0%
HbA1c	MAIN - Teplizumab	5	7	−0.009	−0.062	0.045	0.6921	0.0%
HbA1c	MAIN - Otelixizumab	2	2	0.213	−1.023	1.449	0.2729	0.0%
HbA1c	MAIN - Low-dose IL-2	1	3	−0.514	−0.867	−0.161	0.0245	0.0%
HbA1c	SENSITIVITY - Overall	11	15	−0.154	−0.401	0.092	0.1995	57.6%
HbA1c	SENSITIVITY - Teplizumab	7	9	−0.179	−0.55	0.192	0.2919	59.9%
HbA1c	SENSITIVITY - Otelixizumab	3	3	−0.005	−1.182	1.171	0.9862	78.1%
HbA1c	SENSITIVITY - Low-dose IL-2	1	3	−0.514	−0.867	−0.161	0.0245	0.0%
C-peptide	MAIN - Overall (Excluding key studies)	11	17	0.221	0.069	0.373	0.0072	20.1%
C-peptide	MAIN - Teplizumab	6	8	0.235	0.023	0.446	0.0343	33.6%
C-peptide	MAIN - Otelixizumab	3	5	0.207	−0.199	0.613	0.2294	0.1%
C-peptide	MAIN - Low-dose IL-2	1	3	0.02	−1.803	1.842	0.9674	34.4%
C-peptide	MAIN - Other	1	1	0.699	−0.176	1.575	0.1175	NA%
C-peptide	SENSITIVITY - Overall (All studies)	14	20	0.312	0.111	0.512	0.0042	61.0%
C-peptide	SENSITIVITY - Teplizumab	8	10	0.409	0.139	0.68	0.0075	64.6%
C-peptide	SENSITIVITY - Otelixizumab	4	6	0.078	−0.349	0.506	0.6572	39.9%
C-peptide	SENSITIVITY - Low-dose IL-2	1	3	0.02	−1.803	1.842	0.9674	34.4%
C-peptide	SENSITIVITY - Other	1	1	0.699	−0.176	1.575	0.1175	NA%

2009 (small sample, $n = 6$) representing an outlier. Overall pooled estimate (random effects):

- Proportion = 18.4% (95% CI: 7.9% to 37.4%)
- Substantial heterogeneity: $I^2 = 79.1\%$ (Q = 14.37, $p = 0.0024$)

The wide confidence interval reflects considerable uncertainty. Approximately 1 in 5–6 patients experienced a serious adverse event.

Safety differs by treatment category - critical finding

Subgroup analysis by mechanism of action revealed an important safety signal:

- Treg Enhancement Agents ($n = 2$ studies): 10.8% (95% CI: 6.5%-17.4%)
- WBCs Clearance Agents ($n = 2$ studies): 34.5% (95% CI: 11.0%-69.3%)

The 3.2-fold higher SAE rate in WBCs Clearance agents approached but did not reach statistical significance in random effects models (Q = 3.46, $p = 0.063$), likely reflecting limited power with only 2 studies per subgroup. However, common effect models showed a significant difference (Q = 7.94, $p = 0.005$).

All 5 deaths reported across studies occurred in the WBCs Clearance category (Supplementary Table 6), further supporting the safety distinction. Treg Enhancement agents demonstrated a more favorable safety profile with predominantly diabetes-related adverse events (hypoglycemia, injection reactions), while WBCs Clearance agents showed more systemic immunosuppression effects (cytokine release syndrome, lymphopenia, infections).

Regulatory T-cell expansion

We performed quantitative meta-analysis of Treg expansion using data from Hartemann (2013), which evaluated three doses of low-dose IL-2 (0.33, 1.0, 3.0 MIU/day × 5 days) versus placebo ($n = 33$ total). Each dose comparison was treated as a separate study arm (Supplementary Figure 10).

Pooled effect - striking finding

- SMD = 2.23 (95% CI: 1.70 to 2.75)

- $p = 0.003$ (highly statistically significant)
- $I^2 = 0.0\%$ (no heterogeneity, completely consistent across doses)

This represents the largest effect size in the entire meta-analysis. An SMD of 2.23 is classified as a "very large" effect (Cohen's benchmarks: 0.2 = small, 0.5 = medium, 0.8 = large), rarely seen in clinical research. The zero heterogeneity indicates remarkably consistent Treg expansion across all three dose levels, with no evidence of dose-dependency within this range (Supplementary Figure 11).

The mechanistic paradox

Despite this massive Treg expansion, IL-2 showed:

- NO C-peptide preservation (g = 0.020, $p = 0.967$)
- NO insulin reduction (SMD = 0.554, $p = 0.212$)
- YET significant HbA1c improvement (g = –0.514, $p = 0.025$)

This paradox highlights a fundamental disconnect between impressive immunological effects and clinical outcomes. The expected pathway—Treg expansion → suppressed autoimmunity → preserved beta-cells → improved clinical outcomes was not observed. Possible explanations include insufficient duration of Treg expansion (days-weeks vs months-years needed), Treg numerical expansion without functional suppression, treatment timing too late in disease course, or non-immunological mechanisms underlying IL-2's HbA1c benefit. Leave-one-out sensitivity analysis confirmed robustness of the Treg expansion finding.

Additional immunological outcomes

EBV reactivation EBV reactivation varied by intervention and dose. Teplizumab studies reported 0.2–43% virologic reactivation (mostly asymptomatic and self-limited), with higher rates after first treatment cycle (43%) than subsequent cycles (14%). Clinically significant EBV disease was not increased compared to placebo (Hagopian 2013: 7.7% treatment vs 8.2% placebo). Otelixizumab showed dose-dependent reactivation, with the 27 mg dose producing 71% virologic and 43% clinical reactivation, leading to trial discontinuation. Overall, EBV reactivation represents a real but generally manageable safety concern requiring monitoring protocols.

Anti-drug antibodies ADA development was common, ranging from 19–77% with Teplizumab to 100% with Otelixizumab. Importantly, studies explicitly noted no impact on single-course efficacy, though high-titer antibodies may limit re-treatment potential.

Other immunological markers CD4:CD8 ratio decreased with anti-CD3 treatment (potential biomarker of response). Otelixizumab induced dose-dependent CD8 exhaustion (2.6–6 fold increase in exhaustion markers), which may explain its lack of efficacy. IL-2 showed off-target NK cell activation at higher doses, supporting use of lower doses (0.33–1.0 MIU). Complete immunological results are provided in (Supplementary Figure 12) and (Supplementary Table 3).

Discussion

Summary of findings

This meta-analysis of immunomodulatory interventions in type 1 diabetes revealed three major findings that challenge conventional assumptions about disease-modifying therapy. First, immunotherapy significantly preserved C-peptide (g = 0.221, $p = 0.007$, driven primarily by Teplizumab) but failed to improve HbA1c (g = 0.033, $p = 0.49$), demonstrating a fundamental disconnect between biomarker and clinical outcomes. Second, low-dose IL-2 produced the largest immunological effect observed by massive regulatory T-cell expansion (SMD = 2.23, $p = 0.003$) and was the only intervention significantly improving HbA1c (g = –0.514, $p = 0.025$), yet showed no C-peptide preservation (g = 0.020, $p = 0.967$) or insulin dose reduction (SMD = 0.554, $p = 0.212$) (Table 2),, suggesting a mechanistic pathway independent of beta-cell rescue. Third, treatment benefits on insulin requirements systematically attenuated over time, with follow-up duration explaining approximately half of observed heterogeneity ($R^2 = 49.06\%$, (Supplementary Table 4)), indicating that single-course immunotherapy provides temporary reprieve rather than sustained disease modification. Additionally, safety analysis revealed a 3.2-fold higher serious adverse event rate with WBCs Clearance agents (34.5%) compared to Treg Enhancement agents (10.8%), with all five reported deaths occurring in the WBCs Clearance category. These findings fundamentally reshape understanding of immunotherapy efficacy, mechanism, and clinical translation in type 1 diabetes.

The C-peptide-HbA1c disconnect

The most perplexing finding in our analysis is that immunotherapy preserves beta-cell function without translating to improved glycemic control. This disconnect has four complementary explanations that together reveal fundamental limitations in trial design and endpoint selection.

Intensive insulin management masks therapeutic benefits. Modern clinical trials employ aggressive insulin titration protocols in both treatment and control groups to maintain target HbA1c levels, effectively obscuring metabolic advantages conferred by preserved C-peptide. Mortensen et al. developed the Insulin Dose-Adjusted

HbA1c (IDAA1c) metric specifically to address this problem, demonstrating through multiple regression analysis that stimulated C-peptide showed negative associations with both HbA1c (regression coefficient −0.21, $p < 0.001$) and insulin dose (−0.94, $p < 0.001$), revealing that patients with different beta-cell functions could present identical HbA1c values simply by intensifying insulin treatment [34]. The formula IDAA1c = HbA1c (%) + [4 × insulin dose (units/kg/24 h)] was designed to reduce treatment regimen influence, since increasing exogenous insulin artificially lowers HbA1c regardless of endogenous secretion. Ehlers contextualized this within trial design, explicitly stating that "intervention trials generally include intensive diabetes management for all participants regardless of treatment assignment," meaning that "assessment of differences in glycemic control between treatment arms is not generally a useful outcome" [9]. The Protégé trial exemplifies this paradox—teplizumab significantly preserved C-peptide at 2 years yet was declared a failure because the primary composite endpoint of HbA1c < 6.5% with insulin < 0.5 units/kg/day was not met. When both groups receive identical intensive management protocols, preserved C-peptide enables treatment groups to achieve similar HbA1c as controls but with substantially lower insulin requirement is a critical benefit that HbA1c measurement alone systematically misses.

The observed effect size may be clinically meaningful despite appearing modest. Two landmark DCCT/EDIC analyses establish that C-peptide benefits are continuous across the entire measurable range with no threshold effect. Lachin et al. demonstrated that even a 50% increase in stimulated C-peptide (such as from 0.10 to 0.15 nmol/L) was associated with HbA1c decrease of 0.07% ($p = 0.0003$), insulin dose reduction of 0.0276 units/kg/day ($p < 0.0001$), hypoglycemia risk reduction of 8.2% ($p < 0.0001$), and sustained retinopathy risk reduction of 25% ($p = 0.0010$) [2]. Critically, these benefits persisted even below the previously established 0.2 nmol/L threshold, challenging traditional "responder" classifications. The long-term clinical significance was established by Gubitosi-Klug et al., who followed patients for 35 years and identified that those with peak C-peptide > 0.03 nmol/L sixfold lower than the traditional threshold—demonstrated dramatic reductions in severe hypoglycemia (45% versus 70% in non-responders), with benefits remaining significant after adjusting for mean HbA1c (OR 0.35, 95% CI 0.21–0.57, $p < 0.0001$) [3]. Notably, mean EDIC HbA1c did not differ across responder groups (all approximately 8%), yet clinical outcomes diverged dramatically, proving that C-peptide preservation confers benefits beyond those captured by HbA1c alone. Our observed effect size (g = 0.221) falls within the range expected to produce clinically meaningful long-term benefits, particularly for hypoglycemia prevention and potentially microvascular complications, though these benefits require years to decades to fully manifest.

HbA1c may be fundamentally the wrong primary endpoint for immunotherapy trials. The 2023 international consensus statement endorsed by the American Diabetes Association and European Association for the Study of Diabetes challenges HbA1c primacy, emphasizing that it reflects average glucose over 2–3 months but provides no information about glycemic variability, hypoglycemia exposure, postprandial excursions, or nocturnal glucose patterns—all domains where C-peptide preservation would be expected to confer benefit [35]. Continuous glucose monitoring metrics, particularly time-in-range (TIR, 70–180 mg/dL), capture glycemic excursions and asymptomatic events that HbA1c fundamentally cannot detect. Two patients with identical HbA1c can have vastly different glucose profiles, one with stable control and low variability, another with wide swings and equal time above and below target. For immunotherapy trials where C-peptide preservation improves glucose stability and reduces hypoglycemia risk rather than dramatically lowering average glucose, CGM metrics would theoretically detect benefits that HbA1c systematically misses, particularly when both groups receive intensive insulin management targeting similar HbA1c levels.

Contemporary diabetes management context reduces detectable effect sizes. T1D Exchange registry data from Foster et al. revealed that despite insulin pump use increasing from 57% to 63% and CGM use increasing fourfold (7% to 30%), mean HbA1c levels changed little between 2010–2012 and 2016–2018, with only 17% of youth achieving ADA goals [36]. However, as Petersen et al. demonstrated, recruiting patients to clinical trials produces Hawthorne effect improvements independent of interventions, and control groups receiving enhanced standard care show larger improvements, thereby reducing intervention effect sizes [37]. This suggests that contemporary trials provide intensive monitoring to all participants, narrowing the detectable gap between groups when both receive excellent care, even though most patients in routine practice still have substantial room for improvement.

The IL-2 paradox

The most striking and mechanistically puzzling finding in our analysis is that low-dose IL-2 produced massive regulatory T-cell expansion (SMD = 2.23, the largest effect size observed) and significant HbA1c improvement (g = −0.514, $p = 0.025$), yet showed no C-peptide preservation (g = 0.020, $p = 0.967$) or insulin dose reduction (SMD = 0.554, $p = 0.212$). This paradox fundamentally challenges the assumed biological pathway of immunotherapy: enhanced immune regulation → suppressed autoimmunity → preserved beta-cells → improved

clinical outcomes. Four mechanisms may explain this dissociation.

Treg numerical expansion does not equal functional restoration. Hartemann et al.'s foundational dose-finding study achieved its primary endpoint with dose-dependent Treg increases at all tested doses (0.33–3.0 MIU), yet the Phase 1/2 trial was designed only to establish safe doses for Treg expansion, not to test clinical efficacy [10]. The study explicitly demonstrated that massive Treg expansion can occur without addressing underlying beta-cell destruction. The mechanistic explanation comes from Long et al., who discovered that T1D patients exhibit intrinsic defects in IL-2 receptor signaling—specifically reduced STAT5 phosphorylation upon IL-2 stimulation and elevated PTPN2 expression (a negative regulator)—resulting in diminished FOXP3 maintenance despite IL-2 presence [38]. These signaling defects mean that expanded Tregs retain their functional impairments; maintenance of FOXP3 expression in CD4+CD25+ Tregs was diminished in the presence of IL-2, establishing that T1D Tregs have fundamental defects preventing proper response. Simply increasing Treg numbers cannot overcome intrinsic suppressive dysfunction. The expanded cells lack the functional capacity to suppress autoimmune beta-cell destruction, explaining why trials successfully expanding Treg quantity fail to show clinical beta-cell preservation.

Treg expansion is transient, not sustained. Using deuterium labeling to track Treg persistence, Dong et al. provided precise kinetic data showing that Treg expansion peaks at day 7 after each 5-day IL-2 course, then declines substantially by 91 days post-treatment [39]. The transient expansion lasted only 6–12 weeks after the last IL-2 course. This temporal profile directly explains lack of C-peptide preservation—beta-cell protection likely requires sustained Treg elevation over months to years, not the transient peaks (days to weeks) achieved with current protocols. The combination of high-quality mass spectrometry tracking, multiple patients, and long-term follow-up (104 weeks) demonstrated there was no evidence that combined Treg+IL-2 therapy led to preservation of insulin production, with C-peptide levels decreasing in all patients within the first 30 days despite successful Treg expansion. Short-duration immune modulation appears insufficient for disease requiring chronic suppression.

IL-2 may improve HbA1c through non-immunological metabolic pathways. The most mechanistically innovative explanation comes from Moon et al.'s groundbreaking 2024 discovery that low-dose systemic IL-2 significantly improved insulin sensitivity in obese mice through a novel neuro-immune axis completely independent of beta-cell function [40]. IL-2 receptors are expressed on hypothalamic microglia (80–96% co-expression), and central IL-2 administration triggers hypothalamic microglial activation → POMC neuron activation → sympathetic nervous system stimulation → improved insulin sensitivity in peripheral tissues. The pathway enhances insulin-stimulated pAKT signaling in adipose tissue, increases Tregs while decreasing Th1 cells in fat depots, reduces pro-inflammatory cytokines (IL-1β, IL-6, IL-8), and increases anti-inflammatory mediators (IL-4, IL-10, TGFβ)—all via sympathetic signaling. Sympathetic denervation reversed these beneficial effects, confirming the mechanism. This provides a direct mechanistic explanation for improved HbA1c through enhanced peripheral insulin sensitivity rather than beta-cell rescue. Supporting this framework, Shi et al.'s comprehensive review established that immune-regulatory cytokines directly regulate glucose homeostasis through effects on peripheral tissues—skeletal muscle glucose uptake, hepatic glucose production, adipose tissue metabolism—occurring completely independently of pancreatic beta-cell function [41]. Clinical evidence showed TNF-α inhibition improved insulin sensitivity in insulin-resistant subjects through direct metabolic effects rather than beta-cell preservation, establishing scientific precedent for cytokines acting as metabolic regulators with non-pancreatic mechanisms.

Study design limitations preclude definitive conclusions. Graßhoff et al.'s comprehensive review of low-dose IL-2 trials across autoimmune diseases identified that T1D trials enrolled limited patient numbers (24–48 subjects total) with short follow-up periods (60 days to 1 year)—Phase I/II studies designed for safety and dose-finding, not powered to detect clinical efficacy [42]. While "a biological response in form of an effective expansion of the Treg population could be reliably demonstrated in all studies," clinical benefit varied, suggesting heterogeneous responses requiring patient stratification. The authors noted that "the lack of clinical efficacy could have been due to the short treatment duration in a rather slowly progressing disease," a limitation equally applicable to T1D autoimmunity. Future directions emphasize combination therapies, modified IL-2 formulations with longer half-lives, biomarker development for predicting responsiveness, and patient stratification according to individual immune signatures. The paradoxical IL-2 findings based on 2-month measurements in small studies ($n = 30–60$) may reflect insufficient power and duration to detect disease-modifying effects in chronic autoimmune diabetes.

Time-dependent attenuation of treatment benefits

Our meta-regression analysis revealed that immunotherapy benefits on insulin requirements systematically fade over time, with follow-up duration explaining approximately half of the observed heterogeneity ($R^2 = 49.06\%$,

(Supplementary Table 4)). This finding has profound implications for understanding immunotherapy mechanism and optimizing treatment strategies. The progressive attenuation suggests that single-course treatment slows but does not halt the relentless autoimmune process.

Time-dependent attenuation reflects ongoing autoimmune pressure overcoming therapeutic effects. Natural history data from Greenbaum et al. demonstrated biphasic progressive C-peptide decline in 191 newly diagnosed patients: Phase 1 (months 0–12) showed rapid decline at −0.0245 pmol/mL/month (~50% loss in first year), followed by Phase 2 (months 12–24) with continued decline at −0.0079 pmol/mL/month ($p < 0.001$) [43]. Critically, 93% retained detectable C-peptide at 2 years, demonstrating that autoimmune beta-cell destruction continues progressively and indefinitely without disease-modifying treatment. Our finding that immunotherapy benefits attenuate at quantifiable rates indicates that single-course treatment slows but does not halt this relentless autoimmune process. Sims et al.'s extended follow-up (median 923 days) showed that while teplizumab initially reversed declining insulin secretion and stabilized C-peptide, 50% of treated patients still progressed to diabetes versus 78% placebo, demonstrating that treatment stabilized but did not prevent continued decline [44]. Benefit is real but finite, with ongoing autoimmunity gradually overcoming therapeutic effect.

Earlier intervention may provide more durable benefits. The pivotal trial by Herold et al. demonstrated that a single 14-day teplizumab course in high-risk relatives (stage 2 disease, before clinical diabetes) delayed T1D onset by median 2 years (48.4 vs 24.4 months; HR 0.41, $p = 0.006$), with only 43% of teplizumab-treated versus 72% of placebo developing stage 3 T1D [6]. Greatest effect was observed in the first year (7% teplizumab vs 44% placebo diagnosed), with treatment benefit most pronounced early and showing signs of waning over time. This trial formed the basis for FDA approval and demonstrates that timing of immunotherapy relative to disease stage affects outcomes. Intervening before extensive beta-cell loss produces more meaningful disease delay, supporting the concept that earlier treatment capitalizes on greater remaining beta-cell mass and less entrenched autoimmunity. However, even in this prevention setting, benefits ultimately attenuated, suggesting that maintenance therapy or repeated treatment courses may be necessary for sustained disease control. Current evidence for re-treatment strategies remains limited, representing an important gap requiring systematic investigation in future trials.

Safety considerations

WBCs Clearance agents demonstrated a 3.2-fold higher serious adverse event rate (34.5%) compared to Treg Enhancement agents (10.8%), with all five reported deaths occurring in the WBCs Clearance category (Supplementary Table 6). This safety gradient reflects mechanistic differences between approaches. Herold et al.'s integrated analysis of 1,018 patients across five teplizumab trials revealed that serious adverse events occurred in 12.4% of treated versus 8.2% of controls, with the most common events being transient lymphopenia (79.9% vs 16.7%, nadir day 5, resolved during dosing), leukopenia (63.3% vs 26.5%), rash (34.5% vs 10.2%), and cytokine release syndrome (5.8% vs 1.2%) [45]. Importantly, 7-year follow-up showed no increased long-term infection risk or malignancies, as mechanism spares regulatory and memory T cells rather than causing global immunosuppression. Keymeulen et al.'s dose-escalation study revealed clear dose-dependent safety profiles, with the 27 mg otelixizumab dose producing 71% EBV reactivation (43% clinical) and meeting predefined stopping criteria, while lower 9 mg doses showed 22% reactivation with zero clinical manifestations yet retained C-peptide preservation benefit [11]. This safety gradient—global T-cell depletion creating iatrogenic immunosuppression with attendant infection risk versus selective Treg enhancement—suggests that Treg Enhancement agents should be considered first-line when efficacy is comparable, particularly given the mortality signal exclusively in the WBCs Clearance category.

Comparison to prior reviews

Our findings align with emerging literature establishing the C-peptide-HbA1c disconnect as a consistent biological phenomenon. Lin et al. analyzed 34 clinical trials and explicitly documented C-peptide preservation ($p = 0.0007$) without HbA1c improvement, concluding that nonantigen-based immunotherapies preserve C-peptide and reduce insulin dosage but do not improve HbA1c or fasting plasma glucose [7]. Beese et al.'s comprehensive network meta-analysis of 60 trials involving 4,597 patients identified eleven interventions with significantly higher C-peptide than placebo at 12 months, yet explicitly noted that "benefits to glucose control and insulin dose were not consistent," with only low-dose ATG and CTLA4 showing statistically significant HbA1c reduction [46]. Taylor et al.'s individual participant meta-analysis provided crucial nuance, demonstrating that interventions preserving C-peptide are effective at improving HbA1c (24.8% preservation → 0.55% HbA1c reduction, $p < 0.0001$), but substantial preservation (>76% of baseline) is required to achieve optimal targets, and HbA1c requires 2–3× as many participants as C-peptide to show statistical significance [47]. Our

observed effect size (g = 0.221) may fall below this clinical threshold, explaining lack of HbA1c improvement despite statistically significant C-peptide preservation. Our novel contributions extend this literature by: (1) quantifying time-dependent benefit attenuation through meta-regression (R^2 = 49.06%), demonstrating that benefits systematically fade over follow-up; (2) identifying the IL-2 paradox where HbA1c improvement occurs without C-peptide preservation, suggesting distinct mechanistic pathways; and (3) establishing a safety gradient between treatment mechanisms. Notably, FDA now acknowledges C-peptide as a surrogate endpoint for clinical benefit in teplizumab approval, validating its clinical significance despite the HbA1c disconnect.

Limitations

Several limitations warrant acknowledgment. First, most included studies had relatively short follow-up (12–24 months), potentially insufficient to detect long-term clinical benefits that DCCT/EDIC analyses suggest require years to decades to fully manifest. Second, despite outlier exclusion, substantial heterogeneity remained in insulin requirements (I^2 = 65.1%), reflecting differences in patient populations (age, disease duration), treatment protocols (doses, frequencies), and outcome measurement timepoints. Third, missing data required exclusion of five studies from C-peptide analysis due to absent standard deviations, and poor Grade 3/4 adverse event reporting across studies limited comprehensive safety assessment beyond serious adverse events. Fourth, small subgroup sizes limit definitive conclusions for IL-2 findings based on three studies and Treg expansion from a single study (Hartemann 2013) require replication in larger trials. Fifth, included studies predominantly enrolled pediatric and young adult populations with recent-onset disease, limiting generalizability to adults with established diabetes or diverse demographic groups. Sixth, with limited numbers of studies per outcome, publication bias assessment had insufficient power to detect selective reporting. Seventh, lack of individual participant data precluded identification of responder subgroups or patient-level moderators that might predict treatment benefit, an important direction for precision medicine approaches.

Implications and future directions

For clinicians, these findings indicate that immunotherapy provides modest, temporary benefits rather than curative disease modification. Realistic patient expectations are essential—treatment offers reprieve, not cure. C-peptide preservation does not guarantee improved glycemic control in the short term, and intensive diabetes management remains the cornerstone of care regardless of immunotherapy status. When considering immunotherapy, Treg Enhancement agents offer superior safety profiles compared to WBCs Clearance approaches, with 3.2-fold lower serious adverse event rates and zero mortality signals.

For researchers, future trials should prioritize several key design elements. First, longer follow-up periods (> 5 years) are essential to capture the long-term clinical benefits that DCCT/EDIC analyses demonstrate occur years after C-peptide preservation. Second, mechanistic studies must address both the C-peptide-HbA1c disconnect and IL-2 paradox to understand why biological effects fail to translate consistently to clinical outcomes. Third, combination therapies merit systematic investigation of Haller et al. demonstrated that ATG plus G-CSF preserved beta-cell function in established T1D (4–24 months post-diagnosis) where each agent individually had failed, with 9 of 16 patients maintaining baseline C-peptide at 12 months [20]. Fourth, earlier intervention in high-risk individuals appears more effective than treatment after clinical diagnosis, as demonstrated by the teplizumab prevention trial producing median 32.5-month disease delay. Fifth, international consensus from the American Diabetes Association and European Association for the Study of Diabetes advocates that patient-centered endpoints beyond HbA1c should be prioritized [48]. Time-in-range from continuous glucose monitoring and hypoglycemia rates as co-primary endpoints would better capture benefits of C-peptide preservation, as Agiostratidou et al.'s consensus statement from eight major diabetes organizations explicitly noted that "preservation of β-cell function can reduce hypoglycemia by improving metabolic control and reducing insulin requirements" even when HbA1c remains unchanged [49]. CGM provides temporal resolution to detect glucose stability improvements and asymptomatic hypoglycemia reduction that HbA1c fundamentally cannot capture.

Conclusions

Immunomodulation modestly preserves C-peptide (g = 0.221, p = 0.007) but does not consistently improve HbA1c (g = 0.033, p = 0.49), with treatment benefits attenuating systematically over time (R^2 = 49.06%, (Supplementary Table 4)). The disconnect between biomarker and clinical outcomes, exemplified by the IL-2 paradox where HbA1c improvement occurs without C-peptide preservation through putative non-immunological metabolic pathways, highlights fundamental challenges in translating immunological interventions into sustained patient benefits. These findings do not indicate therapeutic failure but rather reveal that intensive insulin management in both trial arms masks benefits detectable through insulin dose reduction but not HbA1c comparison, and that short-term trials may terminate before long-term clinical advantages of C-peptide preservation

fully manifest. Combination therapies targeting multiple pathways simultaneously, earlier intervention before extensive beta-cell loss, maintenance or repeated treatment strategies to overcome time-dependent attenuation, and better endpoints including continuous glucose monitoring metrics that capture hypoglycemia reduction and glucose stability improvements are essential next steps for advancing disease-modifying therapy in type 1 diabetes.

Abbreviations

AUC	Area under the curve
CI	Confidence interval
DCCT	Diabetes Control and Complications Trial
HbA1c	Glycated hemoglobin
IL-2	Interleukin-2
RCT	Randomized controlled trial
SAE	Serious adverse event
SMD	Standardized mean difference
T1D	Type 1 diabetes
Treg	Regulatory T cell

Supplementary information

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Supplementary Material 1

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Author contributions
SB conceived and designed the study, conducted the literature search, performed study selection, extracted data, conducted statistical analysis, and drafted the manuscript. BD and ST independently performed study selection and data extraction. SN, AK, and NB conducted quality assessment. SR, UBS, IKR, and VPS contributed to data analysis and interpretation. YS, UB, SM, AJ, ZUA, VK, SS, and RH contributed to manuscript revision and critical review. All the authors have read and approved the final manuscript.

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Data availability
All data supporting the conclusions of this systematic review are included in the published article and supplementary materials. The complete dataset and analysis code are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable. This systematic review and meta-analysis was based on published data and did not require ethical approval.

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Competing interests
The authors declare no competing interests.

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