HYDROXYCHLOROQUINE FOR PREVENTION OF ABNORMAL GLUCOSE TOLERANCE AND DIABETES IN INDIVIDUALS AT-RISK FOR TYPE 1 DIABETES MELLITUS

(Protocol TN-22)

VERSION 7.0

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PREFACE

The TrialNet Type 1 Diabetes Protocol TN-22, Hydroxychloroquine for Prevention of Abnormal Glucose Tolerance and Diabetes in Individuals At-Risk for Type 1 Diabetes Mellitus, describes the background, design, and organization of the study. The protocol will be maintained by the TrialNet Coordinating Center (TNCC) over the course of the study through new releases of the entire protocol, or issuance of updates either in the form of revisions of complete chapters or pages thereof, or in the form of supplemental protocol memoranda.

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1. INTRODUCTION

1.1. Study Overview

Title	Hydroxychloroquine for prevention of abnormal glucose tolerance and diabetes in individuals at-risk for type 1 diabetes mellitus (T1D)
IND Sponsor	Type 1 Diabetes Trial Network (TrialNet)
Study Supported by	National Institute of Diabetes, Digestive and Kidney Diseases
Conducted By	Type 1 Diabetes Trial Network (TrialNet)
Protocol Chairs	Dr. Polly Bingley and Dr. Jane Buckner
Accrual Objective	201
Study Design	The study is a 2-arm, double blinded, multicenter, 2:1 randomized, placebo-controlled clinical trial. All participants will receive close monitoring for progression of T1D.
Treatment Description	Participants will receive hydroxychloroquine or placebo and close monitoring for progression to Stage 2 (abnormal glucose tolerance) or Stage 3 (clinically overt) T1D.
Objective	To assess the efficacy, safety and mode of action of hydroxychloroquine to prevent progression from Stage 1 to Stage 2 or Stage 3 of T1D.
Primary Outcome	The primary objective is to determine whether intervention with hydroxychloroquine will prevent or delay the progression from Stage 1 (normal glucose tolerance) to Stage 2 (abnormal glucose tolerance) or Stage 3 (clinically overt) T1D.
Secondary Outcome	Secondary outcomes include: the effect of hydroxychloroquine on the risk of T1D; analyses of metabolic and immune changes over time; safety and tolerability; evaluation of other possible factors on effect (association and effect modification); and mechanistic outcomes.
Major Inclusion Criteria	(1) Individuals at Stage 1 T1D (two or more antibodies and normal glucose tolerance) (2) ≥ age 3 and previously enrolled in TrialNet Pathway to Prevention Study.

1.2. Statement of Purpose

This protocol describes the background, design, and organization of a study of hydroxychloroquine therapy for prevention of progression of Stage 1 (normal glucose tolerance) to Stage 2 (abnormal glucose tolerance) or Stage 3 (clinically overt) T1D. The protocol was written by: the chairs of the TrialNet Hydroxychloroquine Prevention Protocol Committee, Dr. Polly Bingley at the University of Bristol and Dr. Jane Buckner at the Benaroya Research Institute, the TrialNet Chairman's Office at the Benaroya Research Institute, and the TrialNet Coordinating Center (TNCC). Significant changes that occur to this protocol during the course of the trial require the formal approval of the TrialNet Steering Committee. The study protocol, along with the required informed consent forms, will be approved by a Central IRB or each participating institution's Institutional Review Board (IRB) or Ethics Committee/Research Ethics Board (EC/REB).

2. BACKGROUND AND SIGNIFICANCE

2.1. Type 1 Diabetes (T1D)

2.1.1. Definition and Metabolic Characteristics of Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1D) is an immune-mediated disease in which insulin-producing beta cells are completely or almost completely destroyed, resulting in life-long dependence on exogenous insulin. It is a chronic and potentially disabling disease that represents a major public health and clinical concern. The number of patients being diagnosed with T1D is increasing each year and is approaching an epidemic level in many countries[1,2].

For individuals living with T1D, continuous exogenous insulin therapy is needed to prevent ketoacidosis and other catabolic effects of insulin deficiency, and to promote anabolism and to maintain life. Insulin therapy must be combined with frequent blood glucose monitoring to prevent hypo- and hyperglycemia. In addition to the unrelenting financial and psychological burdens on patients and their families from day-to-day management of this disease, individuals with T1D are subject to both short-term and long-term complications. Short-term complications include severe hyperglycemia resulting in diabetic ketoacidosis (DKA), a life-threatening condition. More than 25% of individuals present with DKA at the time of clinical diagnosis; for those under age 5, about 35% have DKA[3-5]. Recurrent DKA after the new onset period remains a significant clinical problem. A study with over 15 years of follow-up recently showed that the presence of DKA at onset of T1D confers worse prognosis, with worse glycemic control on follow-up[6]. Hypoglycemia is a consequence of exogenous insulin therapy which can result in impaired cognition and, if severe, coma and death. Possible long-term complications for those living with T1D include visual impairment and blindness, renal failure, vascular disease and limb amputation, peripheral neuropathy, and stroke. The Diabetes Control and Complications Trial (DCCT) showed that long-term complications could be reduced with near normal control of glucose levels, but at the cost of an increased frequency of severe hypoglycemia[7]. While there have been significant improvements in insulin analogs and insulin delivery systems, such as continuous subcutaneous insulin infusions with wearable pumps, continuous glucose monitoring, and closed loop systems, normal glucose control, particularly in children, is rarely achieved[8] (Figure 1). Moreover, while the frequency of long-term complications is decreasing, individuals with T1D continue to have reduced life expectancy[9,10].

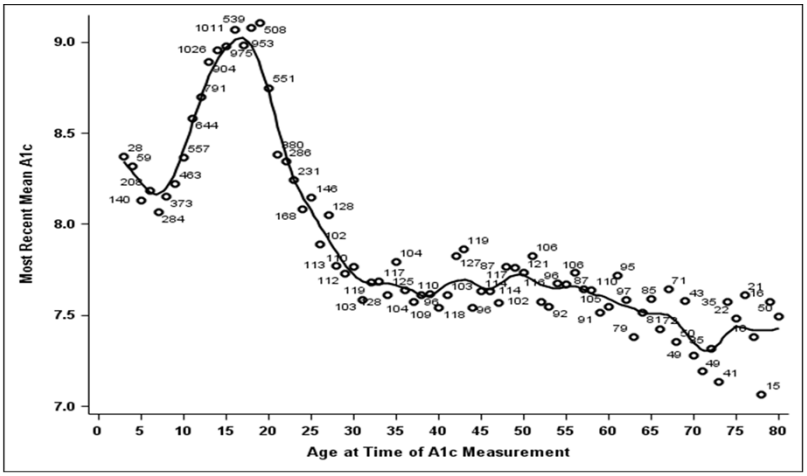

Figure 1. Mean HbA1c in US participants in T1D Exchange Registry. American Diabetes Association recommendation for HbA1c is 7.0%.

As described below, individuals identified for enrollment into this clinical trial are destined to develop clinically overt diabetes. Clearly, delay or prevention of disease progression would represent a significant advance.

2.1.2. Natural History and Stages of the T1D Disease Process

Much is known about the natural history of the T1D disease process[11]. Individuals with T1D have genetic susceptibility for disease; indeed, relatives of individuals with T1D are at much greater risk of developing disease. In the general population, approximately 0.3% of individuals will develop T1D. In contrast, those with a relative with T1D have a 5% risk of disease – a 15-fold increase[12]. Further risk stratification among family members depends upon genetic, immune, and metabolic data[13].

Beta cell destruction is initiated in genetically susceptible individuals years before clinical onset of disease[14]. The autoimmune process that causes beta cell destruction is clinically silent and can only be identified by the detection of autoantibodies in the blood, such as islet cell antibodies (ICA), and autoantibodies to glutamic acid decarboxylase (GAD65Ab), islet antigen 2 (IA-2Ab/ICA512), insulin (mIAA), zinc transporter 8 (ZnT8Ab), and others[15-18]. Continued immune-mediated beta cell destruction involving both B and T cells occurs until physiologic insulin demand cannot be met by the remaining beta cells. Impaired insulin secretion is accompanied by progression from normal to abnormal glucose tolerance (both of which are asymptomatic states), until more severe hyperglycemia occurs associated with classic symptoms of polyuria and polydipsia and thus clinically overt disease[19].

Data gathered from multiple studies during the past 35 years are remarkably concordant with regard to the progression from development of autoantibodies to clinically overt disease. These data, whether from studies of longitudinal cohorts followed from birth[20] or cross-sectionally identified populations at risk,[21-24] indicate that essentially all genetically susceptible individuals with multiple autoantibodies (whether or not a relative) will eventually progress to clinical disease. Thus, T1D is considered to have started with the presence of 2 or more autoantibodies. The presence of two or more autoantibodies with normal glucose tolerance is defined as Stage 1 T1D. Stage 2 T1D is the presence of two or more antibodies with abnormal glucose tolerance, and Stage 3 T1D is clinically overt disease[25].

As illustrated in Figure 2, data also suggest that the rate of progression through each stage is age dependent, with younger individuals progressing more rapidly[21].

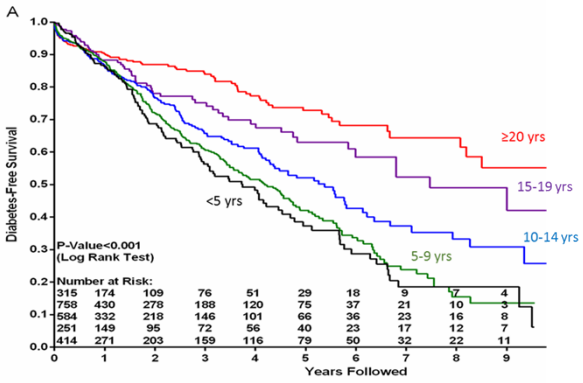

Figure 2. Progression from Stage 1 T1D (two or more antibodies, normal glucose tolerance to Stage 3 T1D (clinical onset). Diabetes TrialNet.

Overall, about 40% of individuals at Stage 1 T1D will progress to Stage 2 within two years (Figure 3), and almost 70% of those at Stage 2 will develop clinically overt T1D (Stage 3) within four years (Figure 4). More children than adults will be expected to progress from Stage 1 to 2 and from Stage 2 to Stage 3 during these time periods.

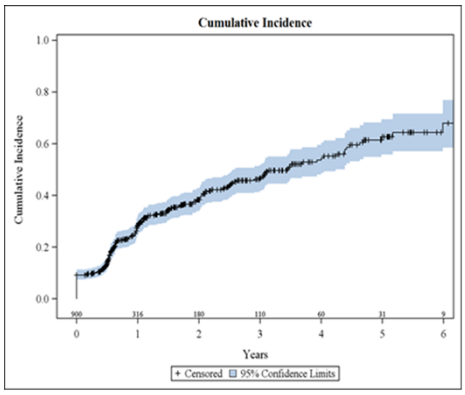

FIGURE 3: Two year risk of confirmed abnormal glucose tolerance is 38% among individuals with multiple autoantibody positivity and normal glucose tolerance at baseline in the TN01 Pathway to Prevention Study.

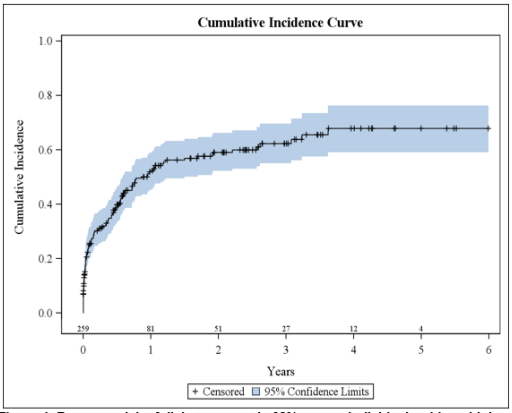

Figure 4: Four year risk of diabetes onset is 68% among individuals with multiple autoantibody positivity and confirmed abnormal glucose tolerance in the TN01 Natural History/Pathway to Prevention Study.

2.1.3 Disease-Modifying Therapy in T1D

Since the first use of insulin in 1922, treatment of T1D has been targeted at controlling the symptoms and consequences of hyperglycemia. This is analogous to symptom control for individuals with rheumatoid and juvenile arthritis, where the aim of therapy was previously to control pain and/or provide devices to adapt to the presence of disability. Yet, control of symptoms of arthritis - and almost all other autoimmune disease - is no longer the primary aim of treatment; instead, the standard of care is disease-modifying therapy with the intent to fundamentally alter the course of the condition. This approach has dramatically changed the lives of those with these autoimmune diseases. The aim of disease-modifying therapy in T1D is the same: to move away from symptom management (e.g., glycemic control) as the mainstay of treatment and find approaches to alter the disease course.

At least four therapies have been shown to have some effect on preservation of insulin secretion at Stage 3 (clinical onset) of disease[26-29]. Two of these (Abatacept or CTLA4-Ig) and Teplizumab (Anti-CD3) are now under trial at Stage 1 and Stage 2 of T1D, testing the concept that treating earlier in disease is likely to have greater benefits. Early treatment has been shown to be useful in other autoimmune diseases, such as juvenile arthritis. Moreover, successful application of disease-modifying therapy at Stage 1 or Stage 2 of T1D would have clear clinical benefit by the delay or prevention of clinically overt disease (Stage 3).

In summary, the rationale for this study is that individuals at Stage 1 T1D, defined as those with immunologic markers and normal glucose tolerance, will inevitably develop Stage 3 or clinically overt

T1D. Prior to development of clinical T1D, they will progress from Stage 1 T1D (normal glucose tolerance) to Stage 2 T1D (abnormal glucose tolerance). Intervention early in the course of disease is likely to be more effective than intervention in those with abnormal glucose tolerance or clinical T1D. As described below, this is particularly likely to be true with the therapy being tested in this trial – hydroxychloroquine.

2.2. Rationale for use of hydroxychloroquine at Stage 1 T1D

2.2.1. Overview

Since T1D results from immune-mediated beta cell destruction, disease-modifying therapy will require approaches that alter the autoimmune process. Both B and T cells are involved in disease pathogenesis, as evident by the efficacy of anti-T cell (Teplizumab), anti-B cell (Rituximab), and co-stimulation blockade (Abatacept) therapies in preserving beta cell function at Stage 3 T1D (clinical onset). Other studies have demonstrated alterations in T and B cell phenotype and function when measured in peripheral blood from those with T1D as compared with unaffected controls.

While the mechanism of action of hydroxychloroquine is poorly understood, it appears to act on autoimmune responses which are important in early pathogenesis of T1D. It has a long history of use in children, is well tolerated, is relatively low cost, and is thought to target B cell responses. In particular, hydroxychloroquine has been shown to decrease cellular activation, including hindering of antigen presentation to CD4+ T cells and reducing the expression of the CD4+ T cell activation marker CD154[30,31]. Hydroxychloroquine is also thought to have a number of other immune actions that may lead to decreased autoantibody production, including (i) alkalinization of intracellular vacuoles leading to inhibition of proteolysis, chemotaxis, phagocytosis, and antigen processing[32], (ii) reduction of macrophage-mediated cytokine production[33] and (iii) inhibition of function of toll-like receptors[34]. Hydroxychloroquine has also been found to inhibit calcium-mediated signaling in T and B cell lines, interfering with downstream effects including TCR-dependent CD69 expression[35].

In a preclinical study, oral hydroxychloroquine was able to partially protect against streptozotocin-induced diabetes in a rat model,[36] with greater preservation of islet structure, β-cell mass and proliferation over the untreated group. Hydroxychloroquine lowered the pancreatic levels of IL-1β, IL-6, TNF-α, and TGF-β1, as well as the serum level of MCP-1, compared to the untreated group, suggesting an anti-inflammatory effect. Lower pancreatic levels of the apoptotic marker caspase-3 were also found in hydroxychloroquine treated animals.

Importantly, the drug not only has been shown to ameliorate other autoimmune diseases, but hydroxychloroquine also abrogates the progression of autoimmunity in at-risk populations[37-41]. This drug is therefore particularly suitable to test early in the T1D process, in this study, targeting those with multiple islet autoantibodies but normal glucose tolerance (Stage 1 of T1D). It also has the potential to be efficacious at an even earlier stage of the disease process, prior to the development of multiple autoantibodies.

Initially developed as a therapy for malaria, hydroxychloroquine is approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for that indication, as well as for rheumatoid arthritis (RA) and discoid and systemic lupus erythematous (SLE). It is widely used in

clinical practice for these indications, including in pediatric populations. Efficacy in autoimmune disease has been well established in RA[42-44], SLE[45-47], antiphospholipid syndrome[48] and Sjögren's syndrome[49,50]. Particularly relevant for this study is that hydroxychloroquine is effective when used early in the course of autoimmune disease, having been shown to slow or halt progression in palindromic rheumatism[37-40] and delay onset of SLE in antibody-positive individuals[41].

Other beneficial actions of hydroxychloroquine have been identified in epidemiologic and randomized studies of other autoimmune diseases, including improvement in cardiovascular risk factors, such as reduction of LDL cholesterol[51], thrombosis[52], glycaemia[52,53], insulin resistance[52,54,55], and reduction of risk of incident diabetes mellitus in SLE[54] and RA[55,56]. A randomized trial of hydroxychloroquine in obese adults demonstrated improved clamp-assessed insulin sensitivity, as well as increased first phase insulin secretion, possibly mediated through anti-inflammatory effects[57]. Because 1/3rd of our pediatric population is currently overweight, and elevated BMI may increase risk of progression to T1D in children[58] and adults[59], these actions may provide an additional benefit to this therapy.

In summary, the rationale for testing hydroxychloroquine as disease-modifying therapy in Stage 1 T1D is that it is a well-established, cost-effective treatment for various autoimmune diseases used safely in both adult and pediatric populations, which may work through multiple mechanisms as an agent to prevent progression of autoimmunity. In addition, hydroxychloroquine has a positive metabolic profile that has the potential to be of particular benefit in the context of the T1D pathogenesis.

2.2.2 Efficacy

2.2.2.1 FDA and/or EMA Approved Indications

Hydroxychloroquine is approved by FDA and EMA for use in malaria, rheumatoid arthritis (including juvenile idiopathic arthritis), and systemic and chronic discoid lupus erythematosus in adults and children. It is also approved for use in dermatological conditions caused or aggravated by sunlight in adults, with unlicensed use for this indication in children.

Table 1 provides a summary of the types of studies, patient populations, and number of patients treated with hydroxychloroquine (or matching placebo) in completed clinical studies in these conditions.

Table 1

Indications	**Exposure N Age groups**	**Notes**
Malaria	Widespread use Adults & children	
No clinical trials published. Usual daily dose: 5 - 6.5 mg/kg		
Rheumatoid arthritis (including juvenile idiopathic arthritis)	Widespread use Adults & children	

HERA study Esdaile J et al, American Journal of Medicine 1995[44]	N=120 Adults	Dose: 7 mg/kg (max 400 mg) vs placebo Duration: 36 weeks Outcome: Significant benefit on synovitis, pain, and physical disability of recent-onset RA
3 year follow up Tsakonas E et al., J Rheumatol 2000[60]	N=104	Early treatment with hydroxychloroquine was associated with less pain and physical disability after 3 years compared with placebo.
Clark P et al. Ann Intern Med 1993[43]	N=126 Adults	Dose: 400 mg/day vs placebo Duration 24 weeks Outcome: Moderate clinical effect in early Rheumatoid Arthritis
Rantalaiho V et al. Arthritis Res Therr 2010 [61]	N=199 Adults	Use of hydroxychloroquine in combination therapy vs single DMARD +/- prednisolone. Duration 2 years Outcome: Combination therapy results in less long-term radiological damage
Das SK et al. Curr Med Res Opin 2007[62]	N= 122 Adults	Dose: 400 mg/day vs placebo (+Nimesulide) Duration 8 weeks Outcome: Improved American College of Radiology (ACR) criteria. Well tolerated.
Lupus Erythematosus (chronic discoid and systemic)	Approved since 1955 Used in adults & children ACR and EULAR guidelines for lupus nephritis accept hydroxychloroquine as adjunctive therapy.	
Costedoat-Chalumeau N et al. Ann Rheum Dis 2013[63]	N=573 Adults	Low hydroxychloroquine levels associated with higher SLE activity, but this was not responsive to changes in dose.
Clowse ME et al. Arthritis Rheum 2006[64]	N=257 Pregnant women	Higher risk of flare if hydroxychloroquine stopped. Steroid sparing Absence of fetal toxicity
Levy RA et al. Lupus 2001[65]	N=20 Pregnant women	Lower SLE activity scores in treatment group. Steroid sparing.
Dermatological conditions caused or aggravated by sunlight	Adults	
Singal AK et al.Clin Gastroenterol Hepatol 2012[66]	N=48 Adults with porphyria cutanea tarda	Dose: 100 mg twice a week versus phlebotomy for 12 months. Hydroxychloroquine as effective and safe as placebo.
Petersen CS et al. J Am Acad Dermatol 1992[67]	N=93 Adults with porphyria cutanea tarda	Dose: 250 mg TDS for 3 days. Efficacious and safe.

2.2.1.2. Other Clinical Situations

Hyperglycemia is a result of the intersection of insulin secretion (impacted by immune-mediated beta cell destruction) and insulin sensitivity. While preserving insulin secretion through immune modulation is the primary rationale for this study, improvement in insulin sensitivity would also impact progression to Stage 2 and 3 of disease. It is assumed that the association of increasing BMI and accelerated progression to T1D in both pediatric and adult relatives at risk is due to obesity-induced insulin resistance[58],[59]. Thus, it is relevant to highlight the salutary effects of hydroxychloroquine on this aspect of disease pathophysiology. Clinical trials of metabolic and other effects have been completed in non-diabetic adult participants with obesity and other risk factors for insulin resistance and these demonstrated improved insulin sensitivity and beta cell function[57,68]. In a further trial in 135 adult participants with type 2 diabetes, treatment with up to 600 mg of daily hydroxychloroquine for 18 months was associated with improved glycemic control[53]. Also of interest, since those with T1D are at risk for cardiovascular complications, is a Phase I trial conducted by Achuthan et al. (2015) comparing the efficacy of hydroxychloroquine (400 mg daily) with aspirin or combined aspirin/clopidogrel as antiplatelet therapy in 20 adult participants which showed that hydroxychloroquine treatment was associated with a reduction in platelet aggregation by arachidonic acid[69].

2.2.3 Safety and Tolerability of Hydroxychloroquine

Hydroxychloroquine has been in clinical use for >60 years. It is generic and its pharmacological characteristics and safety profile have been established in pediatrics, adults, and pregnancy. It is considered a far safer drug than its close relative chloroquine. It is well tolerated, with the most common side effects being transient nausea experienced by 1-10% of patients. Few individuals stop therapy due to side effects, and 'once daily' dosing facilitates adherence. The recommended pediatric dose is 5–6.5 mg/kg once daily (max. dose 400 mg).

The most important adverse effect of hydroxychloroquine is retinal toxicity (see Section 7.2). This is correlated with dose of drug and duration of therapy. There is negligible risk for duration of therapy <6 months,[70] and it is unlikely to occur with less than 5 years of therapy if dose is <5 mg/kg/day up to 400 mg/day[71]. Despite longstanding use of hydroxychloroquine for malaria therapy in very young children, there is a dearth of information about retinopathy, suggesting its rarity[72].

In this study, hydroxychloroquine dose will be targeted at a maximum of 5.0 mg/kg/day actual body weight up to 400 mg/day and individuals will undergo annual ophthalmological exams.

Section 7.2 describes other reported adverse effects.

3. STUDY DESIGN

3.1. Overview

This is a double-masked, multicenter, randomized, placebo-controlled trial to determine whether treatment of participants with Stage 1 T1D (two or more antibodies with normal glucose tolerance) with

hydroxychloroquine results in delay or prevention of progression to Stage 2 T1D (abnormal glucose tolerance) or Stage 3 T1D (clinical onset).

3.2. Objectives

3.2.1. Primary Objective

The primary objective of the study is to determine whether treatment of participants at Stage 1 T1D with hydroxychloroquine results in delay or prevention of progression to Stage 2 T1D or Stage 3 T1D.

3.2.2. Secondary Objectives

- To determine whether treatment with hydroxychloroquine is superior to placebo with respect to the risk of T1D
- To determine whether treatment with hydroxychloroquine is superior to placebo with respect to other measures as obtained from timed collections during longitudinal tests:
    a. metabolic assessment of insulin secretion and insulin resistance calculated from the oral glucose tolerance test (OGTT) data
    b. immunologic measures of disease activity or progression
- To compare the safety and tolerability of hydroxychloroquine to placebo
- To assess the effects of treatment on mechanistic outcomes

3.3. Summary of Inclusion/Exclusion Criteria

Participants must meet all entry criteria for the protocol as outlined below.

3.3.1. Inclusion Criteria

Study participants must be or have:

1. A participant in TrialNet Pathway to Prevention Study (TN01).

2. Age ≥ 3 years at the time of randomization in this trial.

3. Willing to provide informed consent or, if the participant is <18 years of age, have a parent or legal guardian provide informed consent.

4. Normal glucose tolerance by OGTT within 7 weeks (no more than 52 days) prior to randomization. If previous abnormal glucose tolerance, has had two consecutive OGTTs with normal glucose tolerance:
    a. Fasting plasma glucose < 110 mg/dL (6.1 mmol/L), and
    b. 2-hour plasma glucose <140 mg/dL (7.8 mmol/L), and

c. 30, 60, and 90 minute value on OGTT<200 mg/dL (11.1 mmol/L).

5. Two or more diabetes-related autoantibodies present on two separate samples, one of which was drawn within the past six months (210 days). Confirmation does not have to involve the same 2 autoantibodies.

6. Weight ≥12 kg at the time of screening.

7. If a female participant with reproductive potential, willing to avoid pregnancy and undergo pregnancy testing prior to randomization and at each study visit.

8. Anticipated ability to swallow study medication.

3.3.2. Exclusion Criteria

Potential participants must **not** meet any of the following exclusion criteria:

1. Abnormal Glucose Tolerance or Diabetes
   a. Fasting plasma glucose ≥110 mg/dL (6.1 mmol/L), or
   b. 2 hour plasma glucose ≥140 mg/dL (7.8 mmol/L), or
   c. 30, 60, or 90 minute plasma glucose during OGTT ≥200 mg/dL (11.1 mmol/L)

2. History of treatment with insulin or other diabetes therapies.

3. Ongoing use of medications known to influence glucose tolerance, e.g. glucocorticoids, growth hormone, anticonvulsants, thiazide or potassium-depleting diuretics, beta adrenergic blockers, niacin and antipsychotics. Participants on such medications should be changed to a suitable alternative, if available, by their primary care provider, and will become eligible one month after medication is discontinued.

4. Ongoing or anticipated future use of any medications known to impact T1D progression or have untoward interactions with hydroxychloroquine, as described in section 7.2.4.

5. Known hypersensitivity to 4-aminoquinoline compounds.

6. G6PD deficiency.

7. History of retinopathy.

8. Active infection at time of randomization.

9. Serologic evidence of current or past HIV, Hepatitis B (positive for Hepatitis B core antibody or surface antigen), or Hepatitis C infection.

10. Deemed unlikely or unable to comply with the protocol or have any complicating medical issues, including prolonged QT interval, a disease previously or likely in the future to require immunosuppression, or abnormal clinical laboratory results that interfere with study conduct or cause increased risk.

11. Be pregnant or breastfeeding.

3.4. Enrollment

Potential study participants will be identified through the ongoing TrialNet Pathway to Prevention Study. Autoantibody-positive individuals in that study are then further evaluated with the performance of OGTTs, additional blood tests, detailed medical history, and physical exam. The results of these evaluations will be used to determine eligibility for this protocol.

3.5. Description of Study Cohorts

While enrollment for the TrialNet Abatacept Prevention Trial (TN18) is open, enrollment in this study for individuals age 6 or older will be limited to those with mIAA confirmed within 6 months of enrollment as one of their two or more autoantibodies. However, those who are age 3-5 years or who reside in countries not participating in TN18 and have normal glucose tolerance and two or more autoantibodies (regardless of mIAA status) are eligible. Otherwise, after completion of enrollment for TN18, all individuals ≥ 3 years of age with normal glucose tolerance and two or more autoantibodies regardless of mIAA status are eligible to screen for this study.

3.6. Description of Treatment Groups

The intervention will only be administered to those who provide informed consent to participate. Participants will be randomized to receive either oral hydroxychloroquine or placebo along with close monitoring for abnormal glucose tolerance or diabetes. Blood and serum samples for the primary and secondary outcome determinations will be sent to the Core Laboratories for analysis. Clinical laboratory studies may be done at the local sites.

Participants will be randomly assigned in a 2:1 ratio to treatment arms within strata defined by two stratification factors: prior treatment for T1D prevention (prior enrollment in a prevention study and treatment with placebo vs. prior enrollment in a prevention study and treatment with active therapy vs. no prior enrollment in a prevention study) and age group (<8 years old vs. 8 years old or older).

3.7. Treatment Assignment

After participants (or parents/legal guardians as applicable) sign the consent form, complete the screening visit(s), and meet all of the inclusion criteria and none of the exclusion criteria, they will be randomized to receive either hydroxychloroquine or placebo; each treatment arm will include close monitoring for study endpoints.

3.7.1. Procedures for Unmasking

Emergency unmasking occurs as follows: Investigators can break the treatment code by contacting the TrialNet Central Pharmacy via the 24-hour emergency number. No approval or intervention by the study sponsor or TrialNet administration is required in emergency situations. The TrialNet Chair, NIDDK TrialNet program officer and TrialNet Medical Monitor will be notified of the unmasking within 24 hours of its occurrence. Non-emergent unmasking will occur upon notification of the TNCC and approval by TrialNet Chair or NIDDK TrialNet program officer. If unmasking is approved, the study sponsor and appropriate TrialNet committees (e.g. Safety Monitoring) will be notified of the event as soon as reasonably possible; however, they will not be unmasked.

3.8. Study Assessments

During the course of the study, participants will undergo frequent assessments of their glucose tolerance status, insulin production, immunologic status, and overall health and well-being (see Schedule of Assessments).

Samples will be drawn for storage in the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at TrialNet Laboratory Sites for future analysis related to T1D.

3.9. Study Timeline

3.9.1. Study Duration

The study has been designed to provide 83% power to detect a 50% risk reduction in the progression from Stage 1 to Stage 2 or 3 T1D using a two-sided test at the 0.05 level after six years of study duration (approximately 4 years of accrual and a minimum of 2 years of follow-up on all subjects). A total of approximately 201 participants will be allocated in a 2:1 ratio to the two groups.

The assumptions underlying the sample size are: 1) 40% two-year event rate in the placebo group for the progression from Stage 1 to Stage 2 or 3 T1D, 2) detectable hazard ratio of 0.50 (i.e., reducing the hazard rate by 50%, which here translates to 40% vs. 22.5% in the two-year event rates) in the hydroxychloroquine group, 3) a 10% or less dropout rate for a minimum of 2 years of follow-up on all participants in both groups, and 4) assumption that the distribution of time to T1D stage progression is approximately exponentially distributed. Under the above assumptions, the total target number of events that would trigger the final analysis is 80; i.e., the final analysis will be conducted once 80 events (abnormal glucose tolerance or diabetes) have been observed. If the accrual rate is 50 patients per year, with the above assumptions it is estimated that the enrollment period will last approximately 4 years, with a total study duration of approximately 6 years to provide a sufficient number of events to detect the powered detectable difference.

3.9.2. Follow-up Studies

Participants who have progressed to Stage 2 T1D (confirmed abnormal glucose tolerance) will have reached the primary study endpoint. These participants who have developed abnormal glucose tolerance but have not progressed to Stage 3 T1D (clinical onset) will have the option to remain in this study without further therapy, undergoing close monitoring and safety assessments until the development of Stage 3 T1D (clinically overt diabetes) or until they enroll in another clinical trial, whichever comes first.

Once participants reach Stage 3 (diagnosis of clinical diabetes), they will no longer be followed in the context of this protocol; however, they will be offered follow-up in the Long Term Investigative Follow-Up in TrialNet (LIFT) Study (TN16). Alternatively, individuals who progress to Stage 3 may be eligible for interventional studies sponsored by TrialNet or other organizations under separate INDs. In the event that a participant wishes to participate in another investigational study whose entry criteria exclude participants who have previously received immunomodulating therapy and is otherwise eligible for the new study, the participant may request and be told of their treatment group assignment for the hydroxychloroquine prevention study. Every attempt will be made to minimize the potential bias that this may introduce. The TNCC will make treatment assignment information available to the site investigator of the new study after the participant is determined to be willing to participate and not otherwise excluded from the new study. Other study group members will not be informed of the treatment assignment information. Mitigation of bias issues must be balanced against safety and interests of participants.

4. PATIENT MANAGEMENT

4.1. Screening Visit and Eligibility Assessment

This study will draw participants from the TrialNet Pathway to Prevention Study.

The initial testing for islet autoantibodies, HLA type, and OGTT will generally be done as part of Pathway to Prevention Study. Potential participants in the hydroxychloroquine prevention trial are those with two or more autoantibodies present on two separate samples. Initially, for those age 6 or older, enrollment will require the presence of mIAA antibodies unless the individual resides in a country not participating in the Abatacept Prevention Trial (TN18). After completion of enrollment for the Abatacept Prevention Trial (TN18), any two confirmed autoantibodies will meet criteria for this study. The autoantibodies that may be confirmed are mIAA, GAD65Ab, IA-2/ICA512Ab, ZnT8Ab, and/or ICA. The confirmation of two positive autoantibodies must occur within the six months prior to study drug administration, but the confirmation does not have to involve the same two autoantibodies.

Those individuals with two diabetes-related autoantibodies will then be eligible for additional screening tests and possible enrollment into the hydroxychloroquine prevention trial. A participant must have a normal OGTT (done either as part of the Pathway to Prevention Study or during screening for the hydroxychloroquine prevention trial) within 7 weeks (52 days) prior to randomization. If a participant has a previous abnormal glucose tolerance, he/she must have two consecutive OGTTs with normal glucose tolerance in order to be eligible.

Appendix 1 summarizes the flow of participants from the Pathway to Prevention Study into the hydroxychloroquine prevention trial.

4.2. Hydroxychloroquine Trial for At-risk Participants Screening Visit

Prior to the initial visit, the hydroxychloroquine prevention trial will be described to the potential participant. The participant/parent/guardian will be asked to sign an informed consent document, and minors will be asked to sign an age appropriate assent document, describing the purpose, risks, and benefits of screening for the trial. A participant's signature indicates that he/she understands the potential risks and benefits of study participation. During these visits, clinical tests will be done to determine eligibility.

Any antibody positive relative either not eligible or not willing to be randomized into the hydroxychloroquine prevention trial is eligible for continued follow-up as part of the TrialNet Pathway to Prevention Study.

4.3. Randomization and Baseline visits

Participants will be randomized to either the treatment arm or the placebo arm. Randomization must occur within 7 weeks of a normal OGTT in order to ensure that participants have normal glucose tolerance, and study drug administration must occur within 4 weeks after randomization.

4.4. Close Monitoring

During the study period, all participants will receive close monitoring for development of abnormal glucose tolerance and diabetes. OGTT tests will be performed at approximately six-month intervals thereafter. At each visit, participants will be asked questions about the presence or absence of symptoms associated with diabetes. If participants respond affirmatively or have other laboratory or clinical evidence of disease progression, further evaluation will be performed. Individuals in both of the study arms will have laboratory and mechanistic studies performed as detailed in the Schedule of Assessments.

4.5. Administration of Hydroxychloroquine

The hydroxychloroquine used in these human trials is a 4-aminoquinoline compound (hydroxychloroquine sulfate) supplied as a 200mg tablet for oral administration. As described below, these tablets will be prepared in 100mg increments and over encapsulated for this trial.

Hydroxychloroquine must be stored below 25°C/77°F.

4.5.1. Dosing and Dose Withholding

The target dose is 5 mg/kg/day (actual body weight), up to a maximum of 400mg/day, of hydroxychloroquine or placebo. The dose was chosen based on demonstrated safety and efficacy in other human autoimmune diseases. Dose by body weight will be calculated in 100mg increments and will be equivalent to ≤ 5 mg/kg/day averaged over a one-week period.

Dose Withholding

1. Study drug will be withheld in those with 3 or more days of fever >101°F (38.3°C) until resolved.
2. If needed, in individuals who have gastrointestinal or other symptoms limiting their tolerance of study medication, the dose may be temporarily reduced at the discretion of the investigator in consultation with the medical monitor.
3. Any abnormal ophthalmologic examination finding possibly related to study medication, confirmed by repeat measure and central reading within 4 months, will result in stopping the study medication (permanently).
4. Grade 2 anemia or thrombocytopenia, i.e. hemoglobin <10 gm/dl and platelets <75,000/mm3. In consultation with the medical monitor, study medication may be restarted after resolution of the Grade 2 anemia or thrombocytopenia.The complete blood count (CBC) will be repeated no more than one month after restarting study medication. If the abnormality recurs, then medication will be discontinued permanently.
5. Study medication should be withheld if a participant develops an adverse event of severe intensity (grade 3 or higher) or a serious adverse event at least possibly related to study medication

Individuals who are unable to continue on study medication due to adverse effects will remain on study and continue with study visits.

4.5.2. Interruption of Enrollment/Trial Cessation

Section 4.5.1 describes the circumstances for potential withholding or reduction of drug treatment in individual patients. This section lists events that will necessitate interruption of enrollment in the trial as a whole, pending DSMB review. As part of their ongoing safety review, the DSMB will make independent judgments regarding other adverse events requiring trial interruption.

1. Any death that occurs in the study which is assessed as possibly, probably, or definitely related to study treatment regimen by the study site investigator and the medical monitor.
2. Two or more of the first 10 treated participants or ≥20% of all treated participants experience a clinically significant, drug-related adverse event, resulting in the permanent discontinuation of study treatment.
3. If more than one of the first 10 treated participants or ≥10% of all treated participants develop clinically significant retinal changes, the study will be placed on hold pending review by DSMB.

5. STUDY VISIT ASSESSMENTS

The schedule of evaluations and laboratory studies is presented in Appendix 2, Schedule of

Assessments. A summary of assessments for the Protocol is given below.

5.1. General Assessments

General assessments for this Protocol will include:

- Informed consent
- Inclusion/exclusion criteria
- Medical history
- Complete or directed physical examination including height/weight, waist circumference and Tanner staging
- Concomitant medications
- Adverse events
- Treatment compliance
- Ophthalmology examination

5.2. Laboratory Assessments

The following clinical laboratory assessments will be performed during the study as described in the Schedule of Assessments:

- Chemistry (sodium, potassium, chloride, CO2, glucose, BUN, creatinine)
- Liver function tests (ALT, AST, LDH, alkaline phosphatase, total protein, albumin, total and direct bilirubin)
- Hematology (CBC with differential and platelets)
- Urine pregnancy test as appropriate
- Antibodies to HIV, hepatitis B (anti-HBcAb, HBsAg), hepatitis C (HCV)
- G6PD screening

5.3. Mechanistic Outcome Assessments

TrialNet will perform pharmacokinetic, immune, and genetic assays to further understand mechanisms that may be underlying the T1D disease process and response to therapy. For this purpose, samples for PBMC, DNA, RNA, plasma, and serum may be obtained. Islet autoantibodies will be measured at approximately six-month intervals in the study. HLA or other testing for diabetes-related genes may be done under the auspices of either the TrialNet Pathway to Prevention Study or this protocol.

Responses to influenza or other clinically indicated immunizations may be determined through analysis of pre- and post-dose samples. Samples may also be collected for thyroid autoantibodies, virology and other immunization titers. Details of the mechanistic outcome assessments can be found in the mechanistic analysis plan.

5.4. Metabolic Outcome Assessments

Metabolic assessments will consist of:

1. OGTT
   - The OGTT will be performed approximately every 6 months, or more frequently if clinically indicated based on a random glucose level of ≥ 200 mg/dL (11.1 mmol/L).
   - The C-peptide and insulin data from the OGTT will be used to determine insulin secretion with assessment of early and late insulin responses.
   - The insulin, glucose and C-peptide data from the OGTT may be used to determine insulin sensitivity.
   - Other hormones and metabolites such as glucagon and proinsulin may also be measured.
2. HbA1c
   - Measure of glycemic control.

5.5. Visit Windows

Randomization must occur within 7 weeks (no more than 52 days) of the OGTT, and initial treatment administration must begin no more than 4 weeks after randomization. Visit target dates will be determined as of the baseline visit and will not be changed. Subsequent study visits should be +/- 2 weeks of the target date.

6. ADVERSE EVENT REPORTING AND SAFETY MONITORING

6.1. Adverse Event Definition

6.1.1. Adverse Event

In this clinical trial, an adverse event (AE) is any occurrence or worsening of an undesirable or unintended sign, symptom or disease, whether or not related to the treatment and study procedures.

Throughout the study, the investigator must maintain source documentation of all adverse events. Events *not related to diabetes onset* which are Grade 2 or greater per the NCI CTCAE (see Section 6.1.5. Grading Event Severity below) must be reported to TNCC. The investigator should treat participants with adverse events appropriately and observe them at suitable intervals until the events resolve or stabilize.

Adverse events may be discovered through:

- observation of the participant;
- questioning the participant;
- unsolicited complaint by the participant.

Questioning of the participant should be conducted in an objective manner.

6.1.2. Adverse Reaction

An adverse reaction means any adverse event caused by a drug. Since diabetes onset is a study endpoint, this is not considered an adverse reaction unless associated with death. Adverse reactions are a subset of all suspected adverse events for which there is reason to conclude that the drug caused the event. Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction, which means any adverse event definitely caused by a drug. Examples of evidence that suggest a causal relationship (reasonable possibility) between the drug and the adverse event include:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure.
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the populations exposed to the drug.
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

6.1.3. Serious Adverse Event/Reaction

A serious adverse event (SAE) or reaction is defined as any untoward medical occurrence at any dose that suggests a significant hazard, contraindication, side effect, or precaution. This includes but is not limited to any of the following events with the *exception of an adverse event associated with, the initial diagnosis of type 1 diabetes including hospitalization related to that diagnosis. In the event that a DKA related hospital admission occurs, all relevant details of the admission should be reported to the TNCC. The diagnosis of diabetes will be reported as an SAE only in the event of death.*

1. Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment-related or not.
2. A life-threatening adverse event. A life-threatening event is any adverse event that, in the view of the investigator, places the participant at immediate risk of death from the reaction as it occurred.
3. Inpatient hospitalization or prolongation of existing hospitalization, with the exception of hospitalization relating to initial diagnosis of type 1 diabetes.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical

judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

An adverse event or suspected adverse reaction is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the participant at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Regardless of the relationship of the adverse event to study drug, the event must be reported as a serious adverse event if it meets any of the above definitions.

6.1.4. Unexpected Adverse Event/Reaction

An adverse event/reaction is considered unexpected when the nature (specificity) or severity of the event is not consistent with the risks described in the package insert, this protocol, or the informed consent document. Unexpected refers to an experience that has not been previously observed. This includes events that occur more frequently than expected.

6.1.5. Grading Event Severity and Causality

TrialNet has adopted usage of the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) and/or study-specific criteria for classification to describe the severity of adverse events *with the exception of hypoglycemia, hyperglycemia or diabetes onset* (which will be reported as an AE only in the case of death). TrialNet Investigators will also provide an assessment of relationship of AE to study drug as: not, unlikely, possibly, probably, or definitely related.

6.2. Adverse Event Reporting and Monitoring

Adverse events will be reported to the TrialNet Coordinating Center. The investigator will grade their severity according to common toxicity criteria or study-specific criteria and will make a determination of their relation to therapy *with the exception of the initial diagnosis of diabetes which is considered an adverse event only in the case of death.* Events will be assessed and reported consistent with the ICH Guideline for Good Clinical Practice, 21 CFR 312.32 for expedited safety reporting, and per the guidance of the DHHS Office for Human Research Protections (OHRP).

The adverse event case report form for the protocol must be completed for all AEs ≥ Grade 2. In addition to the reporting requirements for the TrialNet network (as described above), the FDA requires reporting of any adverse event, if the event is serious, related, AND unexpected (21CFR312.32 (c)(i)(A)-(B)). When required, the FDA form 3500A (MedWatch form) should also be completed and sent to the TNCC *within 24 hours of when the site was notified of the event.* This will be reviewed by the TrialNet Medical Monitor, the TrialNet Safety Monitoring Committee, and the DSMB, as appropriate. Deaths must be reported immediately. Event outcome and other follow-up information regarding the treatment and resolution of the event will be obtained and reported when available, if not known at the time the event is initially reported. The follow-up information should contain sufficient detail to allow for a complete medical assessment of the case and an independent determination of possible causality.

Adverse events will be assessed by the TrialNet Medical Monitor. The DSMB will conduct regular safety reviews approximately every three to six months (and otherwise as needed) of adverse events by treatment group assignment. Serious adverse events, as well as adverse events leading to study discontinuation, will be reviewed by the DSMB.

For SAEs that are unexpected and considered possibly or probably drug-related, the Medical Monitor will provide information on frequency of similar events, and will review FDA form 3500A reports for distribution to FDA (and equivalent regulatory bodies for non-US sites), NIDDK, DSMB, and site investigators. Expedited safety reports will be submitted to the IND by the TNCC on behalf of NIDDK.

7. PARTICIPANT SAFETY

7.1. Risks, Benefits, and Inclusion of Children

Component analysis: **Monitoring** - There is the prospect of direct benefit to the individual participants for their participation in the study. These potential benefits include the recognized benefits of being in a clinical study, including the close monitoring offered to all participants regardless of group assignment. As noted in the introduction, marked hyperglycemia and DKA occur frequently at the time of clinical diagnosis. Close monitoring of autoantibody positive individuals using an OGTT has been demonstrated to have direct clinical benefit[3-5]. The presence of DKA at diagnosis is reduced from more than 25% to 3-4% when in close monitoring. For those under 5, the frequency of DKA at diagnosis is reduced by two thirds. Moreover, in the general T1D population, the mean HbA1c at diagnosis is 11%. This contrasts with a mean HbA1c of 6-8% when closely monitored, indicating less exposure to hyperglycemia. OGTTs are generally considered a minimal risk procedure, as they just involve an IV placement and the ingestion of oral glucose. Other procedures, venipuncture, laboratory tests, histories, physical examinations, and ophthalmologic examinations are likewise generally considered to be minimal risk. The monitoring aspects of this study are therefore consistent with the United States Department of Health and Human Services, Protection of Human Subjects, Subpart D, section 46.404 (research not involving greater than minimal risk) and with the Food and Drug Administration, Protection of Human Subjects, Subpart D, section 50. 51 (Clinical investigations not involving greater than minimal risk).

Component analysis: Intervention Arm - hydroxychloroquine – The use of hydroxychloroquine offers the prospect of direct benefit. As described above in this protocol, hydroxychloroquine is a Food and Drug Administration (FDA)-approved agent for malaria as well as rheumatoid arthritis (RA) and discoid and systemic lupus erythematous (SLE). It is widely used in clinical practice for these indications, including in pediatric populations. Efficacy in autoimmune disease has been well established in RA[42-44], SLE[45-47], antiphospholipid syndrome[48], and Sjögren's syndrome[49,50]. Particularly relevant for this study is that hydroxychloroquine is effectively used early in the course of autoimmune disease, having been shown to slow or halt progression in palindromic rheumatism[37-40] and in delaying onset of SLE in antibody positive individuals[41]. Other beneficial actions of hydroxychloroquine have been identified with use of the drug in other autoimmune diseases, including improvement in cardiovascular risk factors, such as reduction of LDL cholesterol[51], thrombosis[52], glycemia[52,53], insulin resistance[52,54,55], and reduction of risk of incident diabetes mellitus in SLE[54] and RA[55,56]. Positive effects of hydroxychloroquine on beta cell function and insulin sensitivity have also been demonstrated in healthy volunteers, possibly mediated through anti-inflammatory effects[57]. The risks of

hydroxychloroquine are thus justified by the anticipated benefit to the participants and the relation of the anticipated benefit to the risk is at least as favorable to the participants as that presented by available alternative approaches.

This research proposal in children is therefore consistent with the United States Department of Health and Human Services, Protection of Human Subjects, Subpart D, section 46.405 (research involving greater than minimal risk but presenting the prospect of direct benefit to the individual participants) and with the Food and Drug Administration, Protection of Human Subjects, Subpart D, section 50. 52 (Clinical investigations involving greater than minimal risk but presenting the prospect of direct benefit to individual participants) **for those in the hydroxychloroquine treatment arm**.

Component analysis: Placebo Arm - The placebo intervention has no known risks and is therefore consistent with the United States Department of Health and Human Services, Protection of Human Subjects, Subpart D, section 46.404 (research not involving greater than minimal risk) and with the Food and Drug Administration, Protection of Human Subjects, Subpart D, section 50. 51 (Clinical investigations not involving greater than minimal risk).

Summary: Assent of children, along with consent of both parents/legal guardians, as applicable according to country and IRB or ethics board requirements, will be obtained prior to any study procedures. This research proposal in children is consistent with the United States Department of Health and Human Services, Protection of Human Subjects, Subpart D, section 46.405 (research involving greater than minimal risk but presenting the prospect of direct benefit to the individual participants) and with the Food and Drug Administration, Protection of Human Subjects, Subpart D section 50. 52 (Clinical investigations involving greater than minimal risk but presenting the prospect of direct benefit to individual participants) for those in the active treatment group and is consistent with the United States Department of Health and Human Services, Protection of Human Subjects, Subpart D, section 46.404 (research not involving greater than minimal risk) and with the Food and Drug Administration, Protection of Human Subjects, Subpart D, section 50. 51 (Clinical investigations not involving greater than minimal risk) for those in the placebo group.

7.2. Expected Side Effects and Adverse Events

A full description of the adverse events linked to hydroxychloroquine is available within the prescribing information found in the medication packet insert. The descriptions below highlight the most important and/or common drug-related events and potential adverse events[70].

Gastrointestinal:
Gastrointestinal side effects are common, affecting between 1-10% of users. These include nausea, diarrhea, abdominal discomfort and vomiting. These symptoms are dose-related and stop with cessation of drug. Less than 10% of those with these symptoms choose to discontinue therapy.

Ophthalmologic:
The most important adverse effect of hydroxychloroquine is retinal toxicity. In a retrospective case-control study of 2,361 individuals who had taken hydroxychloroquine for at least 5 years, retinopathy was seen in 7.5% of users when assessed with spectral domain optical coherence tomography and 10 degree standard automated perimetry visual fields; as duration of use increases, an unknown, but

higher percentage that varies with the daily dose was seen. The risk of retinopathy was duration- and dose-dependent, and was less than 2% within 10 years in those taking 4-5 mg/kg/day[71]. There is negligible risk when duration of therapy <6 months[70] and it is unlikely to occur if dose is <5 mg/kg/day actual body weight (generally equivalent to 6.5 mg/kg/day ideal body weight) up to 400 mg/day. Moreover, retinopathy detected by these sensitive techniques is not equivalent to clinically apparent or significant visual effects. Despite longstanding use of hydroxychloroquine for malaria therapy in very young children, there is a dearth of information about retinopathy, suggesting its rarity[72]. Current practice recommendation by the American Academy of Ophthalmology is annual examination after 5 years of drug exposure in those on daily doses ≤5.0 mg/kg with no major risk factors[73].

Dermatologic AEs (pruritus, allergic rashes, and increased skin pigmentation), neuromuscular AEs (muscle aches), and insomnia each occur 1% of the time and resolve within 1-2 weeks with continued therapy.

Cardiovascular: Hydroxychloroquine has the potential to cause QT prolongation and should not be administered with other drugs that have a potential effect on the QT interval *(see Drugs Interactions; section 7.2.4.2)*. In addition, rare cases of cardiomyopathy have been reported in those over age 50 who received high doses of hydroxychloroquine over a long duration.

Thrombocytopenia and hemolysis are rare and are probably associated with high doses, other drugs, or G6PD deficiency.

There are case reports of allergic reactions (urticarial, angioedema), abnormal liver function tests, and skeletal muscle myopathy, which are reversible over time.

7.2.1. Immunizations

With widespread clinical use, there is no evidence that hydroxychloroquine has an impact on the safety or efficacy of vaccines. As such, all participants will be instructed to maintain their standard clinically indicated immunization schedules. However, the mechanism of action of the drug raises the theoretical possibility that live cellular vaccines could be less effective while receiving hydroxychloroquine.

7.2.2. Pregnancy

Hydroxychloroquine is widely used in pregnancy without safety concerns. However, due to the impact of pregnancy on study outcome measures and the fact that the drug is being tested for a new indication, female participants with reproductive potential will be instructed to avoid pregnancy and use birth control while on study. They will undergo urine pregnancy testing at the start of every study visit. All pregnancies that are identified during the study must be requested to be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy occurring in a female participant. Monitoring of the participant should continue until the conclusion of the pregnancy. Participants who are found to be pregnant while on this study shall have treatment

withheld, but will still be followed for safety. Treatment may only be resumed when these participants are no longer pregnant or breast-feeding.

7.2.3. Risk of Overdose

A number of fatalities have been reported in children following the accidental ingestion of the 4-aminoquinoline compound chloroquine, sometimes in relatively small doses. However, hydroxychloroquine is a different drug. It has less toxicity overall, and a higher therapeutic ratio.

7.2.4. Prohibited Medications

Participants will be asked to consult with their study team before taking any new therapy.

7.2.4.1. Drugs that may impact T1D progression or effect of Hydroxychloroquine

Participants will be instructed not to use prednisone, other immunosuppressive agents, or chronic inhaled or nasal corticosteroids during this trial in order to prevent possible impact on progression to diabetes. They will also be instructed not to use proton pump inhibitors or antacids, which may inhibit accumulation of hydroxychloroquine in lysosomes by decreasing the pH of these organelles, and thus potentially antagonizing immunomodulating effects. However, as an intention-to-treat study, no individual will be withdrawn from analysis if this occurs.

7.2.4.2. Hydroxychloroquine drug interactions

Hydroxychloroquine has known drug interactions with therapies that are unlikely to be used by the population enrolled in this study. Upon review by the TrialNet medical monitor, participants who have a clinical indication to take these medications after enrollment may be withdrawn temporarily from study treatment while on these other medications, or permanently, but with continued monitoring and safety assessments for this trial. These medications include monoamine oxidase inhibitors, anti-arrhythmogenic drugs such as amiodarone, cyclosporine, digoxin, halofantrine, other anti-malarials, anti-epileptic drugs, praziquantel, agalsidase, CYP34A inhibitors such as cimetidine and ketoconazole (which may increase hydroxychloroquine levels), penicillamine, bosutinib, droperidol, lanthanum, laronidase, cholinesterase inhibitors, and kaolin. Hydroxychloroquine potentially prolongs the QT interval, and therefore should not be co-administered with other drugs that potentially prolong the QT interval, including SSRIs.

7.3. Protecting Against or Minimizing Potential Treatment Risks

Participants who have other active serious medical problems will not be enrolled. Frequent monitoring of participants with history, physical examination, and laboratory studies will allow for early identification of adverse events. Every attempt will be made to minimize the number of venipunctures. Participants will be screened for G6PD deficiency and excluded if present.

To mitigate potential ophthalmologic risk, a fundus photograph or examination will be attempted at Baseline, and OCT and visual field examinations will be attempted annually in all participants. Participants with a successful OCT examination may continue with the study per protocol. If neither the OCT nor visual field examination can be successfully completed (e.g., in very young children), these

examinations will be re-attempted on an annual basis while the participant continues on study drug. The target dose of hydroxychloroquine will be 5.0 mg/kg/day to a maximum of 400mg. This is below the dose associated with ophthalmologic risk. Moreover, individuals who reach study endpoint (abnormal glucose tolerance) will be considered to be a non-responder and withdrawn from therapy, thus decreasing duration of exposure.

Individuals will be informed of potential impact on skin, sleep, gastrointestinal symptoms, and muscle aches. If symptoms continue or are intolerable, drug dose may be temporarily reduced or stopped, at the discretion of the investigator in consultation with the medical monitor. If signs or symptoms are consistent with infectious mononucleosis, evaluation may include measurements of EBV serology and viral load as clinically indicated.

8. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

This is a randomized, double-blinded, placebo-controlled clinical trial designed to assess the impact of hydroxychloroquine to reduce the risk of progression from Stage 1 T1D (multiple autoantibodies, normal glucose tolerance) to Stage 2 (abnormal glucose tolerance) or Stage 3 (clinical diagnosis of diabetes). Eligible participants will be randomized in a 2:1 allocation ratio to treatment with hydroxychloroquine vs. placebo. Randomization will be stratified by two factors to ensure balance in the proportions who are randomized to each of the treatment arms: (1) prior enrollment on a T1D prevention trial and (2) age (<8 vs. ≥8 yrs. old). Specifically, participants will be randomized to hydroxychloroquine vs. placebo with stratification on whether they: (1) had prior enrollment on a T1D prevention trial and received placebo vs. had prior enrollment on a T1D prevention trial and received active therapy vs. had no prior enrollment on a T1D prevention trial, and (2) are younger than 8 years old vs. 8 years old or older. Age is a known significant factor in relation to clinical outcomes of interest even in the T1D prevention setting. Further, this cutoff of 8 years old at study entry was selected based on analyses that showed a significant effect modification on clinical outcomes based on this[74].

Analyses of study data will be conducted to address the primary and secondary objectives of the trial, other stated objectives, and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study. Analyses by age, gender, and race/ethnicity, as appropriate, are also planned.

All analyses will be conducted under an intention-to-treat principle, whereby all outcome data in all randomized participants will be included in all analyses, as appropriate. Details of the analysis can be found within the statistical analysis plan.

8.1. Primary Outcome

The primary outcome is the elapsed time from random treatment assignment to the development of confirmed abnormal glucose tolerance or clinical diabetes among those enrolled in the primary analysis cohort consisting of participants with islet cell autoimmunity and absence of metabolic abnormalities (normal OGTT). The date of the confirmatory abnormal OGTT or diabetes will be considered as the date of reaching these study endpoints. It is expected that abnormal glucose tolerance will be detected prior to diabetes onset by OGTT; however, the presence of diabetes, without a previous abnormal

OGTT, is also considered an endpoint.

While the hydroxychloroquine prevention study is ongoing, any participant who has progressed to abnormal glucose tolerance will have met the primary endpoint criteria. These participants who have developed abnormal glucose tolerance but have not progressed to Stage 3 T1D will have the option to remain in this study without further therapy; they will receive close monitoring and safety assessments until the development of Stage 3 T1D (clinical diabetes) or until they enroll in another clinical trial, whichever comes first.

The study endpoint is realized with either confirmed OGTT criteria for abnormal glucose tolerance or diabetes or clinical criteria for diabetes.

OGTT criteria for abnormal glucose tolerance or diabetes:

The presence of an OGTT consistent with impaired glucose tolerance or diabetes on two sequential dates. A participant with abnormal glucose tolerance or diabetes on an OGTT should undergo a repeat OGTT as soon as possible, but no less than one day apart. Aim to repeat within one month.

The definition of abnormal glucose tolerance is:

a. Fasting plasma glucose ≥ 110 mg/dL (6.1 mmol/L) and < 126 mg/dL (7.0 mmol/L), or
b. 2 hour plasma glucose ≥ 140 mg/dL (7.8 mmol/L) and < 200 mg/dl (11.1 mmol/L), or
c. 30, 60, 90 minute plasma glucose during OGTT ≥ 200 mg/dL (11.1 mmol/L).

Criteria for Diabetes Diagnosis:

A. Diabetic Ketoacidosis (DKA) or unequivocally symptomatic* AND at least one of the following**:**
    1) Random glucose ≥ 200 mg/dl (11.1 mmol/L) OR
    2) Fasting Glucose ≥ 126 mg/dl (7.0 mmol/L) OR
    3) OGTT = diabetic** OR
    4) HbA1C ≥ 6.5%.

*Unequivocal symptoms: Severe/persistent polyuria, polydipsia and/or significant unexplained weight loss

**A diabetic OGTT is defined as:

a. Fasting plasma glucose ≥ 126 mg/dL (7.0 mmol/L) AND/OR
b. 2 hour plasma glucose ≥ 200 mg/dL (11.1 mmol/L)

B. If not unequivocally symptomatic, two separate objective measures are required for diagnosis. These tests must occur within 1 year of each other and must be consecutive, defined as without an intervening non-diabetic OGTT.
    - **The preferred objective measures for diagnosis are:**
        * 2 diabetic OGTTs, not on the same day (the confirmatory OGTT is the date of

diagnosis)

- However, if an individual is diagnosed without the consecutive OGTTs, the following also constitute diagnosis in TrialNet:
    * Diabetic OGTT + FPG ≥ 126 mg/dl [7 mmol/L] (not on the same day)
    * Diabetic OGTT + HbA1c ≥ 6.5% (these may be on the same day)
    * FPG ≥ 126 mg/dl [7 mmol/L] + HbA1c ≥ 6.5% (these may be on the same day)

The TrialNet Eligibility and Events Committee will review and adjudicate any cases with only a single objective measure, cases which do not meet the criteria above, and/or cases which have uncertain results of tests and/or the test procedures. Qualifying glucose values should be from laboratory tests, rather than capillary blood glucose meter readings.

8.2. Primary Analysis

The primary objective of the study is to assess the effect of hydroxychloroquine therapy versus placebo on the risk of progression to Stage 2 or 3 T1D, which is measured from an OGTT or clinically diagnosed with confirmation.

Time to progression to Stage 2 or 3 T1D will be calculated across participants within each treatment group. Time to T1D stage progression will be estimated based on Kaplan-Meier methodology, where participants who were "free of abnormal glucose tolerance and/or T1D" at their last follow-up will be censored at that time point.

This time-to-event outcome will be analyzed using a Cox proportional hazards regression model that stratifies by prior T1D prevention treatment (none vs. placebo vs. active therapy) and age at the time of randomization (<8 yr vs ≥8 yr), where treatment effect in this model will be tested using the Wald statistic. Incorporating these as stratification factors in the Cox regression model will allow separate baseline hazard functions to be fitted for each stratum. We will further evaluate differences in event rates in those in the prior treatment stratification factor; specifically, we will assess if there are differential event rates in those enrolling after participation in another prevention trial and ensure that this is accommodated by corresponding stratification in the Cox regression model.

In addition to stratifying on these factors, we will also evaluate if they are effect modifiers on any differences observed in the time to T1D stage progression between these prior treatment groups; i.e., we will formally test for interactions of treatment effect with prior treatment for T1D prevention as well as age on time to T1D stage progression (i.e. progression from Stage 1 to Stages 2 or 3 T1D).

Proportional hazards can be tested to ensure that we can assume that these effects will be parallel. If the assumption of proportional hazard is not appropriate, the data will be examined to determine the cause of non-proportional hazard, such as the presence of a decaying, diverging, or crossing effect of hazard ratios over time. Based on the cause of the non-proportional hazard, post-hoc analyses such as frailty models, parametric models, or models with interactions and time-dependent covariates may be employed.

Additional factors may be defined before unmasking of the study data to the investigators. The analyses will distinguish between factors specified prior to unmasking, and those identified post-hoc during

analysis. Data from the TrialNet Pathway to Prevention Study may be used to determine participant eligibility. Outcomes from other studies may be used in meta-analyses, but not in the evaluation of this study's efficacy.

8.3. Secondary Outcomes and Analyses

A variety of secondary analyses are planned, including the following:

Time to progression to Stage 3 T1D (clinical diabetes) will also be evaluated. Those participants who progress to Stage 2 T1D (abnormal glucose tolerance) prior to developing clinical diabetes will also be included in the time to (Stage 3) T1D analyses. Any participants who develop abnormal glucose tolerance and enroll in another treatment trial or receive other prevention therapy prior to development of Stage 3 T1D will be censored at that time. The treatment arms will be compared in terms of the corresponding distributions of time to progression to Stage 3 T1D (clinical diabetes) using the same methods described for the primary endpoint. Subgroup analyses analogous to those described for time to the composite event definition of progression to Stage 2 ***or*** 3 T1D will be conducted on the endpoint of time to progression to Stage 3 T1D.

Longitudinal analyses will assess the effects of hydroxychloroquine versus placebo treatment on immunologic and metabolic markers over time up to the onset of progression to Stage 2 or 3 T1D. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures. Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures. Generalized estimating equations may also be employed for the analysis of quantitative factors if the assumption of multivariate normal random errors is violated.

Immunologic and metabolic markers will be modeled to determine the effects of hydroxychloroquine versus placebo treatment while adjusting for participant characteristics for each follow-up time point of interest. For continuous endpoints that lend themselves to normal error linear models, ANOVA and ANCOVA models will be employed. Generalized linear modeling will be employed for dichotomous and categorical endpoints by using the most appropriate link functions. Longitudinal analyses may be employed in order to characterize the relationship among the repeated measures during the treatment period and possibly beyond. Due to the exploratory nature of the longitudinal modeling, treatment effect hypothesis testing will not be conducted.

The association of demographic, genetic, immunologic, metabolic, lifestyle factors, the presence of illness and concomitant medications, among others, both at baseline and over time, with the risk of T1D stage progression will be assessed in Cox regression models over time. The effects of changes in longitudinal factors on risk of progression will be assessed using time-dependent covariates for these factors. Analyses will be conducted separately within the hydroxychloroquine and placebo groups, and differences between groups in covariate effects (group by covariate interactions) will be assessed. Models will then be assessed within the two groups combined, taking account of any group by covariate interactions. Subgroup analyses will be conducted comparing the effects of hydroxychloroquine versus placebo on the risk of progression to Stage 2 or 3 T1D with a test of the group by subgroup factor interaction in a Cox proportional hazard (PH) Model. Specifically, we will evaluate potential differential effects of treatment in relation to other factors at baseline, including: age (both continuous as well as

categorical: children < 8 years of age vs. ≥ 8 years, as well as < 8 years vs. 8 to 12 years vs. adolescents [13-17 years] vs. adults [≥ 18 years]), gender, race/ethnicity, whether or not they have a first degree relative (FDR) with T1D, and specific autoantibody status. Similar analyses will be conducted using the values of quantitative baseline metabolic and anthropometric factors such as weight, BMI, and the immunologic and metabolic factors described in Section 6 that include the autoantibody titers, basal C-peptide, stimulated C-peptide (peak and AUC mean), and measures of insulin resistance modeled from the OGTT. These potential effect modifiers, or interaction effects, will be evaluated both using tests of interaction in the Cox regression model as well as stratified analyses. In addition, we will evaluate differences in time to T1D stage progression across all randomized participants using multivariable Cox regression models with tests of interaction and stratified analyses to assess potential differential effects, based on whether or not participants were previously treated for T1D prevention.

Toxicity is defined as adverse events that are classified as either possibly, probably, or definitely related to study treatment. The maximum grade for each type of toxicity will be recorded for each participant, and frequency tables will be reviewed to determine toxicity patterns. In addition, we will review all adverse event data that is graded as 3, 4, or 5 and classified as either “unrelated” or “unlikely to be related” to study treatment in the event of an actual relationship developing. The incidence of severe (grade 3+) adverse events or toxicities will be described for each treatment arm, but will also be compared between the arms. Fisher’s exact tests will be used to quantitatively compare the incidence of severe as well as specific toxicities of interest between the treatment arms, and we will graphically assess differences in maximum grades observed for toxicities between the arms. These analyses will be useful in identifying potential toxicity patterns of interest and in planning future trials. We will also assess tolerability of hydroxychloroquine through assessing the proportion of patients who discontinue treatment due to adverse reactions or even those who refuse further treatment for lesser toxicities that inhibit their willingness to continue participation on the trial. These tolerability measures will be assessed within each of the treatment arms and we will explore differences in these measures between the arms. All participants who have received at least one dose of any of the therapeutic agents in a treatment arm will be evaluable for toxicity and tolerability.

8.4. Study Power and Accrual Target

This study has been designed to provide 83% power to detect a 50% risk reduction in the hazard rate for progression to Stage 2 or 3 T1D (abnormal glucose tolerance or diabetes diagnosis) using a two-sided test at the 0.05 level after a minimum of two years of follow-up on all participants and an expected total study duration (accrual and follow-up) of six years. We note that if accrual is more rapid than expected, minimum follow-up on participants may need to be extended in order to meet the number of events required for the final analysis.

A total of 201 participants will be randomized in a 2:1 allocation to treatment with hydroxychloroquine (n=135) vs. placebo (n=66). Randomization will be conducted using block randomization with variable block sizes with stratification on (1) whether or not participants have been previously treated for T1D prevention, and (2) age group (< 8 years old vs. 8 years old or older).

The assumptions underlying the estimated sample size for this design are: (1) 40% two-year event rate in the placebo group, (2) detectable hazard ratio of 0.50 (i.e. 22.5% two-year event rate) in the hydroxychloroquine group, (3) less than a 10% two-year dropout rate in both groups, and (4) survival curves which are consistent with exponential survival distributions. Even testing for variable proportions of each stratum and with slight variation in the expected hazard rates for the placebo group across the strata, our proposed sample size of 201 participants will provide at least 83% power to detect a hazard ratio of 0.50 in the time to T1D stage progression between the two treatment groups using a stratified Cox regression model[75].

Under the above assumptions, the target total number of events is equal to 80; i.e., the final analysis will be conducted once 80 events (confirmed abnormal glucose tolerance, diabetes) have been observed. If the accrual rate is 50 patients per year, with the above assumptions it is estimated that the enrollment period will last approximately 4 years with a total study duration of approximately 6 years (4 years of accrual + minimum of 2 years of follow-up) to provide a sufficient number of events to detect the assumed difference.

The final test of significance up to the six-year time point will employ group sequential critical values to protect against inflation in the type I error probability due to interim assessments of the emerging data for review by the DSMB (see Section 8.5).

Note the accrual period and the study sample are only projections, since the actual accrual rate and the loss-to-follow-up rate are unknown. As the study progresses, more accurate projections of the study end date will be computed based on the observed rate of enrollment, the observed number of events, and the observed rate of loss-to-follow-up. These data will be provided to the DSMB and the TrialNet governing body, and if need be, this document will be amended.

8.5. Interim Monitoring Plan

A formal interim analysis of efficacy will be conducted when 50% of events (i.e., information fraction = 0.50) have been observed; i.e., we will conduct an interim analysis to compare the two treatment arms when 40 participants enrolled on this trial have a reported progression to Stage 2 or 3 T1D. To preserve the Type I error rate control for each of these comparisons on superiority, the Lan-DeMets error spending rate function with the O'Brien-Fleming boundaries is utilized. If these boundaries are crossed, then the TrialNet DSMB will determine if accrual to that arm should be suspended and/or if treatment of participants should be modified based on these results[76]. The interim and final analysis boundaries and characteristics were generated using the East 6 clinical trial software program (version 6.3, Cytel Inc).

Information fraction	Cumulative events	Alpha spent	Efficacy boundary (p-value)
0.50	40	0.0031	2.963 (or -2.963)
1.00	80	0.00153	1.969 (or -1.969)

The DSMB may terminate the trial prematurely if a statistically significant effect is observed and it is considered that all major trial objectives have been met.

In lieu of a formal futility rule at the interim analysis (i.e., 50% information), we will utilize conditional probability methods to assess the probability that hydroxychloroquine will delay time to T1D stage progression based on the data observed at that point if the observed hazard ratio is >1.05 (i.e., in favor of placebo).

The DSMB will also consider early termination due to absence of a treatment effect (i.e., futility) based on computations of conditional power conducted both under the initial study design and under the current trend of the data[77].

8.6. Withdrawal Criteria – Individual Participants

An intention-to-treat approach will be used in these analyses. Participants will not be replaced. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless a participant withdraws consent for the use of those data. Every effort will be made to conduct a final study visit with the participant, and participants will be followed clinically until, if applicable, all potentially study-related adverse events resolve.

- Withdrawal of consent by the participant
- Withdrawal of the participant by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease.

9. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

9.1. Statement of Compliance

This study will be conducted in compliance with the protocol and consistent with current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements (*ICH E6, 45CFR46, and FDA 21CFR sections 11, 50, 56, 312)*.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by a Central Institutional Review Board (CIRB) or an appropriate Independent Ethics Committee/Research Ethics Board (IEC/REB) or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are implemented.

9.2. Participating Centers

Participating TrialNet clinical sites must have an appropriate assurance, such as a Federal-wide Assurance (FWA) or an Unaffiliated Investigators Agreement (UIA), with the Office for Human Research Protections (OHRP), since they are actively engaged in research and obtain informed consent. The protocol and consent forms will be approved by a Central Institutional Review Board (CIRB) or Institutional Review Boards or Ethics Committees/Research Ethics Boards at each of the participating clinical sites. HIPAA and applicable local regulations will be followed by each participating institution in accordance with that institution's requirements. The participating international sites will obtain

approval from their corresponding review boards in accordance with their local procedures and institutional requirements.

The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The investigator is required to ensure that all source documents are legibly completed for every participant entered in the trial.

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants in this study. When a participant participates in this study at more than one TrialNet site, sharing of this information is required. Sharing of information obtained during this study between the TrialNet Hub, TrialNet Clinical Centers and affiliates will occur whenever necessary to assure participant understanding and consent, safety, and adherence to protocol according to country-specific guidelines.

9.3. Informed Consent

The process of assuring that individuals (and parent/guardian if less than 18 years of age) are making an informed decision about participating in this study includes both verbal and written communication. Written materials include a Volunteer Handbook, Volunteer Understanding Assessment, and written or electronic consent forms. The consent form describes the procedures, risks and benefits for the study. The consent forms will be reviewed with participants (and their parent/guardian in the case of participants under 18 years of age) and the participant will be given time to review the written or electronic consent form and ask questions. An assent form has also been developed for participants less than 18 years of age (unless local IRB/REB requirements differ in procedure).

As part of the informed consent process, prior to treatment, the participant and/or parent or guardian (if the participant is less than 18 years of age) will also be required to complete a short, written Volunteer Understanding Assessment that is designed to ensure that the participant understands the study, as well as what is being asked of him/her. The participant will have access to a copy of their consent/assent forms.

The consent process will be conducted by qualified study personnel (the Trial Investigator or Study Coordinator and/or other designee). All participants (or their legally acceptable representative) must read, sign and date a written orm prior to participation in the study, and/or undergoing any study-specific procedures.

Remote informed consent may be used as needed when a participant is unable to travel to a TrialNet site. When providing remote consent (via phone or video conference) the POC will review the checklist and long-form informed consent document with the subject/parent or guardian. After the subject/parent or guardian is provided the printed long form informed consent document, the POC will address any questions the subject/parent or guardian may have before the informed consent form is signed and any procedures are performed. The informed consent document may be provided to the subject/parent or guardian using any method that the complete content can be available in print. Signed and completed documentation must be available to the subject/parent or guardian and the study team, by any method that assures that the complete content can be available in print.

Written assent for participants <18 years old may be waived if enrolled using the remote informed consent process. Written assent will be obtained at the participant's next in-person visit.

When a participant turns 18 they are permitted to re-consent remotely should they be unable to return to the study site for an in-person visit. Re-assent when a participant reaches age 12 is waived until the participant returns for an in-person visit at the study site.

The informed consent form must be updated or revised whenever there is new, clinically significant information applicable to the safety of the participants, when indicated for a protocol amendment, and/or whenever any new information becomes available that may affect a participant's participation in the study.

Participants will be re-consented if they reach the age of 18 years while enrolled in the study.

9.4. Study Participant Confidentiality

The investigational sites participating in this study will maintain the highest degree of confidentiality possible for the clinical and research information obtained from participants. Participant-identifying clinical and research information will be shared between TrialNet sites to support participants and assure protocol compliance as applicable. As a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (obscuring any personally identifying information). Authorized representatives, as noted above, are bound to maintain the strict confidentiality of medical and research information that may identify individuals. The investigational site will normally be notified in advance of auditing visits. Study records with the study participant's information for internal use at the clinical sites will be secured at the study site during the study. At the end of the study, all records will continue to be kept in a secure location. There are no plans to destroy the records.

Study participant data, which is for reporting purposes, will be stored at the TrialNet Coordinating Center. Case report forms sent to the Coordinating Center will identify participants by their unique TrialNet Identification Number. The data entry system at the Coordinating Center is a secured, password-protected computer system. At the end of the study, all study databases will be archived at the Coordinating Center, and the data collection forms will be electronically scanned and saved in electronic format for long-term storage.

Stored samples including genetic samples could be utilized to learn more about causes of T1D, its complications (such as eye, nerve, and kidney damage) and other conditions for which individuals with diabetes are at increased risk, and how to improve treatment. The results of these future analyses, and any mechanistic studies, will not be made known to the participant.

9.5. Risks and Benefits

The risks of this study are presented in this protocol, the prescribing information package insert, and informed consent form. There is no guaranteed benefit to participants for their participation in the study. This study will examine whether intervention with hydroxychloroquine will delay or prevent the development of abnormal glucose tolerance, but there is no guarantee that this will occur. However, all participants will benefit from close monitoring for the development of diabetes. This close monitoring significantly reduces the morbidity typically associated with clinical onset of disease.

Special consideration regarding risks and benefits for children is described in section 7.1.

9.6. Ethics

The study protocol, along with the required informed consent forms, will be approved by a Central Institutional Review Board (CIRB), and/or each participating institution's Institutional Review Board (IRB) or Ethics Committee/Research Ethics Board (EC/REB) at international sites prior to the initiation of any research procedures (at the site). In addition to details described in the sections above (informed consent, confidentiality, and risks and benefits), the investigators have reviewed and considered ethical ramifications in the design and development of this protocol. The investigators have made every effort to minimize and monitor risks and discomforts to participants throughout the course of the study.

10. STUDY ADMINISTRATION

10.1. Organizational Structure

This study is part of Type 1 Diabetes TrialNet, which is funded by the National Institutes of Health. Funding will cover the costs of administration and laboratory tests associated with this study.

10.2. Groups and Committees

10.2.1. Hydroxychloroquine Prevention Study Chair Committee

The Study Chair and TrialNet Executive Committee will receive periodic reports from the TNCC on the progress of the study. These will include accrual rates and baseline demographic characteristics. Criteria and results of ongoing monitoring of the TrialNet labs in terms of reproducibility will also be provided on a routine basis and reported on during the Joint Study Chair Committee meetings, as scheduled.

10.2.2. TrialNet Chairman's Office and TNCC

The TrialNet Chairman's Office, the TNCC, and the TrialNet Hub will collaboratively provide leadership to the TrialNet study group; this will include protocol and manual preparation, training for clinical sites, development of statistical design for each study, and analysis of study results. The TNCC will also coordinate interactions among the participating TrialNet Clinical sites, laboratories (including TrialNet core Laboratories and other subcontract laboratories), NIDDK, and other sponsoring agencies.

10.2.3. Clinical Sites

Principal Investigators at each participating TrialNet clinical site will oversee all operations at that site. The clinical sites will forward all laboratory and data collection form information to the TNCC for analysis. Direct communication and site visits, as needed, will facilitate evaluation of the trial management.

10.2.4. Eligibility and Events Committee

A TrialNet Eligibility and Events Committee will review all relevant information for each participant in which there is uncertainty related to eligibility or to diagnosis of diabetes according to criteria stated in section 9.1. The Committee will determine whether the diagnosis of diabetes or abnormal glucose tolerance in each of these participants is sufficiently sound so as to include that participant among the cases who have reached the primary outcome in the statistical analysis. The Committee will be masked to treatment assignment as it reviews each case.

10.2.5. Clinical Site Monitoring

In order to conduct this study with established research principles, site visits may be conducted during the study to evaluate study conduct and ensure participant safety. All sites will be monitored by the TNCC and appropriate TrialNet committees for patient enrollment, compliance with protocol procedures, completeness and accuracy of data entry, the occurrence and reporting of adverse events (AEs) and serious adverse events (SAEs), site pharmacy accountability/operations, and to confirm the presence of appropriate IRB/REB regulatory approvals/documents.

10.3. Medical Monitor and Data Safety and Monitoring Board (DSMB)

All adverse events will be recorded on source documents; adverse event forms will be sent to the local IRBs/REBs, per their reporting requirements, and, for AEs ≥ Grade 2, to the TNCC.

An independent physician will be designated to serve as the medical monitor for this study who will maintain regular contact with the study and the Study Chair. (S)he will review all adverse event reports, masked to treatment assignment, and will file event reports with regulatory authorities as appropriate.

The DSMB will meet approximately every 3 months and as needed to review indicators of safety. In addition, they will meet every 6 months to review the interim effectiveness and potential toxicity of the study treatments based on interim analyses of indicators of effectiveness and safety (separately by treatment group) prepared by the TNCC. The DSMB will independently evaluate whether there are grounds to modify or discontinue the study.

10.4. Sample and Data Storage

Samples to be stored for research purposes will be located at the NIDDK Repository and at TrialNet Laboratory Sites. While TrialNet is active, the use of the samples will be restricted to TrialNet

researchers, unless researchers from outside of TrialNet obtain approval from the TrialNet Steering Committee and the NIDDK to utilize the samples. All samples will be coded with unique study numbers, but TrialNet researchers will be able to identify samples if it is necessary to contact participants for reasons of health or to notify them about future studies. Approval from the TrialNet Steering Committee and the NIDDK would be required before such linkage could occur. Researchers from outside of TrialNet will not be permitted to identify samples.

Data collected for this study will be sent to the TNCC. De-identified data will be stored at the NIDDK Repository, under the supervision of the NIDDK/NIH, for use by researchers including those outside of TrialNet.

With permission of the participant, when TrialNet is completed, samples will continue to be stored at the NIDDK Repository. Since the stored data will be fully de-identified upon the completion of TrialNet, it will no longer be possible to identify samples. Thus, whereas a sample can be destroyed upon a participant's request during the existence of TrialNet, it can no longer be destroyed once TrialNet is completed. However, there will still be the potential to link data derived from the samples with data that was derived from TrialNet studies. Once TrialNet is completed, researchers will only obtain access to samples through grant proposals approved by the NIDDK. The NIDDK will convene an external panel of experts to review requests for access to samples.

10.5. Preservation of the Integrity of the Study

The scientific integrity of the trial dictates that results be reported on a study-wide basis; thus, an individual Center will not report the data collected from its site alone. All presentations and publications using TrialNet trial data must protect the main objectives of the study. Data that could be perceived as threatening the study outcome will not be presented prior to release of the primary study outcomes. Approval as to the timing of presentations of data and the meetings at which they might be presented will be granted by the TrialNet Publications and Executive Committees. Study results should be discussed with the news media only upon authorization of TrialNet Executive Committee, and never before the results are presented. Any written statements about this study that are shared with national media must be approved by TrialNet Executive Committee before release.

10.6. Participant Reimbursement and Compensation

Participants may be compensated for each visit attended in the study. In compliance with ICH Guidance E6, the amount and method of payments to participants shall be designed to avoid coercion or undue influence on the study participants. Payments to participants will be prorated and not wholly contingent on completion of the trial by the participant.

APPENDIX 1: Hydroxychloroquine in At-Risk Individuals: Study Flow Chart

TrialNet Pathway to Prevention Study Results

	Antibody results meet eligibility criteria[1]. Confirmation of 2 positive autoantibodies must occur within six months prior to randomization, but the confirmation does not have to involve the same 2 autoantibodies.
	OGTT within 52 days of Baseline: Fasting Plasma Glucose <110 mg/dL (6.1 mmol/L) AND 2 hour Plasma Glucose <140 mg/dL (7.8 mmol/L) AND 30, 60 and 90-minute glucose < 200 mg/dL (11.1 mmol/L)

Hydroxychloroquine Prevention Study <u>Screening</u>

Procedures	Consent to study participation is reviewed and signed. Laboratory assessments, History, Physical Examination, Volunteer Understanding Assessment, Study Education. Autoantibodies and/or OGTT if not performed during Pathway to Prevention Monitoring Visit, or if repeat testing is needed to meet entry criteria windows.
Results to move on to Hydroxychloroquine Randomization	Meets all study inclusion criteria Does not meet any exclusion criteria[2, 3]

Hydroxychloroquine Prevention Study <u>Randomization and Baseline</u>

Procedures	Confirmation of eligibility, randomization, baseline laboratory assessments, and administration of hydroxychloroquine study drug/placebo.

[1]If autoantibodies are not confirmed positive on the second test a tiebreaker draw will be required. While TN18 (Abatacept Prevention) is open for enrollment, if the individual is ≥ 6 years of age and eligible for TN18, at least one of the antibodies must be mIAA.

[2]If previous abnormal glucose tolerance, two most recent consecutive OGTTs must show normal glucose tolerance.

Note, individuals are permitted to undergo OGTT and autoantibody testing to determine eligibility for hydroxychloroquine during a screening visit if not yet eligible through OGTT and autoantibody testing in TN01; Pathway to Prevention Monitoring Visit. Data obtained from screening tests for this study may be used if needed for eligibility for other TN studies.

[3]If participant is not eligible or unwilling to participate in this study but remains eligible for monitoring, the participant may be followed in TN01 Pathway to Prevention Study as applicable.

APPENDIX 2: Schedule of Assessments

		Double-Masked Treatment				
	Screening	Baseline	Month 1	Month 3	Month 6 & every subsequent 6 months [1]	Month 9 & every subsequent 6 months
Visit number	**-1**	**0**	**1**	**2**	**3+**	**4+**
Informed Consent	X					
Eligibility including G6PD screening	X					
Medical history	X	X				
Complete Physical Examination [2]	X				X (annual visits only)	
Limited/Directed Examination [2]		X		X	X (six month visits only)	
CBC with differential and platelets	X	X		X	X	
Chemistries and liver function tests	X	X			X	
Urine pregnancy test (as appropriate)	X	X		X	X	
HIV, Hep B and C serology	X					
Adverse Events		X	X	X	X	
Prior/ Concomitant Meds	X	X	X	X	X	
Dosing & Drug Dispensing [3]		X		X	X	
OGTT [4]	(X[5])				X	
HbA1c	X				X	
Islet autoantibodies	(X[5])	X		X	X	
Mechanistic assessments [6]	X	X		X	X	
Ophthalmology examination [7]		X[7]			X (annual visits only)	
Study med accountability				X	X	
Interim contact [8]			X			X

1= Participants will be followed every six months until development of study endpoint, diabetes, or the end of the trial.

2= Interim medical history and directed/limited physical examination every six months. Complete examination at screening and annually thereafter. Once Tanner Stage assessed as ≥ 3, no further genitalia examination required. All visits include adverse event assessment and collection of concomitant medications.

3= Initial dosing and dispensation must occur during the baseline visit.

4= If OGTT consistent with abnormal glucose tolerance or DM, repeat as soon as possible, but no less than one day apart. Aim to repeat within 1 month. Mechanistic samples, including CBC/differential, will be collected at the confirmatory OGTT visit. In order to maintain the masking of the study outcome, a random subset of participants with a normal OGTT will also be brought back for an additional OGTT after undergoing a regularly scheduled OGTT. Individuals who report signs or symptoms of diabetes may undergo further testing as indicated including OGTT evaluation.

5= If not done as part of the TrialNet Pathway to Prevention study, OGTT and Islet Autoantibodies may be done at the screening visit.

6= May include samples for pharmacokinetic assessment, RNA, plasma, serum, DNA, measures of B and T cell number and function to understand the effect of therapy on the immune system and infectious disease. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the participant's age and body weight (For participants <18 years, 5 mL/kg per visit, 9.5 mL/kg in an 8 week period). Responses to clinically indicated immunizations may be determined through analysis of pre- and post-dose samples.

7= Ophthalmology examination: baseline examination may occur any time from screening until three months after randomization. Subsequent examinations will occur annually for all participants, starting at the 12 month visit.

8 = All subjects may have interim contact with study personnel for formal inquiry about adverse events and presence or absence of diabetes related symptoms. This interim contact does not require an in-person visit.

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