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REVIEW

Treatment of type 2 diabetes by targeting interleukin-1: a meta-analysis of 2921 patients

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Abstract

With obesity and type 2 diabetes prevalence steadily increasing and no effective means in sight to support the population in obtaining and maintaining stable weight loss, there is an imminent need for pharmacological therapy to treat and prevent type 2 diabetes. Current anti-diabetic treatment is symptomatic, and very few drugs have both a strong preclinical rationale and clinical proof-of-principle as therapies targeting pathogenic processes in type 2 diabetes. The emerging appreciation of low-grade inflammation as a significant cause of insulin resistance and beta cell failure warrants exploring anti-inflammatory compounds as drug candidates. Since recent studies have demonstrated considerable phenotypic heterogeneity in the type 2 diabetic syndrome, the concept of one drug fits all is naïve, and biomarkers for the selection of type 2 diabetes subtypes for differentiated treatment based on genetic and pathogenic stratification are urgently needed. Biologics antagonizing the master pro-inflammatory cytokine interleukin-1 is one of the few principles specifically targeting low-grade inflammation in type 2 diabetes. Although early phase II studies were encouraging, subsequent underpowered studies and phase III studies designed primarily with cardiovascular endpoints have discredited the potential of anti-interleukin-1 approaches to treat the subgroup of patients that may benefit from this treatment. In this meta-analysis of 2921 individuals from eight phase I–IV studies, we demonstrate a significant overall HbA1c-lowering effect of interleukin-1 antagonism. Meta-regression analyses demonstrated a significant correlation between baseline C-reactive protein and C-peptide, and HbA1c outcome. The identification of further biomarkers for future clinical trials to define the potential of anti-interleukin-1 therapies in type 2 diabetes is urgently needed.

Keywords Anti-cytokine biologics · Immunometabolism · Inflammation · Innate immunity · Meta-regression

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Introduction

Obesity and type 2 diabetes (T2D) are serious threats to global health, welfare, and medicare budgets—and a problem frankly out of control [1]. Although 85% of type 2 diabetes is associated with overweight, and despite convincing evidence that lifestyle intervention is effective in preventing incident type 2 diabetes and in improving glycemia and cardiovascular risk in patients with overt type 2 diabetes, measures at the public health and individual levels that can effectively induce and stably preserve weight loss have yet to be devised [2–4].

In the absence of effective and acceptable lifestyle corrections, patients are left with symptomatic polypharmacy aiming at cardiovascular risk reduction, or, for high-risk groups, bariatric surgery, an expensive, low-capacity, and resource-requiring procedure associated with long-term adverse effects [5, 6]. There is currently no marketed drug targeting the fundamental pathogenic processes in type 2 diabetes.

Type 2 diabetes arises when insulin secretion fails to compensate for insulin needs [7]. In the case of T2D, this mismatch is most often unmasked by obesity leading to insulin resistance. However, drugs primarily targeting insulin resistance have largely been abandoned due to their side-effects, and drugs boosting beta cell secretory compensation act merely by stimulating insulin secretion, not by halting the underlying molecular pathogenesis of the secretory defect [8].

A further persistent impediment to effective anti-diabetic treatment has been the notion of type 2 diabetes as a homogenous disease entity. With the realization of the significant heterogeneity in type 2 diabetic phenotype, the avenue is open for individualized tailor-made treatments of subpopulations based on genetic and pathogenic biomarkers [9].

Likely, sub-phenotypes of type 2 diabetes exist where low-grade inflammation is a prominent pathogenic factor. Evidence to support this notion is the variation in plasma high-sensitivity assayed C-reactive protein (hsCRP) levels and other inflammatory markers in type 2 diabetic patients [10]. It is therefore critical to extract information from existing intervention trials that would support the design of clinical trials to investigate the efficacy and safety of anti-inflammatory drugs in such a sub-phenotype.

A limited number of anti-inflammatory approaches have been tested in clinical trials in T2D, including small molecules such as the salicylic acid derivative, salsalate and specific anti-cytokine biologics [11, 12]. Anti-inflammatory properties have been assigned to many anti-diabetic drugs such as angiotensin I–converting enzyme inhibitors, angiotensin II receptor blockers, statins, DPPIV inhibitors, Glp-1 agonists, and even insulin [13]. However, for these classes of drugs, the beneficial effects of the anti-inflammatory action are difficult to tease apart from the anti-diabetic properties and are generally considered to be modest. Currently, the only specific anti-inflammatory biologic for which a sufficient number and quality of trials have been reported to justify meta-analysis is antagonists against the key pro-inflammatory cytokine interleukin-1 (IL-1).

The purpose of this paper is therefore to conduct an updated meta-analysis of all available trials employing IL-1 antagonists and a meta-regression to identify suitable clinical biomarkers useful for selection of patients for future trials.

Immunometabolism in type 2 diabetes: metabolites as danger-associated molecular patterns

A central pathophysiological rationale supporting IL-1 as an interventional target in type 2 diabetes is the notion that metabolites are sensed by the innate immune system and possibly even by the pancreatic beta cells as danger-associated molecular patterns (DAMPS) that activate the inflammasomes [14]. This class of NOD-like receptors processes pro-IL-1 and pro-IL-18 into biologically active IL-1 and IL-18 [15].

Pioneering work demonstrated that glucose-mediated human islet cell apoptosis is mediated by IL-1 and that high glucose concentrations induce beta cell IL-1 secretion [16]. Later work demonstrated that many metabolites elevated in insulin resistant (free fatty acids, minimally modified LDL, adipocytokines, LPS) or overtly diabetic patients (glucose, islet amyloid polypeptide) stimulate pro-IL-1 mRNA and protein expression and/or inflammasome activation, leading to IL-1 release from intra-islet macrophages or to a lesser extent beta cells themselves [17]. Taken together, the preclinical findings suggest that these metabolites are drivers of low-grade inflammation and may be pathogenic in beta cell failure causing T2D, or, in the case of glucose, contribute to a progressive decline in functional beta cell mass in overtly diabetic subjects. It follows from this argument that the type 2 diabetic sub-phenotypes expected to benefit most from anti-IL-treatment are those with the highest metabolic drive on IL-1 expression, and/or with the lowest endogenous level of IL-1 antagonism, both associated with the highest net pro-inflammatory load. A further consequence of this notion is that biomarkers of pronounced baseline dysmetabolism, deficient IL-1 receptor antagonist (IL-1Ra) production, and elevated IL-1 or its downstream proxies, IL-6 and CRP should be predictive of response to anti-IL-1 therapies and as such provide suitable selection criteria for future trials aimed at individualized treatment.

Clinical trials and biomarkers of response to anti-IL-1 therapies

Evidence from clinical trials to support this hypothesis is scarce. In the pioneering clinical trial, the following biomarkers were associated with clinical response: low body surface as a likely surrogate of distribution volume and thereby drug exposure, low endogenous IL-1Ra, a 5′ IL-1Ra promoter polymorphism coding for low endogenous circulating IL-1Ra levels, high age, previous smoking, and cardiovascular disease (CVD) load, the latter possibly reflecting either an aggravated low-grade inflammatory state due to vascular wall inflammation or a common pathway priming for both diabetes and CVD [18, 19]. A positive correlation between baseline and endpoint glycemia rested on one outlier with extremely high HbA1c, and the omission of this individual eliminated significance (unpublished analysis) [18]. It should be noted that due to the inclusion criteria (baseline HbA1c > 7.5%), the spread in entry glycemia in this study was narrow. Very little information on biomarkers of response is available from other anti-IL-1trials. Subsequent reviews with tabulation of individual trial outcomes have pointed to an apparent association between baseline and outcome glycemia, but a formal meta-regression analysis is lacking [11, 20].

A previous meta-analysis published in 2018, based on five trials identified from a search in 2017, concluded that anti-IL-1 therapies had a significant but modest glucose-lowering effect (−0.25% reduction in HbA1c); two of the five studies also reported a significant improvement in beta cell secretory function [21]. Since the publication of the Huang meta-analysis, three major and important studies have been added as follows: (1) the phase IIb canakinumab pilot development program by Ridker et al. that was utilized to guide the design of the large-scale cardiovascular outcomes trial using canakinumab; (2) the trial subsequent to the pilot and to date the largest anti-IL-1 study with almost 4000 type 2 diabetic patients, the *Canakinumab Anti-Inflammatory Thrombosis Outcome Study* (CANTOS) pre-specified diabetes analysis; and (3) a study reporting the so far most potent effect of anti-IL-1 treatment in patients with rheumatoid arthritis and type 2 diabetes as comorbidity [22–24]. These studies (1) add significant statistical power to the overall meta-analysis and (2) provide an appropriate spread in baseline and outcome glycemic endpoints to make meta-regression meaningful.

The aim of this study was therefore to conduct a more comprehensive meta-analysis of the effects of anti-IL-1 treatments on available relevant endpoints in type 2 diabetes and in particular to perform meta-regression analyses not performed before to identify predictors of response as biomarkers for future more targeted clinical trials of anti-IL-1 treatment of T2D phenotypic subpopulations. We hypothesize that the clinical response to IL-1 blockade depends on the baseline dysmetabolic status, as such a greater response observed in individuals with a more metabolic imbalance at baseline.

Methods

Study strategy

The study was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines.

A systematic search of studies published in PubMed up until February 5, 2019, was conducted. The following search was designed to capture records for randomized clinical trials (RCT) investigating the effect of IL-1 antagonist on HbA1c in patients with type 2 diabetes: ("interleukin 1 receptor antagonist protein" [MeSH Terms] OR "canakinumab" [Title/Abstract] OR "Anakinra" [Title/Abstract] OR "Kineret" [Title/Abstract] OR "AntiIL" [Title/Abstract] OR "Anti-interleukin-1" [Title/Abstract] OR "Interleukin-1 antagonism" [Title/Abstract] OR "IL-1Ra" [Title/Abstract] OR "interleukin 1 receptor antagonist" [Title/Abstract] OR "IL-1beta antibody" [Title/Abstract] OR "interleukin-1 inhibition" [Title/Abstract] OR "interleukin-1beta" [Title/Abstract]) AND ("diabetes mellitus, type 2" [MeSH Terms] OR "type 2 diabetes mellitus" [Title/Abstract] OR "type 2 diabetes" [Title/Abstract]).

Additional articles were searched by references and citations in the original search and from the PubMed option "Similar Articles." ClinicalTrials.gov was also searched for registered and published randomized clinical trials.

Inclusion criteria

RCT studies were selected that reported the effects of IL-1 antagonists on HbA1c in the intervention group and placebo (with standard of care treatment) in patients with type 2 diabetes classified according to the American Diabetes Association (ADA) (either fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL)), an oral glucose tolerance test 2-h plasma glucose ≥ 11.1 mmol/L (200 mg/dL, or HbA1c ≥ 6.5% (48 mmol/mol)), or World Health Organization (WHO) criteria (either fasting plasma glucose ≥ 7.0 mmol/L or an oral glucose tolerance test 2-h plasma glucose ≥ 11.1 mmol/L) [25]. IL-1β antagonist therapy was defined as IL-1 receptor antagonist (IL-1Ra) or anti-interleukin-1 monoclonal antibody therapy. The unit for HbA1c was percent glycated hemoglobin A1c.

In addition, the studies should fulfill one of the following criteria for eligibility:

(A) Arithmetic mean pre- and post-treatment, standard deviation (SD) pre- and post-treatment, and the number of study subjects (N) pre- and post-treatment of the placebo and intervention group, respectively [23, 26]
(B) Arithmetic median pre- and post-treatment, interquartile range pre- and post-treatment, and N pre- and post-treatment of the placebo and intervention group, respectively [22]
(C) Arithmetic mean pre- and post-treatment changes (baseline to end of follow-up) in placebo and IL-1 antagonist group, respectively, and the associated 95% CI or p value or standard error (SE) or standard deviation (SD), and N of the placebo and intervention group pre- and post-treatment [18, 26, 27]
(D) Geometric mean pre- and post-treatment, standard deviation (SD) pre- and post-treatment, and N pre- and post-treatment of the placebo and intervention group, respectively [28]
(E) ANCOVA least squares mean pre- and post-treatment change (baseline to end of follow-up) in placebo and intervention groups, respectively, standard error (SE), and N of the placebo and intervention group pre- and post-treatment [24, 29]
(F) Arithmetic, geometric, and least square means can be assumed to be of similar although not identical dimensions if the baseline values for intervention and placebo do not differ markedly, and the correlation coefficient is moderate (0.65) to high (above 0.80) [30, 31].

IL-1 antagonists and equipotency

Several studies had multiple intervention groups parsed by different concentrations of different IL-1 antagonists. Written correspondence with Novartis helped elucidate the equipotency between anakinra (IL-1receptor antagonist (IL-1Ra)), canakinumab (anti-interleukin 1β monoclonal antibody), LY2189102 (anti-interleukin 1β monoclonal antibody), and gevokizumab (anti-interleukin 1β monoclonal antibody). The following doses were therefore selected for this meta-analysis: 100 mg Anakinra, 150 mg of Canakinumab, 180 mg of LY2189102, and 0.03–0.1 mg/kg (intermediate group) for Gevokizumab.

End of follow-up

We utilized the end of follow-up time point for the analysis. Everett et al. [22] had the longest follow-up time (48 months) which was not comparable to the other studies. We subsequently did the analysis utilizing a more comparable time point for the analysis shown (6 months).

Exclusion criteria

We excluded abstracts, reviews, commentaries, studies with inadequate data, missing HbA1c endpoint, and duplicate publications from the same cohorts with the same data [32–34]. "Inadequate data" is defined as data that were presented such that it was impossible to extract or calculate the necessary values for inclusion into a meta-analysis. Studies registered in ClinicalTrials.gov (NCT01276106, NCT00605475) which were completed but not published on PubMed or in preprint, were not included in the meta-analysis.

Study selection process

One author (TMPO) first examined the study titles and abstracts. All studies identified as potentially relevant to the topic were eligible for a full-text review. The study selection process is shown in Fig. 1. A list of selected studies can be found in Table 1. A total of eight eligible studies were included.

Risk of bias and study quality assessment

The quality of each study was evaluated and scored using the risk-of-bias tool of the Cochrane collaboration [35]. This was done independently by two authors (YK and CE). We assessed the methodological quality of these studies. They were evaluated on the five following domains describing different causes of bias: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in the measurement of the outcome, and bias in the selection of the reported result. These domains were assessed by categorical variables of low, unclear, and high bias. Discrepancies were discussed between the two authors until an agreement was reached.

Data extraction

Two authors (YK and CE) extracted information from the included studies from the articles or from information listed on ClinicalTrials.gov. The following variables were obtained from each study: author, year of publication, name of study, ClinicalTrial.gov number, size of study, size of intervention group and placebo group, drug administration method (subcutaneous, IV, oral), dosing frequency (single or repeated), exact dose in milligrams or milligrams per kilogram for intervention group, length of follow-up time, baseline clinical and biochemical characteristics of placebo and intervention groups, diabetes classification, placebo comparator, standard of care treatment, and data as described above to calculate mean HbA1c difference between the mean pre- and post-treatment changes in intervention and placebo groups. Our main endpoint for the meta-analysis was HbA1c decrement which was also the dominant primary outcome measure of the individual trials. For the included studies, we extracted similar data for pre-and post-treatment data for placebo and intervention groups for CRP, fasting plasma glucose (FPG), and C-peptide area under the curve (AUC) following meal test or oral glucose tolerance test (OGTT) + IV bolus. Extracted data for fasting glucose was converted to milligrams per deciliter.

Data synthesis and meta-analysis

For each study, we present the HbA1c (%) mean difference with standard error (SE), which is calculated as the difference between the absolute mean pre- and post-treatment change in intervention and placebo groups, respectively, ((using the data in the format A–E as listed in the Inclusion criteria section): intervention change from baseline – placebo change from baseline). A two-sided p value less 0.05 was considered significant.

If the studies did not present the HbA1c (%) mean difference, we used the statistical program Comprehensive Meta-analysis (CMA) to calculate this from the various data presented in the articles in the formats listed in the inclusion criteria above [36]. If median (interquartile range, IQR) was presented in the articles, we took the median for the mean and calculated the SD by IQR/1.35. SD to SE conversions and vice versa were calculated based on formulas in Cochrane Library Handbook and the book "*Introduction to Meta-analysis*" by Michael Borenstein who also developed the CMA statistical program [35, 36]. A detailed description of each study is provided in Supplementary Table 1. We also

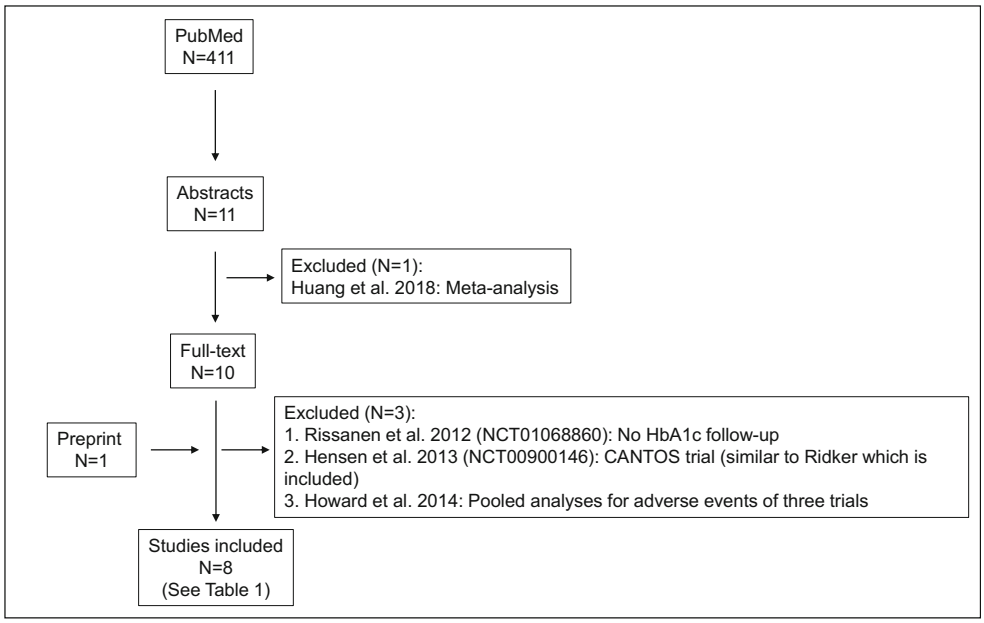

Fig. 1 Flowchart of study selection

provide the data set we composed and utilized in the supplemental material (Appendix Table 1). The same considerations applied for CRP, FG, and C-peptide.

Final meta-analysis was performed using STATA statistical software version 15.1. We used the *metan* command to calculate random effects summary estimates due to heterogeneous study protocols among trials. Comparing the placebo group with individuals randomized to IL-1 antagonist, we calculated weighted mean differences for HbA1 (%), CRP (mg/L), fasting blood glucose (mg/dL), and C-peptide (nmol/L min). We reported the fixed and random effects estimates. The fixed effects meta-analysis assumes all studies are estimating the same treatment effect implying no between-study heterogeneity but only variability due to chance. The random effects meta-analysis estimates the average treatment effect by accounting for between-study heterogeneity due to different treatment effects between studies [37]. After a random effects meta-analysis, the 95% prediction interval can be calculated, which provides a "predicted range for the true treatment effect" in "95% of similar (exchangeable) studies that might be conducted in the future" [37, 38]. We also used the *metareg* command to conduct a meta-regression analysis to identify the causes of heterogeneity by estimating the effects of baseline parameters (baseline HbA1c, placebo baseline HbA1c, BMI, baseline FPG, and baseline CRP levels) on the change in HbA1c at the end of follow-up time. Statistical heterogeneity was assessed by the I^2 statistic, which is a measure of between-study variability, and the corresponding Cochrane's Q-statistic p value ($p_{\text{heterogeneity}}$). I^2 statistic ranges from 0 to 100%; higher values indicate increased between-study heterogeneity. To investigate the validity and robustness of the meta-analysis of the mean differences in HbA1c, we explored heterogeneity in sensitivity analyses by stratifying by HbA1c at baseline, drug type, and frequency of drug administration. To account for differences in scales (arithmetic, geometric, least squares) used in the studies, we performed a sensitivity meta-analysis of the standardized mean differences between the paired pre-post mean differences in the intervention and placebo groups. Publication bias was measured by Egger's test and by visually assessing the funnel plot. Leave-one-out analysis investigated if any study had an exaggerated impact on the pooled effect size.

Results

We included eight studies in the meta-analysis on the effects of anti-IL-1 treatment on type 2 diabetes (Fig. 1). The selected studies were published from 2007 to 2018. In total, 2921 individuals were included (mean age range of 50–62.5 years). Four studies investigated the IL-1β antibody canakinumab, two studies investigated the IL-1 receptor antagonist anakinra blocking both IL-1α and β action, one study investigated the IL-1β antibody gevokizumab, and one study investigated the IL-1β antibody LY2189102. Two studies administered anti-IL-1 as a single dose and six studies as a repeated dose. Most studies administered the drug subcutaneously. Seven studies were double or triple-blinded, and one study was open-label [23]. Length of follow-up for our analysis varied from 8 weeks to 12 months. Table 1 shows details of the selected studies.

HbA1C

In the random effects meta-analysis, the average mean difference in HbA1c compared with the placebo was − 0.32% (95% CI − 0.51 to − 0.13, $p = 1.16 \times 10^{-7}$) with a 95% prediction

Table 1 Study characteristics [a]

Study	Year	Total *N*	Total *N* drug	Total *N* placebo	Drug	Drug administration frequency	Study design	ClinicalTrials.gov	Drug administration route	Length of follow-up	Drug dose	Type of mean	Age (years)	Baseline HbA1c (%)	Baseline BMI	Mean CRP (mg/L)
Larsen et al.	2007	67	34	33	Anakinra	Repeated	Phase II	NCT00303394	Subcutaneous	13 weeks	100 mg	Arithmetic	60.6	8.7	31.5	NR
Cavelti-Weder et al.	2012	20	10	10	Gevokizumab	Single	Phase I	NCT00541983	Subcutaneous and IV	8–12 weeks	Intermediate [b]	Arithmetic	50	9.1	31	2.5
Ridker et al.	2012	271	92	179	Canakinumab	Repeated	Phase IIb	NCT00900146	Subcutaneous	4 months	150 mg	LS	54.3	7.4	29.3	1.9
Sloan-Lancaster et al.	2013	42	19	23	LY2189102	Repeated	Phase II	NR	Subcutaneous	24 weeks	180 mg	LS	52.9	7.9	32.5	6.1
Noe et al.	2014	57	23	34	Canakinumab	Single	NR	NCT00900146	IV	24 weeks	1.5 mg/kg	Arithmetic	55.1	7.7	32.8	2.5
Choudhury et al.	2016	129	60	69	Canakinumab	Repeated	Phase II	NCT00995930	Subcutaneous	12 months	150 mg	Geometric	61.9	6.85	30.3	1.85
Everett et al.[c]	2018	2303	961	1342	Canakinumab	Repeated	Phase III	NCT01327846	Subcutaneous	48 months	150 mg	Arithmetic	61	7.1	29.1	4.3
Ruscitti et al.	2018	32	17	15	Anakinra	Repeated	Phase IV	NCT02236481	Subcutaneous	6 months	100 mg	Arithmetic	62.53	7.83	28.37	10.78

NR not reported, *LS* least square means

[a] Baseline values are presented for the placebo group

[b] Intermediate group = 0.03 mg and 0.1 mg/kg

[c] Baseline values for the patients with pre-diabetes as no baseline characteristics were presented for the diabetes subgroup

interval (95% PI) from − 0.92 to 0.29. The variability in treatment effect estimates was 84% due to real study differences (heterogeneity) and only 16% due to chance ($p_{\text{heterogeneity}} = 2.00 \times 10^{-7}$), which explains the difference between the random and the fixed effects estimates [− 0.14% (95% CI − 0.19 to − 0.10)] (Fig. 2). There was no publication bias ($p_{\text{Egger}} = 0.10$) (Appendix Fig. 1). Leave-one-out analysis revealed that the open-label study by Ruscitti et al. was a major driver of the pooled effect size and heterogeneity, as by removing the Ruscitti study, the heterogeneity decreased ($I^2 = 18\%$, $p_{\text{heterogeneity}} = 0.29$) (Appendix Fig. 2), and the average mean difference in HbA1c became more precise [− 0.14% (95% CI − 0.22 to − 0.06) (95% PI − 0.30 to 0.02)] and similar to the fixed effect estimate. To account for the differences in the scales across studies, the random effects standardized average mean difference (unit-less) in HbA1c compared with the placebo was − 0.50 (95% CI − 0.78 to − 0.21, $p = 6.2 \times 10^{-4}$; 95% PI − 1.37 to 0.37) and I^2 was 78% ($p_{\text{heterogeneity}} = 2.97 \times 10^{-5}$) (Appendix Fig. 3).

Stratification by drug removed heterogeneity and showed that canakinumab had smaller Hba1c (%) mean difference with a narrow confidence interval compared with the other IL-1β antagonists (Appendix Fig. 4). Stratification by drug administration regimen revealed that the single dose studies had a larger and more variable mean difference in placebo-adjusted HbA1c compared with repeated drug dosing regimen (Appendix Fig. 5). Stratification by subjects who had baseline HbA1c greater or less than 7%, the fixed and random effects estimate was − 0.36% [(95% CI − 0.61 to − 0.12) (95% PI − 1.15 to 0.42)] and − 0.13% [(95% CI − 0.29 to 0.04) (95% PI: Not Applicable, since this is based only on one study)], respectively, p difference = 0.12. (Appendix Fig. 6).

To investigate if the size of the mean differences in HbA1c was influenced by baseline HbA1c levels, we performed an unweighted and weighted meta-regression by baseline and intervention HbA1c (Fig. 3, Table 2). The HbA1c decrement increased with increasing baseline placebo HbA1c in unweighted (beta(SE) − 0.35 (0.11), $p = 0.021$) and weighted regressions (beta(SE) − 0.37(0.16), $p = 0.056$), respectively, with a similar although not significant tendency with increasing intervention HbA1c in unweighted (beta(SE) − 0.27(0.13), $p = 0.09$) and weighted regressions (beta(SE) − 0.26 (0.16), $p = 0.16$), respectively; however, none of these four values differed statistically from each other. HbA1c decrement was also dependent on baseline CRP in weighted but not in unweighted regressions, but notably this relation was observed both in the placebo and intervention groups (Appendix Table 2). HbA1c decrement was not dependent on baseline BMI or FG (Appendix Table 2).

The risk of bias assessment revealed moderate biases in the randomization process, the measurement of outcome, and the selection of reported results by Ruscitti and Cavelti-Weder (Appendix Figs. 7 and 8). For Cavelti-Weder et al. their

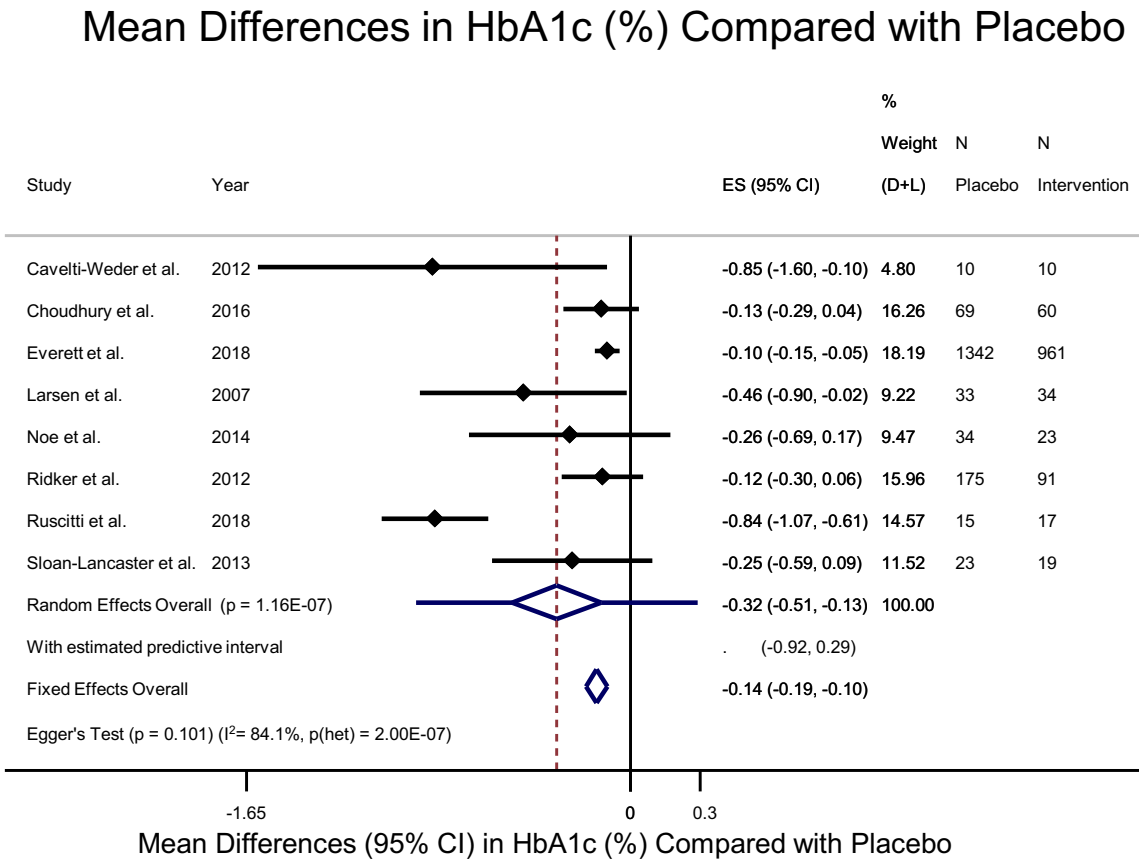

Fig. 2 Mean differences in HbA1c (%) compared with placebo

primary and secondary endpoint was to assess the safety profile and pharmacokinetics of gevokizumab. The effects of gevokizumab on glycemic biomarkers were assessed as an ancillary analysis. They also found that the baseline differences between intervention groups were slightly different in glycated hemoglobin (intervention 8.6% vs. placebo 9.1%)

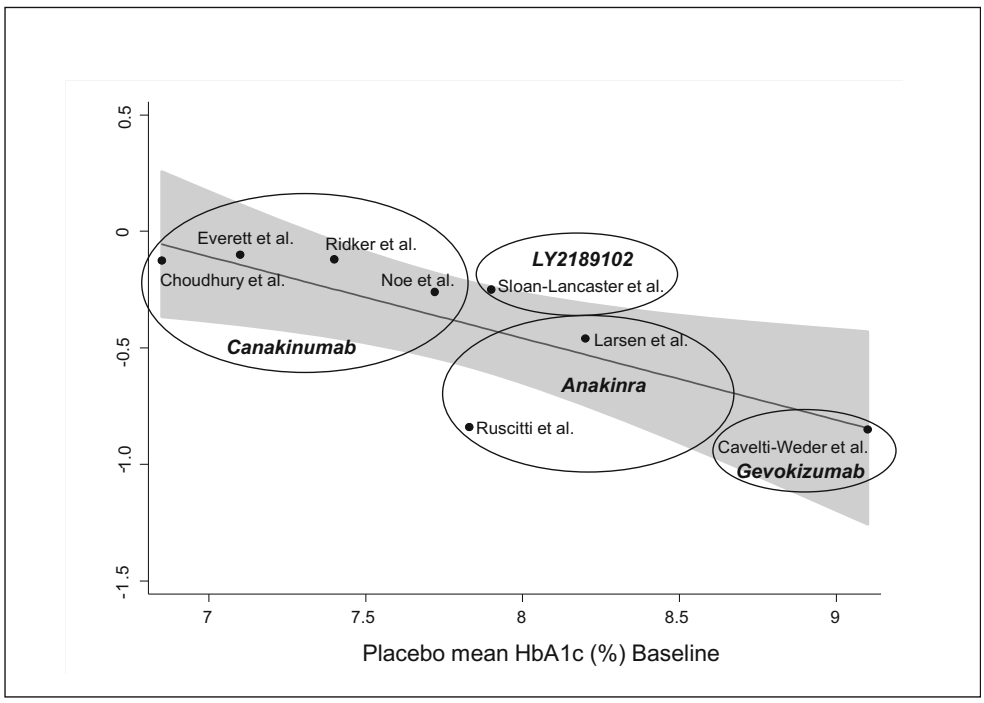

Fig. 3 Meta-regression of mean difference in HbA1c (%) compared with placebo by baseline HbA1c (%)

Table 2 Regression analysis for mean difference in HbA1c (%)

		Unweighted			Weighted		
	n	Beta	SE	*p* value	Beta	SE	*p* value
Baseline placebo HbA1c	8	− 0.350	0.113	0.021	− 0.372	0.158	0.056
Baseline intervention HbA1c	8	− 0.272	0.132	0.085	− 0.255	0.159	0.159
Placebo HbA1c—studies restricted to repeat drug administration regimen	6	− 0.357	0.216	0.175	− 0.396	0.223	0.150
Intervention HbA1c—studies restricted to repeat drug administration regimen	6	− 0.180	0.190	0.396	− 0.204	0.202	0.370

albeit not statistically significant. The Ruscuitti et al. study is an open-label design, which makes it susceptible to biases compared with a double-blind controlled trial, and as such the results could have been influenced by knowledge of intervention received. By removing these studies, the pooled fixed and random effects Hba1c mean differences were identical [− 0.11 (95% CI − 0.16 to − 0.07, 95% PI − 0.18 to − 0.05; $p = 3.21 \times 10^{-6}$)]. The between-study heterogeneity was also removed ($I^2 = 0\%$, $p_{\text{heterogeneity}} = 0.60$), so that variability in the pooled fixed effect Hba1c mean difference compared with placebo was only due to chance.

Fasting glucose

In the random and fixed effects meta-analyses, the mean difference of fasting glucose was − 3.13 mg/dL [(95% CI − 6.56 to 0.30) (95% PI − 13.79 to 7.53)] and − 0.94 mg/dL (− 1.91 to 0.02) with $I^2 = 79.7\%$ ($p_{\text{heterogeneity}} = 5.74 \times 10^{-4}$), respectively (Fig. 4), demonstrating that fasting glucose levels mediated by IL-1 antagonists did not significantly decrease.

C-peptide

The random and fixed effects mean difference of AUC C-peptide was 12.15 nmol/L min [(95% CI − 2.46 to 26.77, $p = 0.10$) (95% PI − 92.36 to 116.67)] and 12.09 nmol/L min (95% CI − 1.78 to 25.96) with $I^2 = 6.1\%$ ($p_{\text{heterogeneity}} = 0.345$), respectively (Fig. 5), demonstrating that C-peptide levels mediated by IL-1 antagonists did not significantly change. However, there was a positive linear association between baseline HbA1c levels (%) and mean difference in AUC C-peptide ($p = 0.04$) (Appendix Fig. 9); thus, removing the study with the lowest baseline HbA1c resulted in a fixed effect mean difference of AUC C-peptide of

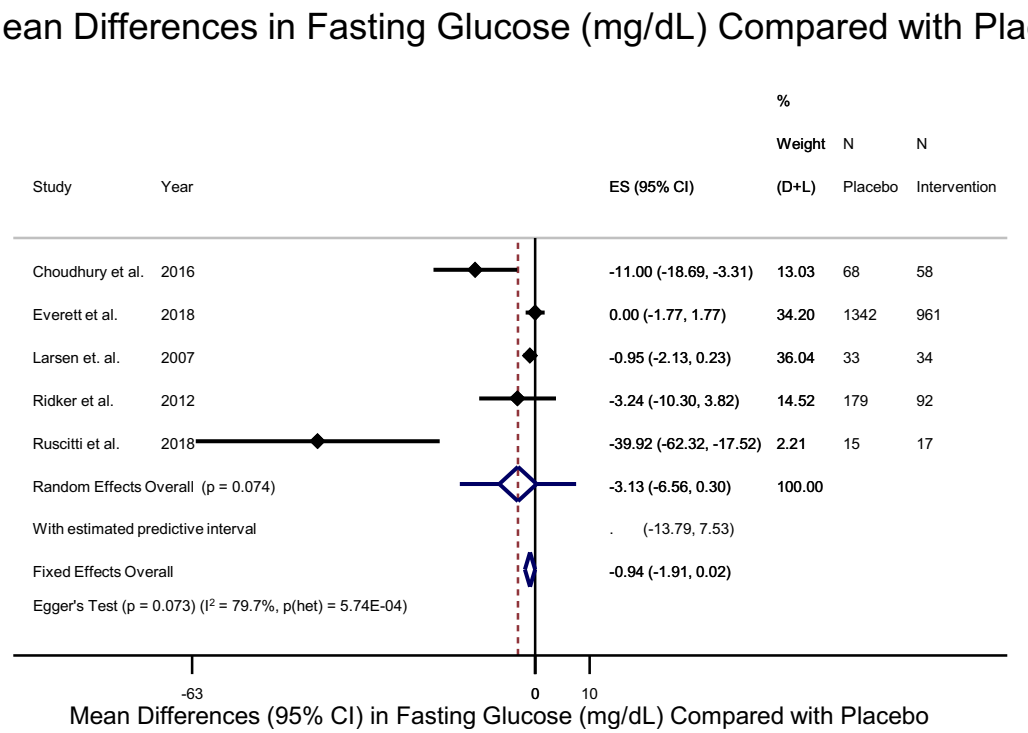

Fig. 4 Mean differences in fasting glucose (mg/dL) compared with placebo

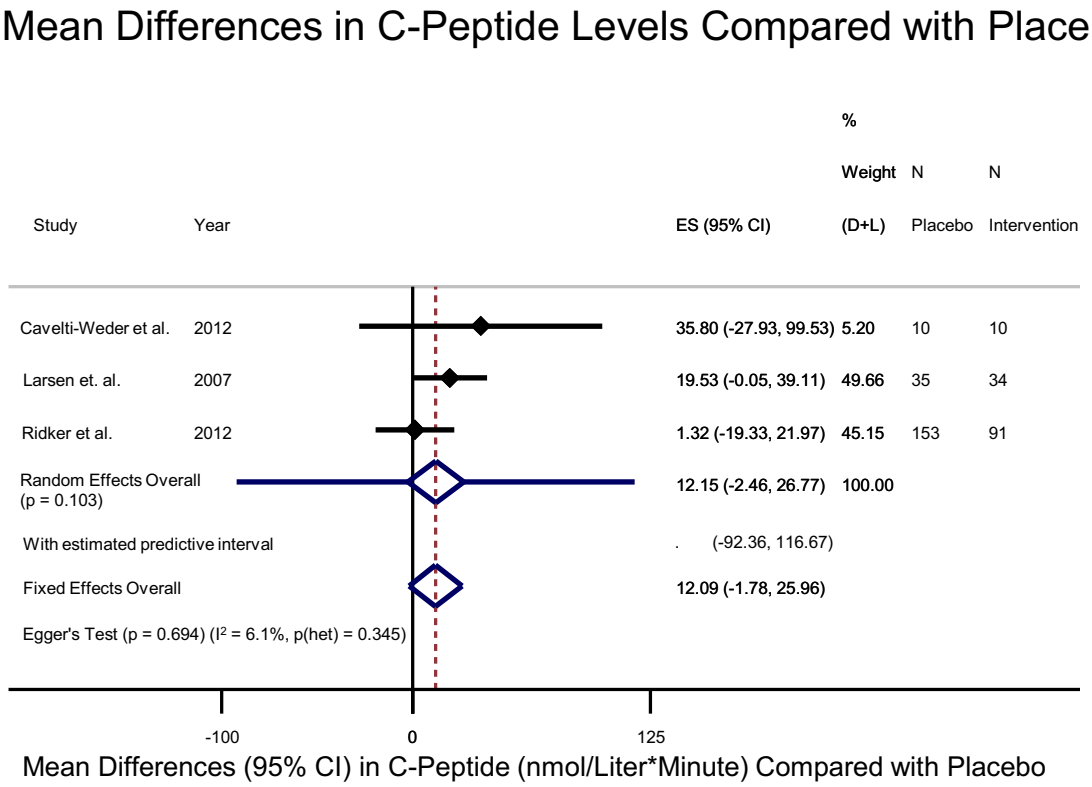

Fig. 5 Mean differences in C-peptide levels compared with placebo

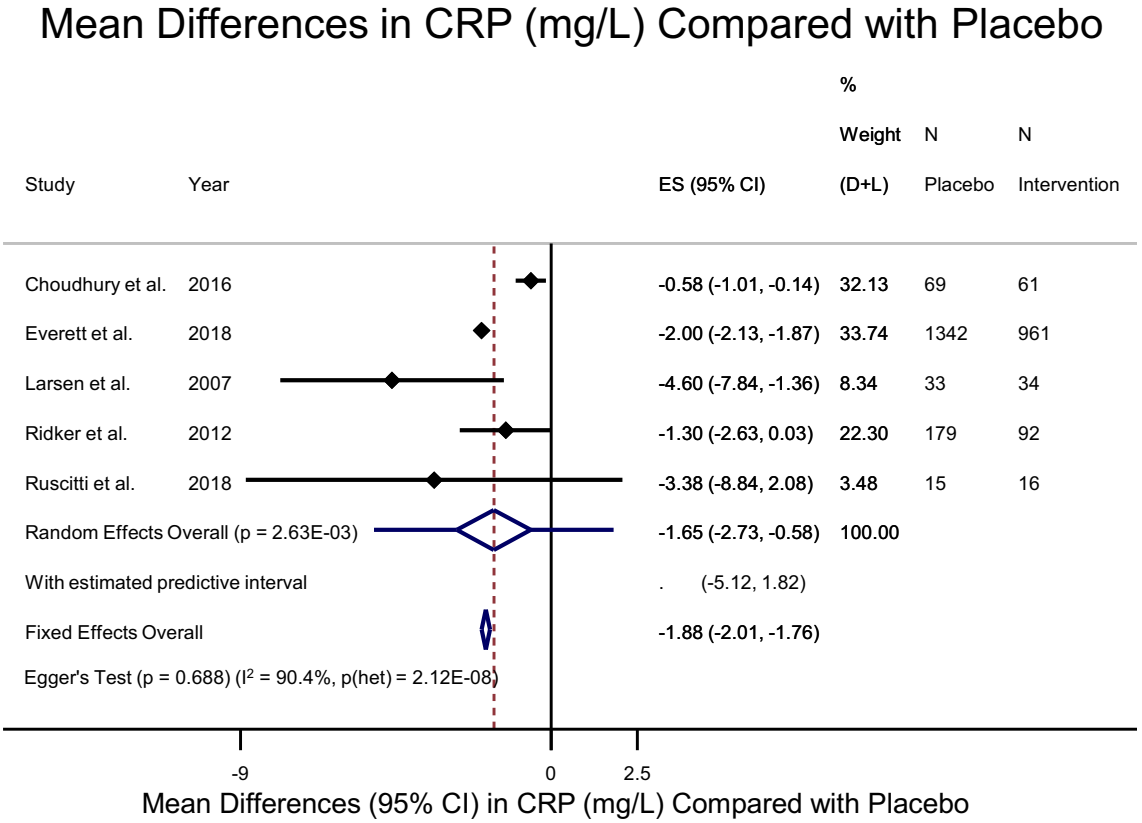

Fig. 6 Mean differences in CRP (mg/L) compared to placebo

20.93 nmol/L min [(95% CI 2.21 to 39.65, $p = 0.03$)] with no heterogeneity.

CRP

The random and fixed pooled effects mean difference in CRP levels was − 1.65 mg/L [(95% CI − 2.73 to − 0.58) (95% PI − 5.12 to 1.82)] and − 1.88 mg/L (95% CI − 2.01 to − 1.76 with $I^2 = 90.4\%$, $p_{\text{heterogeneity}} = 2.12 \times 10^{-8}$), respectively (Fig. 6), showing that IL-1 antagonist treatment significantly reduced CRP concentrations.

Discussion

Summary of key findings

This meta-analysis, the largest hitherto performed, of the impact of anti-IL-1 biologics on glycemia and inflammatory markers in patients with type 2 diabetes demonstrated a significant decrement in HbA1c in random average effect analysis, associated with a reduction in the inflammatory biomarker CRP, as also found by Huang et al. [21]. With the scope of designing more targeted clinical trials by selecting patients with the largest anticipated benefit, we performed further analysis focusing on two key aspects: the inflammatory state and the baseline glycemia and its relation to the outcome.

Inflammation and treatment effects

The largest effect sizes of the intervention on HbA1c were obtained with anakinra and gevokizumab, compared with both canakinumab and LY2189102. If the Cavelti-Weder et al. study, assessing gevokizumab, is excluded due to the limited power and large heterogeneity likely caused by its complex design, it would appear that combined blockade of IL-1α and IL-1β by the receptor antagonist anakinra is more efficacious than neutralizing only IL-1β [27]. However, a major driver of this difference was attributable to the remarkable lowering observed in patients with T2D as comorbidity to rheumatoid arthritis (RA) [23]. Interestingly, this was unrelated to the effect of anakinra on CRP, indicating that factors other than systemic inflammation are responsible for the larger treatment effect in that study, because dosing was identical to what was used in the study of T2D without RA as comorbidity [18].

Since a high CVD burden was predictive of response in the Larsen study, and since RA patients, especially with T2D comorbidity, have a higher CVD risk, one possibility is that risk factors other than those linked to systemic inflammation may control treatment responses [19]. Indeed, a serum proteome analysis of anakinra-treated T2D patients without comorbidity identified both inflammatory and non-inflammatory biomarkers [39]. Thus, increases in transthyretin (TTR), a protein binding thyroxine and retinol-binding protein and the iron transport protein transferrin (Tf) were identified as surrogates for clinical outcome. When considering that TTR may prevent β-amyloid formation and that islet amyloid polypeptide drives local islet inflammation in T2D by inflammasome activation, it is possible that IL-1 antagonism reduces islet amyloidogenesis [14, 40]. Tf binds circulating ferric iron thereby reducing the bioavailable iron pool. By increasing Tf, IL-1 blockade may reduce cellular labile iron pools that catalyze reactive oxygen species (ROS) formation, and thereby protect directly β cell mass and function.

Retinol-binding protein 4 (RPB4) and a protein tentatively identified as modified apolipoprotein-A1 (m-apo-AI) increased expression as a consequence of anakinra treatment [39]. RBP4 was associated with improved β cell function and increased TTR, RBP4, and m-apo-AI with reduced inflammation [39]. RPB4 has generally been positively associated with insulin resistance and CVD, albeit in a non-causal manner, and was recently reported to impair β cell function by unknown mechanisms [41–43]. Apo-A1 has anti-atherogenic, and m-apo-A1 has anti-inflammatory properties [44]. Thus, although the functional implications of these serum proteome biomarkers are far from clear, these findings may support that IL-1 antagonism has actions on common pathological pathways in diabetogenesis and atherogenesis.

Glycemia and treatment effects

A priori, we hypothesized that treatment effect depended on baseline HbA1c level, suggested to be a mechanistic driver of islet inflammation [16]. Due to the dispersion in baseline HbA1c levels across studies, we elucidated the effect of baseline glycemic control by first dichotomizing the studies into those where patients at study entry had HbA1c less and greater or equal to 7%, chosen as the accepted target for non-pregnant T2D according to ADA guidelines [25]. As shown in Appendix Fig. 6, the study enrolling patients below this threshold did not attain a statistically significant treatment effect of canakinumab, in contrast with studies recruiting patients above this limit [28]. The study was well-powered, being the third largest trial to date. The lack of effect could either be due to a lower glycemic drive on local islet inflammation or due to the study drug used, since canakinumab had the lowest efficacy across all four studies using this compound (Appendix Fig. 4).

Next, we performed meta-regression analyses. As shown in Fig. 4, there was an apparent increased decrement in HbA1c with increasing levels of baseline HbA1c. Unweighted as well as weighted (accounting for variances of treatment effect and residual heterogeneity between studies) analyses showed

borderline significant regression coefficients in the intervention group and significant regression in the placebo group. Since most studies did not ensure stable lifestyle and conventional anti-diabetic therapy by a run-in period before the start of specific intervention, the possibility that improved glycemia in the placebo group is at least in part a study effect cannot be ruled out. In summary, these analyses did not confirm the demonstrated dichotomized relationship.

Finally, we performed a leave-one-out analysis, demonstrating that only exclusion of the study of anakinra in patients with comorbid RA and T2D attenuated the overall effect size (Appendix Fig. 2). Patients in this study had entry HbA1c in the very mid-range of all studies (Fig. 3), and yet displayed the largest treatment effect, also suggesting that baseline glycemia does not predict the outcome.

Taken together, these analyses do not provide clinical evidence for the concept that glycemia by driving islet inflammation is a predictive biomarker of clinical response to anti-IL-1 therapies. However, the option of reverse causation, i.e., that the level of inflammation controls glycemia via adverse effects on β cell function and insulin sensitivity, must be kept in mind. Thus, although based only on three studies, it is of potential interest that β cell secretory function improved the most in response to IL-1 blockade in patients with high baseline HbA1c (Appendix Fig. 9). Considering that we found no overall evidence for baseline glycemia predicting the HbA1c improvement outcome, this observation may support the notion that the metabolic derangement at the start of treatment correlates with local islet inflammation and impaired insulin secretion, which would be expected to dictate glycemia. However, since systemic metabolic derangement may impede also on whole-body insulin sensitivity, and since no study has so far demonstrated effects of anti-inflammatory biologics on human insulin sensitivity, we hypothesize that the improved β cell function is incapable of compensating for persistent insulin resistance, masking the correlation between baseline glycemia and HbA1c outcome.

Study limitations

Our study has limitations that should be considered. There was tremendous variability observed in the way the data was reported among the studies. Potential confounders (i.e., age, disease duration, etc.) are presumably eliminated, because all the patients were subjected to random allocation. The included RCTs were clinically heterogeneous in terms of comorbidities, type of IL-1 antagonist intervention, drug administration frequency, blinding, and follow-up; these factors could attenuate the effect sizes and regression coefficients observed. More data are needed, in particular from the CANTOS diabetes sub-study, for which there is very limited biomarker information available currently [22]. In addition, trial duration in the meta-analysis also varied from 6 weeks to 12 months, and drug effects may change over time, as was seen in the Everett study, where canakinumab had short-term but no long-term beneficial effect on HbA1c. However, we had decided to use the 6-month data from this study, which may have inflated the pooled effect size, yet excluding the Everett study did not influence the overall pooled effect estimate. It is also important to note that for patients with established CVD (as in the Everett study), target HbA1c (%) is 7.5 to 8.0. Thus, most patients were already within target at entry, which may mask treatment effects of the intervention.

Variability due to differences between studies accounted for 84%, which was also reflected in the prediction interval (PI) that quantifies the distribution of the estimates of the interventions; thus, in 95% of cases, the effect will fall within the limits of the prediction interval in a hypothetical new, but similar study. The PI was mostly below zero, but also overlapped zero, indicating anti-IL-1 biologics will be beneficial in most settings, but in some settings anti-IL-1 biologics may be ineffective [37]. Due to the heterogeneity in the studies, future studies should be designed to target these heterogeneities to identify optimal patient and study design characteristics for intervention. Our sensitivity analysis demonstrated that the type of IL-1 antagonist modified the magnitude of the effect observed, but all agents lowered HbA1c levels. Perhaps unsurprisingly, the HbA1c decrement was more robust among studies that administered the drug repeatedly. As alluded to in the results, there was moderate bias in the studies by Cavelti-Weder and Ruscitti, the latter also having a major impact on the pooled estimates in the leave-one-study-out analysis. This could be attributable to the size of the study, the comorbidities present among the patients, and the study being an open-label design. Excluding Cavelti-Weder and Ruscitti resulted in an overall homogenous estimate of the true effect size in the mean difference in HbA1c (%) of −0.11, with 95% confidence and prediction intervals below zero, indicating that anti-IL-1 biologics could be beneficial in 95% of future study settings if conducted without any biases.

Conclusions, gaps, and perspectives

The three studies recently added to the list of anti-IL-1 trials in T2D and published since the last meta-analysis provided interesting novel information, each representing extremes in the efficacy span [21]. A caveat for both of these studies should be noted, as T2D patients were recruited with either CVD or RA as a comorbidity [22, 23]. Contrary to our expectation, the wider effect-range did not alter conclusions of the previous meta-analysis and did not allow identification of predictive biomarkers, suitable for patient selection for more targeted trials [21].

This meta-analysis illustrates a current dilemma in the future development of anti-inflammatory therapies for T2D. On one hand, the overall effect sizes of both anti-inflammatory

biologics and small molecules appear modest, and yet selected patient populations (T2D and RA) and subgroups (patients with low body surface, low endogenous IL-1Ra encoded for by a common (allelic frequency in the background population 44%) 5′-IL-1Ra gene promoter SNP, anamnestic evidence of high CVD burden) had robust responses with HbA1c decrements between 0.8 and 1.2% points comparable with effect sizes of current anti-diabetic block-busters [11, 18, 19].

This dilemma calls for trials designed specifically for a number of current pertinent research questions [Box 1]. Although these knowledge gaps in themselves would warrant more trials, industry and many clinicians may ask: given the costs and potential adverse effects of anti-inflammatory therapies, why would we want to invest effort in answering these questions, when we already have effective and safe drugs obtaining the desired effect sizes? What needs to be considered here is the fact that treatment failure of current symptomatic drugs due to progressive β cell functional inhibition and apoptosis is one of the most pressing current clinical problems in diabetology. Further, although not systematically reported in the included studies, hypoglycemia is not a complication to anti-IL-1 therapy, and the safety of IL-1 receptor blockade has been proven in the > 100,000 patients treated for rheumatologic disorders. In randomized controlled studies of anakinra, a marginal increase in trivial infections did not attain statistical significance. This remarkable safety profile is partly related to the short half-life of the compound, allowing rapid reversibility of innate immune inhibition at withdrawal. As expected when using anti-IL-1 antibodies which exert their inhibitory action for weeks, more adverse effects are observed. In the CANTOS study, there was a significant increase in fatal infections. Thus, an appraisal of the safety concerns raised by CANTOS requires more clinical studies with short-lived anti-inflammatory agents with rapidly reversible actions, such as anakinra, inflammasome inhibitors, or salsalate.

Box 1Pertinent research questions requiring clinical trials

- What is the most safe and efficient way of blocking low-grade inflammation in T2D? Anti-cytokine agents? Cytokine receptor blockers? Inflammasome inhibitors? NF-κB inhibitors?
- What is the optimal dosing regimen (continuous or intermittent)? As remission induction therapy to break an inflammatory vicious cycle at glycemic flares?
- What is the relevant outcome measure(s)? Glycemia? β cell function? Insulin sensitivity? CVD risk markers?
- What patient subsets should be included? Patients with inflammatory comorbidities such as RA, gout, inflammation, heavy CVD burden?
- When in the disease course should treatment be instituted? Early, with prominent residual β cell function? Later, when inflammatory progression sets in?
- Are current suggested biomarkers suitable for selection of patients and monitoring of response? Should future biomarkers reflect metabolic or inflammatory status? Comorbidity?

We should be ready for a paradigm shift where conventional glycemic endpoints should not disqualify novel treatments targeting the underlying pathogenetic processes, and where symptomatic and causative therapies go hand-in-hand in combinations. Preservation of functional β cell mass must have priority, since, after all, diabetes arises from the lack of adequate β cell function. In the absence of non-invasive monitoring of human β cell mass, efforts should be made to extract as much data from already completed studies to guide the future trials required to reach the logical goal of keeping the β cells healthy.

Compliance with ethical standards

Disclosure The authors declare that they have no conflicts of interest.

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