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Hydroxychloroquine in Stage 1 Type 1 Diabetes

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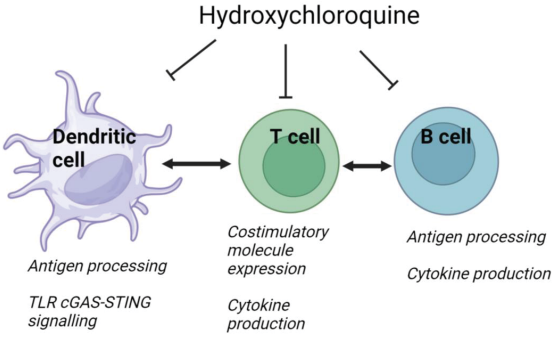
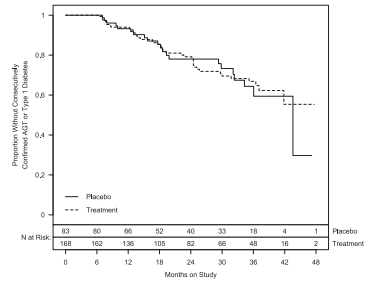
Hydroxychloroquine for Delaying Progression of Type 1 Diabetes in Patients With Stage 1 Disease



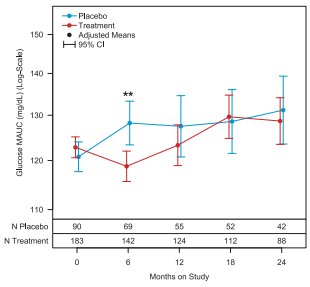
Hydroxychloroquine targets multiple immune activation functions that may be involved in type 1 diabetes. Individuals with stage 1 type 1 diabetes from the TrialNet Pathway to Prevention Study were randomly assigned to hydroxychloroquine or placebo. The end point was confirmed abnormal glucose tolerance or clinical diabetes.

Hydroxychloroquine did not delay the disease progression

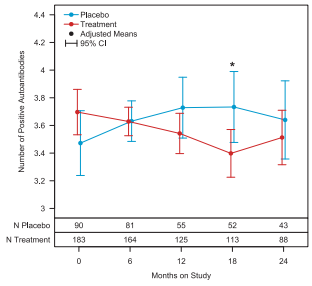


The hydroxychloroquine-treated group had a transient decrease in the glucose average area under the curve (AUC) to oral glucose at month 6 and a reduced change in the frequency of positive autoantibodies

Glucose AUC to OGTT



N of autoantibodies



ARTICLE HIGHLIGHTS

- Hydroxychloroquine inhibits antigen-presenting cell function and may improve metabolic responses. We therefore tested whether it would affect early stages of progression of autoimmune type 1 diabetes (T1D).
- We treated individuals with stage 1 T1D with hydroxychloroquine or placebo. After a median follow-up of 23.3 months, the trial was stopped because of futility. There were no safety concerns.
- Hydroxychloroquine did not delay progression to stage 2 T1D. However, it reduced the acquisition of additional autoantibodies and the titers of autoantibodies to GAD and insulin. Glucose responses to oral glucose tests were improved with hydroxychloroquine at month 6.

Hydroxychloroquine in Stage 1 Type 1 Diabetes

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OBJECTIVE

Innate immune responses may be involved in the earliest phases of type 1 diabetes (T1D).

RESEARCH DESIGN AND METHODS

To test whether blocking innate immaune cells modulated progression of the disease, we randomly assigned 273 individuals with stage 1 T1D to treatment with hydroxychloroquine (*n* = 183; 5 mg/kg per day to a maximum of 400 mg) or placebo (*n* = 90) and assessed whether hydroxychloroquine treatment delayed or prevented progression to stage 2 T1D (i.e., two or more islet autoantibodies with abnormal glucose tolerance).

RESULTS

After a median follow-up of 23.3 months, the trial was stopped prematurely by the data safety monitoring board because of futility. There were no safety concerns in the hydroxychloroquine arm, including in annual ophthalmologic examinations. Preplanned secondary analyses showed a transient decrease in the glucose average area under the curve to oral glucose in the hydroxychloroquine-treated arm at month 6 and reduced titers of anti-GAD and anti-insulin autoantibodies and acquisition of positive autoantibodies in the hydroxychloroquine arm (*P* = 0.032).

CONCLUSIONS

We conclude that hydroxychloroquine does not delay progression to stage 2 T1D in individuals with stage 1 disease. Drug treatment reduces the acquisition of additional autoantibodies and the titers of autoantibodies to GAD and insulin.

Type 1 diabetes (T1D) results from an immune-mediated destruction of β-cells (1). The causative events are thought to begin years before clinical diagnosis in most individuals, and a recent redefinition of T1D recognizes a point of no return with the appearance of multiple islet autoantibodies (stage 1). This is followed by deterioration in β-cell function, leading to abnormal glucose tolerance (AGT; stage 2), before the onset of clinical diabetes (stage 3) (2). Data from preclinical models and successful immune therapy trials have indicated that adaptive immune cells, including T and B lymphocytes, are required for the disease to progress. There is also evidence that innate immune cells, including pancreatic macrophages, mucosal-associated invariant T cells, and others, are involved in the presentation of antigens that lead to the adaptive

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responses (3). Neoantigens, released by stressed β-cells, may be taken up by pancreatic macrophages and presented, after lysosomal degradation, to adaptive immune cells in the pancreatic lymph nodes or even in the pancreas (4,5).

In addition, metabolic studies of individuals at risk of T1D have identified quantitative and qualitative abnormalities in insulin secretion, even when glucose tolerance is normal (6). B-cell dysregulation may serve as a biomarker of the pathologic process but also itself contributes to disease progression in uncertain ways.

The antimalarial drug hydroxychloroquine has been evaluated in randomized controlled trials and is widely used for the treatment of rheumatoid arthritis (7), systemic lupus erythematosus (8), and other inflammatory rheumatic diseases (9), including use in very young children, and is particularly effective in the early stages of the disease process. The mechanism of action is thought to be through interference with lysosomal activity and autophagy, thereby affecting membrane stability and altering signaling pathways and transcriptional activity. These actions can result in inhibition of cytokine production and modulation of costimulatory molecules (10). The alkalinization of intracellular vacuoles that is induced by hydroxychloroquine leads to inhibition of proteolysis, chemotaxis, phagocytosis, and antigen processing. In addition, Toll-like receptor activation is impeded. Consistent with this mechanism, oral hydroxychloroquine partially protected against toxin-induced diabetes with streptozotocin in a rat model (11). The treatment lowered pancreatic levels of interleukin-1β, interleukin-6, tumor necrosis factor-α, and transforming growth factor-β1 expression and serum levels of MCP-1 and caspase-3. Moreover, hydroxychloroquine may improve metabolic impairments, as evidenced by reduced rates of diabetes in patients with rheumatoid arthritis and systemic lupus who are treated with the drug (12–15). Hydroxychloroquine was also associated with improved insulin sensitivity and β-cell function over a 13-week period in a placebo-controlled double-blind study in overweight/obese adults without diabetes (16).

These observations suggest that hydroxychloroquine treatment may be beneficial for preventing the progression of T1D in its earlier stages in humans. We therefore conducted a randomized placebo-controlled trial (2) to determine whether hydroxychloroquine treatment would delay or prevent the progression from stage 1 T1D (two or more diabetes-related autoantibodies and normal glucose tolerance) to stage 2 (two or more diabetes-related autoantibodies and AGT) or stage 3 T1D (clinical diagnosis) (2).

RESEARCH DESIGN AND METHODS

Trial Participants

The trial was conducted between August 2018 and 31 October 2022. A total of 45 sites enrolled or observed trial participants: 33 in the U.S. and Canada and 12 in Australia, the U.K., Finland, Italy, and Sweden. Institutional or central review board approval was obtained at each participating site. Informed consent was obtained from participants age >18 years or from one legally authorized representative for minors, and assent was obtained from minors age 7 to <18 years, before any study procedures were performed.

Relatives (without diabetes) of individuals with T1D had been screened for diabetes-related autoantibodies in the TrialNet Pathway to Prevention Study (TN01), and other autoantibody-positive individuals were enrolled in TN01 and eligible for participation (17). Inclusion criteria included age ≥3 years at the time of random assignment and stage 1 T1D, defined as two or more diabetes-related autoantibodies detected in two serum samples, one of which was obtained within 6 months of enrollment, with normal glucose tolerance. The remaining inclusion and exclusion criteria are listed on ClinicalTrials.gov. Potential participants underwent an oral glucose tolerance test (OGTT; 1.75 gm/kg glucose to a maximum of 75 gm) within 7 weeks of random assignment to confirm normal glucose tolerance, defined as fasting plasma glucose ≤110 mg/dL (6.1 mmol/L), 2-h plasma glucose <140 mg/dL (7.8 mmol/L), and plasma glucose at 30, 60, or 90 min ≤200 mg/dL (11.1 mmol/L). Individuals with AGT or glucose levels fulfilling the diagnosis of T1D (2) on two occasions within 52 days of randomization, other clinically relevant medical history, abnormal laboratory chemical values, or abnormal blood counts were excluded.

Trial Design and Intervention

Participants were randomly assigned at a ratio of two to one to receive either hydroxychloroquine (5 mg/kg per day to a maximum dose of 400 mg per day) or placebo. Daily doses were rounded to the nearest 100 mg but were varied over the week to ensure an average dose ≤5 mg/kg per day adjusted for current weight. Participants underwent assessment of glucose tolerance status, insulin production, immunologic status, and overall health every 6 months. Treatment assignments were generated using PROC PLAN in SAS and stored in a secure file maintained by the TrialNet Coordinating Center.

End Points and Assessments

The primary end point was the elapsed time from random assignment to either consecutively confirmed AGT (i.e., stage 2 T1D, as defined previously [18]) or stage 3 T1D (clinical onset). The time to diagnosis of AGT or stage 3 diabetes was defined as that from the date of random assignment to the first of the two diagnostic tests. Participants and study sites remained masked to the diagnosis of AGT. Masking was maintained for a confirmatory OGTT by the inclusion of random requests for repeat OGTTs for quality control. Scheduled OGTTs were performed every 6 months (± 2 weeks) after random assignment. The study end point was realized with either confirmed OGTT criteria for AGT or diabetes or clinical criteria for diabetes (as

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*A complete list of Type 1 Diabetes TrialNet Study Group members can be found in the APPENDIX.

defined by the American Diabetes Association [18]). Study medication was stopped in participants who reached the study end point, but those who had stage 2 T1D were observed until they were diagnosed with stage 3 T1D.

Safety and Metabolic Studies

Known adverse effects of hydroxychloroquine include retinal toxicity. The risk of retinopathy is considered to be duration and dose dependent (19). In addition to general medical examinations, participants underwent ophthalmologic examinations, including fundus photography, visual field testing, and spectral-domain optical coherence tomography within 3 months of random assignment and annually, while receiving the study drug. All photographs and scans were read and interpreted centrally. Complete blood count with differentials and blood chemistry analytes were measured in local laboratories. Plasma glucose and C-peptide levels were measured in a central laboratory (20), the latter using the Tosoh Bioscience assay. Autoantibodies were measured by radiobinding assay at the Barbara Davis Diabetes Center (21).

Statistical Design and Analysis

The efficacy end point, the cumulative incidence of AGT or stage 3 T1D over time after random assignment within each group, was estimated using the Kaplan-Meier method, applying the intention-to-treat principle. AGT was defined as fasting glucose $\geq$110 mg/dL (6.1 mmol/L), with 120-min glucose $\geq$140 mg/dL (7.8 mmol/L) and $<$200 mg/dL (11.1 mmol/L), or 30-, 60-, or 90-min glucose $\geq$200 mg/dL (11.1 mmol/L) confirmed on two consecutive OGTTs. The diagnosis of stage 3 diabetes was defined using American Diabetes Association criteria (22), with the exception that two consecutive OGTTs meeting the threshold for diabetes were required in the absence of unequivocal symptoms.

The mean area under the curve (AUC) for glucose and C-peptide was calculated by summing the area between the data points over the 120-min interval using the trapezoidal rule and then dividing by the 120-min interval, which represents the mean concentration during the OGTT.

The difference between treatment groups was estimated by the hazard ratio (HR), and hypothesis tests used the likelihood ratio test based on the Cox proportional hazards model (23,24). Time-to-AGT was discretized to 6-month times in keeping with the OGTT schedule. In planning, the predicted rate for the development of AGT was 40% in 2 years. The original study design was to conduct the final analysis once 80 events (confirmed AGT, diabetes) had been observed. The study was designed to provide 83% power to detect a 50% risk reduction in the hazard rate for progression to stage 2 or 3 T1D using a two-sided test at the 0.05 level after a minimum of 2 years of follow-up on all participants and an expected total study duration (accrual and follow-up) of 6 years. All comparisons are two sided and not adjusted for multiple comparisons.

Adverse events (Common Terminology Criteria for Adverse Events [version 4]) of grade $\geq$2 were reported. Because of the known retinal toxicity of hydroxychloroquine, ophthalmology examinations were reviewed under the supervision of an ophthalmology expert advisory panel (25). Data on safety and efficacy were evaluated twice yearly by an independent data safety monitoring board (DSMB). Lan-DeMets stopping rules were used for the primary end point, with a type I spending function patterned after the O'Brien-Fleming method (26). In October 2022, the DSMB declared that study enrollment be terminated because of a lack of efficacy.

Prespecified secondary longitudinal analyses were also performed to assess the effects of hydroxychloroquine versus placebo treatment on immunologic and metabolic markers over time. Log transformations were applied to glucose measures, C-peptide measures, and autoantibody titers to ensure that the variables fit well in the normal random error models (Supplementary Table 1). The C-peptide and glucose AUCs were compared with ANCOVA, regressing on baseline level and age. The means and treatment group effect are adjusted for age and baseline constituents using the predicted value from the fitted model. The mean responses at 6-, 12-, 18-, and 24-month time points were compared between the two groups using ANCOVA, adjusted for baseline measures and age. Additionally, linear mixed-effects models with random intercepts and slopes were used to compare the average change in measures after intervention between the two groups. The adjusted means were calculated based on the observed data without imputation. In the mixed-effects models, individual-level variation was captured based on the observed data.

An interim analysis was planned when 50% of events (40 cumulative events) had been observed, and the conditional probability method was used to assess the futility of treatment. The actual number of events observed in the interim analysis was 44. At the time of the interim analysis, 253 participants had been randomly assigned in this trial, 170 to hydroxychloroquine and 83 to placebo.

Trial Oversight

The Type 1 Diabetes TrialNet Network/National Institute of Diabetes and Digestive and Kidney Diseases was the study sponsor. TrialNet investigators designed the trial. An independent medical monitor (who was unaware of the treatment group assignments) reviewed all safety data. Hydroxychloroquine and placebo tablets were purchased by the TrialNet Coordinating Center. National Institute of Diabetes and Digestive and Kidney Diseases representatives participated in the design and conduct of the trial; interpretation of the data; preparation, review, and approval of the manuscript for submission; and decision to submit the manuscript for publication. The authors wrote the manuscript and reviewed the data. A complete list of TrialNet study team investigators is attached in the Appendix.

RESULTS

Participants and Medication

From a potential pool of 493 participants in TN01 screened for eligibility, 273 were randomly assigned to receive either hydroxychloroquine (n = 183) or placebo (n = 90) (Fig. 1). Enrollment in the trial was significantly affected by the COVID-19 pandemic. In the pre–COVID-19 period (before 15 March 2020), the rate of enrollment was 126 participants per year, whereas during COVID-19, it was 49 participants per year (Supplementary Fig. 1). Fifteen participants in the treatment arm and seven in the placebo arm withdrew or were lost to follow-up within the first month, and 168 and 83, respectively, were included in the time-to-event analysis.

There were not significant differences in the demographic characteristics or baseline metabolic or immunologic features of the study groups (Table 1). More than 50% of participants were siblings of individuals with T1D. Most participants were

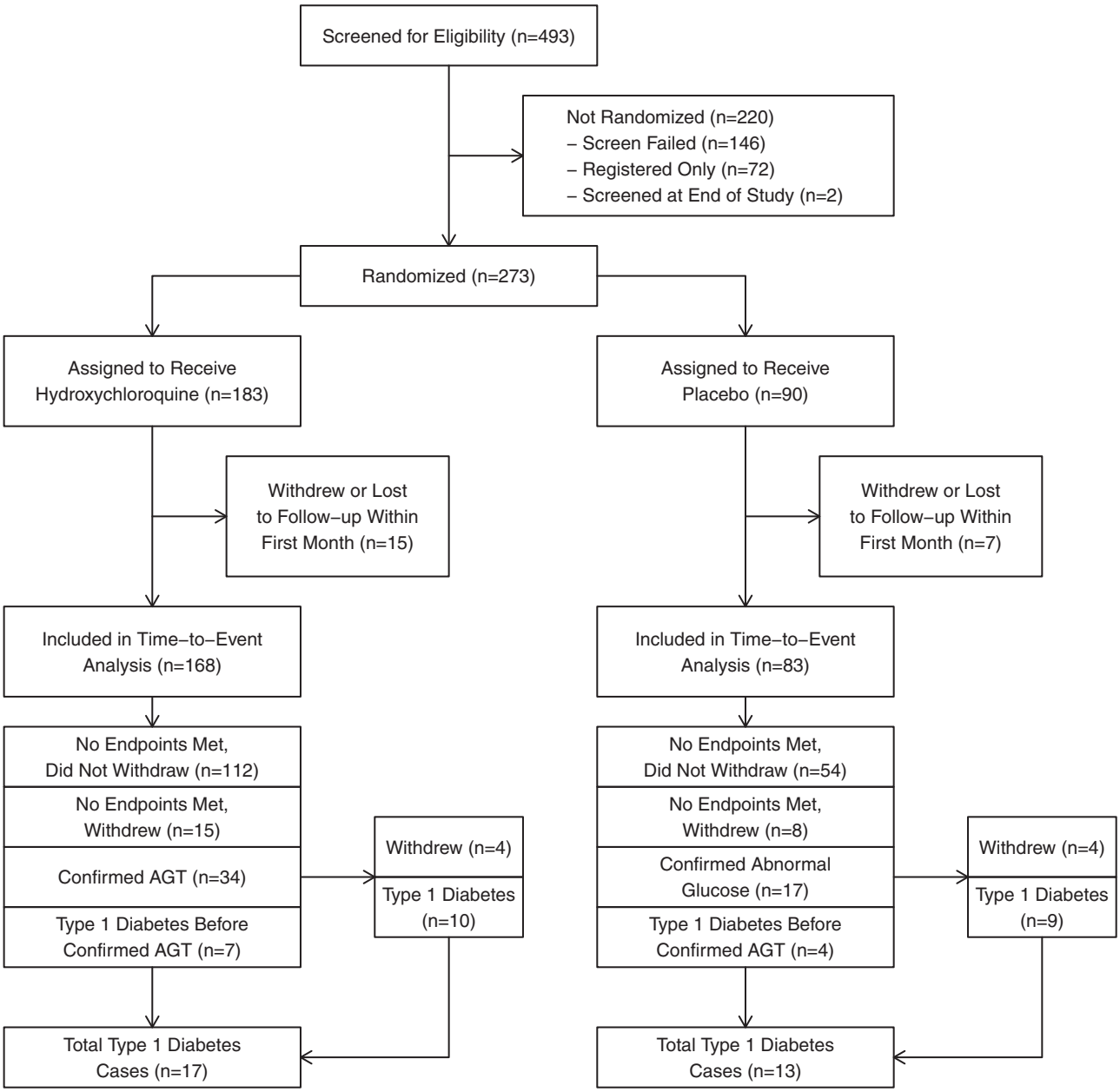

Figure 1—CONSORT diagram showing study conduct.

positive for autoantibodies to GAD ($GADA^+$) and insulin (IAA^+). Three, 58, 63, 64, and 85 participants had one, two, three, four, and five or more autoantibodies, respectively, before receiving treatment. The three participants with a single autoantibody at the baseline visit had two confirmed autoantibodies at the screening visits.

The median study medication compliance (number of pills taken/number expected to be taken) was ∼90%. Pill reports were not available in 17 participants.

Efficacy

The study participants were observed for a median of 23.3 months. After conducting the interim analysis and the DSMB review, study enrollment was stopped because of futility. Thirty-four hydroxychloroquine-treated participants and 17 placebo-treated participants had confirmed abnormal OGTTs. Ten of the 34 with AGT in the hydroxychloroquine group and nine of the 17 placebo group participants with AGT subsequently developed stage 3 diabetes. Eight participants with abnormal OGTTs (four in each treatment arm) withdrew after meeting the study end point. Seven and four participants in the active drug and placebo groups, respectively, were diagnosed with T1D without a preceding AGT test. The unadjusted HR, describing the time to confirmed abnormal OGTT or T1D, was 0.95 (95% CI 0.56–1.61) (Fig. 2). Participants who experienced progression to stage 2 or 3 T1D had a median follow-up time of 18.6 months, whereas those who did not experience progression had a median follow-up time of 24.8 months. An additional eight hydroxychloroquine-treated participants and four placebo-treated participants had abnormal OGTTs that were not confirmed within 1 year.

The COVID-19 pandemic affected the study conduct. In general, there was a similar impact of missed study visits on both study arms. Of the 1,065 follow-up OGTT visits, 1,028 occurred within 1 year of the

Table 1—Demographics

Variable	Hydroxychloroquine (n = 183)	Placebo (n = 90)
Age, years	11.7 (8.3–15.6)	10.9 (8.2–15.7)
Sex		
Female	61 (33.3)	33 (36.7)
Male	122 (66.7)	57 (63.3)
Race		
White	171 (97.2)	85 (96.6)
Black or African American	3 (1.7)	1 (1.1)
Other	2 (1.1)	2 (2.3)
Missing	7	2
Ethnicity		
Non-Hispanic	168 (91.8)	86 (95.6)
Hispanic	15 (8.2)	4 (4.4)
BMI, kg/m^2	18.9 (16.4–21.7)	18.0 (16.1–22.6)
BMI z score	0.377 (−0.298 to 1.002)	0.286 (−0.212 to 0.938)
Family history		
>1 first-degree relative	15 (8.2)	6 (6.7)
Sibling(s)	100 (54.6)	48 (53.3)
Offspring	6 (3.3)	5 (5.6)
Parent(s)	42 (23.0)	22 (24.4)
HbA_{1c}, %	5.20 (5.00–5.30)	5.20 (5.00–5.30)
C-peptide mean AUC, pmol/mL	1.60 (1.18–2.04)	1.66 (1.10–2.10)
Fasting glucose, mg/dL	90.0 (83.5–95.0)	90.0 (85.2–94.0)
Glucose mean AUC, mg/dL	124.1 (113.2–133.4)	119.6 (111.9–131.2)
N of positive autoantibodies*		
1	2 (1.1)	1 (1.1)
2	35 (19.1)	23 (25.6)
3	39 (21.3)	24 (26.7)
4	46 (25.1)	18 (20.0)
5	61 (33.3)	24 (26.7)
HLA DR3 present	41 (36.6)	26 (42.6)
HLA DR4 present	72 (64.3)	42 (68.9)
HLA type DR3/DR4	20 (17.9)	18 (29.5)

Data are given as n (%) or mean (95% CI). *Data reflect n at time of enrollment. Participants needed to have two or more diabetes-related autoantibodies present in two separate samples, one of which was drawn within the past 6 months (180 days). Confirmation did not have to involve the same two autoantibodies.

previous visit, and 37 occurred after 1 year of the previous visit. We determined that, if all confirmatory tests were performed within 1 year as prescribed by the protocol, a maximum of 13 events could have occurred, of which a maximum of six observed end points could have occurred earlier than recorded (Supplementary Table 2). If all the treatment group participants who missed tests did not meet an end point, and all the placebo group participants who missed tests did meet an end point, the study outcome would not have differed.

We also investigated whether a hydroxychloroquine effect might have been different in any subgroups predefined by demographic and mechanistic factors. However, neither age, BMI z score, autoantibodies, nor HLA type had a significant effect on the primary outcome measure (Supplementary Fig. 2).

Safety

Adverse events were reported in 27.9% and 26.1% of the hydroxychloroquine- and placebo- treated participants, respectively. A total of 11 and one event of grade ≥2 in the drug treatment and placebo arms, respectively, were judged to be possibly, probably, or definitely related to the study drug (Supplementary Table 3). (Those categorized as nervous system disorders included responses to IV placement or removal but did not include retinal events.) Of the 738 ophthalmologic examinations that were performed in 251 participants (166 in the drug treatment group and 85 in the placebo group), 39 were considered abnormal (27 in the active treatment group and 12 in the placebo group), but none of the abnormal examinations led to a diagnosis of hydroxychloroquine-induced retinal toxicity.

Effects of Hydroxychloroquine Treatment on Metabolic Measures and Autoantibodies

We used data from the OGTTs to assess the impact of hydroxychloroquine on metabolic responses and measured the HbA_{1c} levels. The glucose mean AUC increased over the course of the study, but the slopes were not significantly different between the study arms by a mixed-effects model (0.054 log-scaled units per year for placebo; $P < 0.0001$ and 0.033 log-scaled units per year for hydroxychloroquine; P = 0.0003; comparison of slopes P = 0.199). There was, however, a reduction in the glucose mean AUC in response to the OGTT in the hydroxychloroquine treatment group at month 6 (P = 0.002) (Fig. 3*A*), but the glucose mean AUC increased over the next 12 months. There were no differences in the adjusted means of BMI over time by group, and no reduction was seen in the hydroxychloroquine group at month 6 (Supplementary Fig. 3). The slopes of the C-peptide mean AUC were not significantly changed during the course of the study (0.0041 log-scaled units per year for placebo; P = 0.688 and −0.0073 log-scaled units per year for hydroxychloroquine; P = 0.308; comparison of slopes P = 0.362) (Fig. 3*B*). The slopes of the ratio of C-peptide to glucose mean AUC decreased minimally over the course of 12 months but were not significantly changed by drug treatment (placebo −0.00049 units per year; P = −0.035 and hydroxychloroquine −0.00032 units per year; P = 0.045; comparison of slopes P = 0.559). The HbA_{1c} levels increased in both groups over time (slope 0.15% per year for placebo; $P < 0.0001$, and 0.136% per year for hydroxychloroquine; $P < 0.0001$; comparison of slopes P = 0.552) (Fig. 3*C*).

We evaluated the number of positive autoantibodies and their titers in those that were present at study entry. The number of positive autoantibodies was

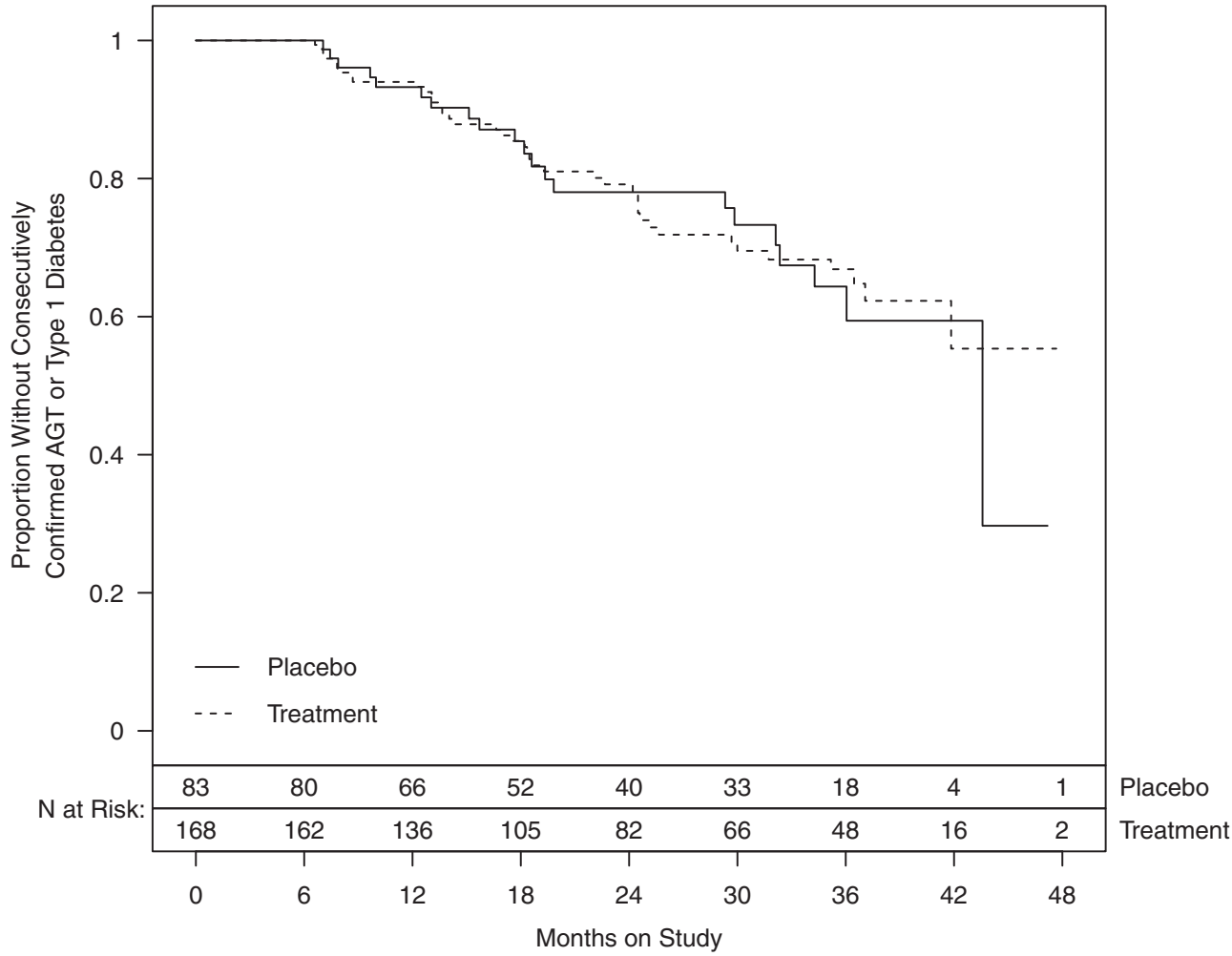

Figure 2—Effects of hydroxychloroquine treatment on primary study outcome. Time to the first consecutively confirmed AGT or stage 3 T1D diagnosis is shown for the two treatment arms. Unadjusted HR, describing time to confirmed abnormal OGTT or T1D, was 0.95 (95% CI 0.56–1.61; *P* = not significant).

compared in the study arms with a linear model. In the drug treatment group, there was a reduction in the number of positive autoantibodies, whereas in the placebo group, the number of positive autoantibodies increased with time (*P* = 0.034) (Fig. 3*D*). The titers of GADA and IAA declined in the hydroxychloroquine group among participants positive at baseline (*P* = 0.032 and 0.026 vs. placebo, respectively), whereas there were no significant differences in the titers of autoantibodies to zinc transporter 8 or autoantibodies to IA2 (Supplementary Fig. 4*A* and 4*B*).

CONCLUSIONS

We tested the hypothesis that hydroxychloroquine, a drug known to inhibit antigen-presenting cell function, would delay the progression of stage 1 T1D to stage 2 or 3 T1D. Our study was well powered based on predictions of the rates of progression of disease from our TrialNet studies of the natural history of stage 1 T1D. The safety experience in this trial, which enrolled mostly children, did not identify any significant concerns. The decision by the DSMB to prematurely stop study enrollment indicated a lack of efficacy of the treatment on the clinical outcomes. However, we found that the treatment did have effects on the acquisition of autoantibodies, as had been postulated from its presumed mechanisms of action. These secondary findings suggest that an intervention that can prevent the generation of autoantibodies is not sufficient to prevent disease progression once two or more autoantibodies are present (i.e., stage 1 T1D).

The HbA_{1c} levels increased with time in both treatment arms. The broad range of time to progression to stage 2 T1D suggests that the study may have not observed the participants for a sufficient period of time to accurately estimate the median time to event. We observed that 40% of the placebo group met the study end point at 4 years, twice as long as expected. Nonetheless, there was no evidence of efficacy with a median follow-up time of 23.3 months. The COVID-19 pandemic and lower rates of recruitment and follow-up in the study centers may have accounted for the prolonged time to acquire the events needed to fulfill our statistical planning parameters. COVID-19 did not seem to affect the primary outcome. COVID-19 infection (defined by a positive test) was self-reported. Although not prespecified, in an age-adjusted Cox model comparing rates of progression between those who reported infection with SARS-CoV-2 (*n* = 154) and those who did not, we did not find significant differences (HR 1.596; 95% CI 0.772–3.296; *P* = 0.207).

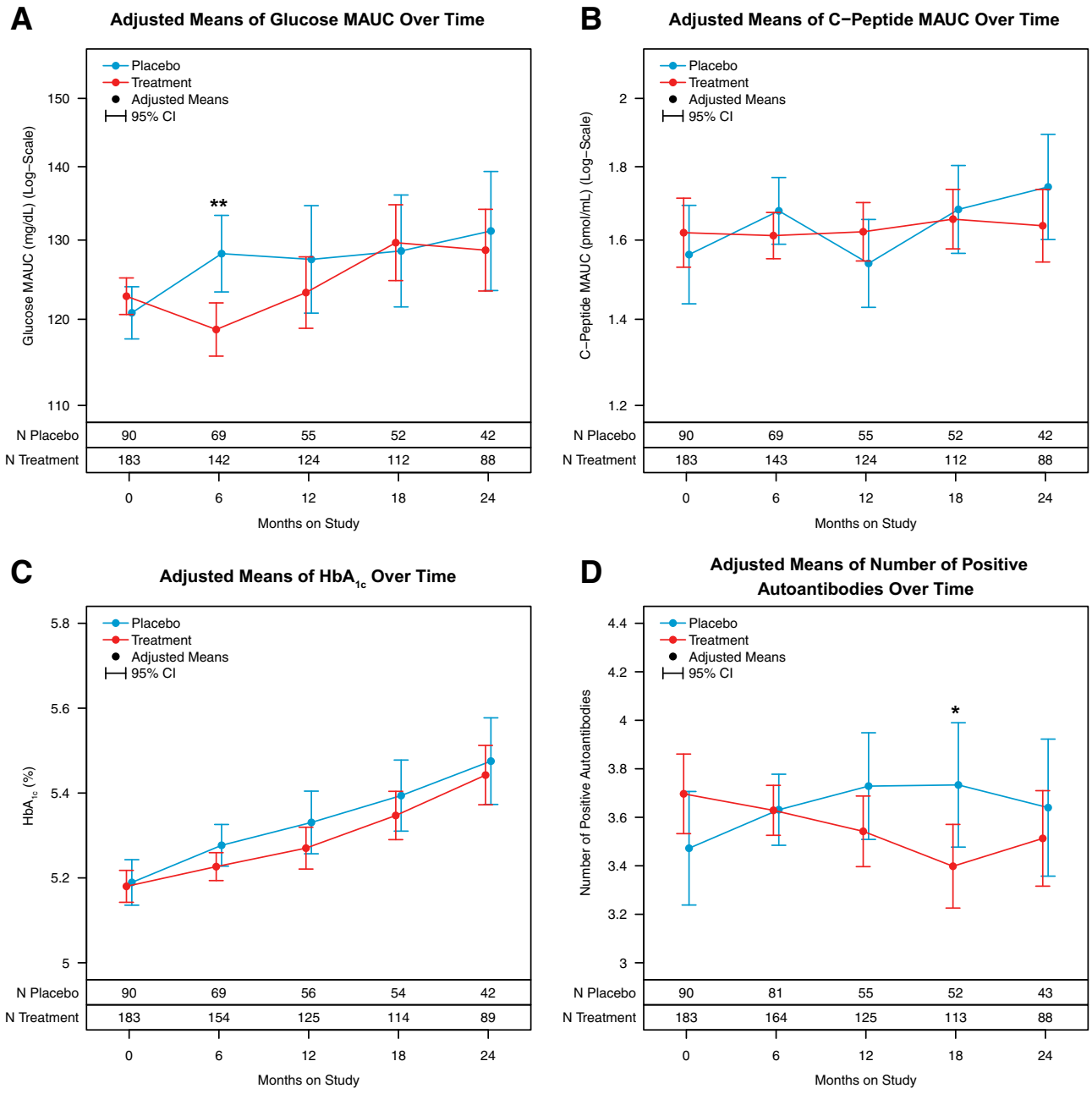

Figure 3—Effects of hydroxychloroquine treatment on metabolic and immunologic measures. *A*: Glucose mean AUC (MAUC) was analyzed by linear mixed model. MAUC increased significantly over 24 months in the hydroxychloroquine and placebo groups (slope for placebo 0.054 mg/dL per month; $P < 0.0001$; hydroxychloroquine 0.0334; $P = 0.0003$; comparison $P = 0.199$). Individual time points were compared based on likelihood ratio tests. *B*: C-peptide levels were unchanged over course of the study (slope for placebo 0.0041 pmol/mL per month; $P = 0.688$; hydroxychloroquine −0.0073 pmol/mL per month; $P = 0.31$; comparison $P = 0.362$). *C*: HbA_{1c} levels increased in both groups over time (slope for placebo 0.15% per month; $P < 0.0001$; hydroxychloroquine 0.136% per month; $P < 0.0001$), but slopes were not significantly different between the treatment arms (comparison $P = 0.552$). *N* of participants in each treatment arm is shown. For all measures, mean ± 95% CI is shown. *D*: *N* of positive autoantibodies at each study visit was determined. Slope describing change in *n* of positive autoantibodies increased in placebo group (0.042 per month) but declined in hydroxychloroquine group (−0.090; $P = 0.032$). *N* of participants at each study time point is reported at bottom of the graph. For each graph, red = drug and blue = placebo. *$P = 0.034$, **$P = 0.002$.

The number of positive of autoantibodies increased over time in the placebo arm, and hydroxychloroquine treatment prevented this progression. This was found for the titers of IAA and GADA, but not zinc transporter 8 or IA2 autoantibodies, among those who were seropositive at study entry. Interestingly, among the biochemical autoantibodies, IA2 autoantibodies have been identified as biomarkers of active disease progression. Our finding that hydroxychloroquine was associated with a reduction in autoantibodies but had no impact on disease progression is consistent with studies in NOD mice and with our previous clinical trial of rituximab, suggesting that antigen presentation rather than the ability to produce autoantibodies is the key mechanism of B cells for T1D progression (27,28). We cannot determine, from our studies, the

effects of hydroxychloroquine on antigen presentation. It is likely that events, including antigen presentation, initiating progression of T1D occur before the appearance of stage 1 T1D.

We found improvement in glucose levels during the OGTTs after 6 months of therapy. This is consistent with several reports in the rheumatologic literature describing reduced rates of diabetes risk in individuals with rheumatoid arthritis, lupus, and psoriasis who were treated with hydroxychloroquine (12–15). It is possible that this reflects insulin sensitivity, because at least two randomized placebo-controlled trials using euglycemic clamps and intravenous glucose tolerance tests have demonstrated that 400 mg hydroxychloroquine per day can improve insulin sensitivity in adults without diabetes (16,29). In the current study, the C-peptide mean AUC during the OGTTs was unaffected, and we did not find a significant improvement in the ratio of C-peptide to glucose. More investigation is needed to address the effects of hydroxychloroquine on insulin sensitivity in this population that was, for the most part, not overweight and was normoglycemic at baseline. Consistent with a metabolic effect that is independent of insulin secretion, Gerstein et al. (30) reported that hydroxychloroquine may be effective in individuals with T2D who are refractory to sulfonylureas, but the improvement in insulin sensitivity based on the ratio of C-peptide to glucose in the hydroxychloroquine group was transient. Our study participants had BMI *z* scores that were generally low, suggesting that obesity was not a prerequisite for this effect of hydroxychloroquine.

There are limitations to this clinical study. We tested a single dose of hydroxychloroquine that is on the low end of dosing for other diseases but was selected to minimize the risk of retinal toxicity. Most importantly, COVID-19 affected both study recruitment and study visits during the pandemic, thus prolonging the assessment of time to AGT tests. Although this would have affected both treatment arms equivalently, if the drug efficacy waned with continued drug administration, as suggested by experience with other biologics in T1D, this may have had a greater impact on the hydroxychloroquine study arm. Our analysis of secondary end points was prespecified but not adjusted for multiple testing. In addition, our study population was predominantly White, but T1D has incidence in more diverse populations (31).

In summary, in this randomized placebo-controlled trial of hydroxychloroquine in individuals with stage 1 T1D, we did not identify an effect of treatment on progression to stage 2 disease, but we found that the drug had biologic activity on progression of the autoantibody repertoire, with a reduction in glucose during the first 6 months of therapy. Our findings are consistent with the concept that inhibiting autoantibody production in humans does not affect disease progression once two or more autoantibodies are present, suggesting that treatment with hydroxychloroquine earlier in the disease process may be effective.

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APPENDIX

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