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Study protocol

A randomized, double-blind, placebo-controlled, Phase 1/2a trial protocol to assess the safety and efficacy of TAK-101 administered by microneedles in patients with celiac disease

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ABSTRACT:

Introduction: Celiac disease (CD) affects 0.2 to 2.0% of the worldwide population, causing significant enteropathy and systemic symptoms. There is no effective treatment for CD, and patients must follow a strict gluten-free diet lifelong. Recent studies investigated the efficacy of the non-immunosuppressive agent TAK-101 delivered intravenously in reducing immunological response to gluten in CD patients. However, no study has evaluated transdermal delivery, which initiates different immunological pathways.

Objective: In a population of CD patients, we aim to assess the safety and pharmacokinetics of TAK-1 delivered by a Hollow Microstructured Transdermal microneedle (MN) (Phase 1) and the efficacy of MN-TAK-101 compared to placebo (Phase 2).

Methods: This will be a Phase 1/2a trial lasting 180 days for each phase. Phase 1 will be an open-label, 2-part multicenter study: 1A, a group of patients will be divided into escalating dose cohorts. While, in 1B, another group of patients will be divided into repeated ascending dose cohorts until the maximum tolerated dose is reached. Phase 2 will be a double-blind multicentered randomized (stratified, block) placebo-controlled trial comparing TAK-101 and placebo, using different subjects than those in Phase 1. The primary outcome is the change in Interferon-γ production from baseline after MN-TAK-101 or MN-placebo administration. Secondary outcomes will assess the changes in the ratio of villous height to crypt depth, in the number of intestinal intraepithelial lymphocytes, in CD4, CD8, and γδ cells frequency, in the Celiac symptom index-modified questionnaire and safety measurements. The sample size is between 27-49 patients for phases 1A and 1B; 76 patients in phase 2. For statistical

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analysis, only descriptive analysis will be done for phase 1, while for Phase 2, the choice of parametric vs. nonparametric tests will be considered according to the normality of the data. This is a first Phase 1/2a placebo control randomized trial to assess the safety and efficacy of TAK-101 applied transdermally for patients with celiac disease.

Keywords: Celiac Disease; TAK-101; Microneedle; Gluten intolerance; Gluten desensitization.

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Abreviations

(A.E.s): Adverse Effects
(APCs): Antigen-Presenting Cells
(AUC): Area Under the Curve
(CD): Celiac Disease
(Clast): Last Measurable Concentration
(Cmax): Maximal Observed Concentration
(CTCAE) : Common Terminology Criteria for Adverse Events
(DGP-IgG): Deamidated Gliadin Peptide Immunoglobulin G
(DLT): Dose-Limiting Toxicity
(DMC): Data Monitoring Committee
(DRAEs): Drug-Related Adverse Events
(ECG): Electrocardiogram
(EDC): Electronic Data Capture
(ELISpot): Enzyme-Linked Immunospot
(GFD): Gluten-Free Diet
(HIV): Human Immunodeficiency Virus
(HLA): Human Leukocyte Antigen
(HMO): Health Medical Organization
(hMTS): Hollow Microstructured Transdermal System
(IELs): Intestinal Intraepithelial Lymphocytes
(IFN-y): Interferon-Gamma
(IRB): Institutional Review Board
(MN): Microneedle
(MN-PLBO): Microneedle Placebo
(NYHA): New York Heart Association
(OGC): Oral Gluten Challenge
(PBMCs): Peripheral Blood Mononuclear Cells
(PLGA): Poly-Lactic-co-Glycolic Acid
(SAEs): Significant Adverse Events
(SD): Standard Deviation
(SEM): Standard Error of the Mean
(S.F.U.s): Spot Forming Units
(TEAEs): Treatment-Emergent Adverse Events
(TG2): Tissue Transglutaminase 2
(Tlast): Time of Last Measurable Concentration
(Tmax): Time of Maximal Observed Concentration
(ULN): Upper Limits of Normal
(Vh:Cd): Villus Height to Crypt Depth

Introduction

Celiac disease (CD) affects 0.2 to 2.0% of the population and is common among adults, especially in females (Choung et al., 2015; King et al., 2020; Serena et al., 2019; Singh et al., 2018). Affected patients have genetic predisposition due to the presence of human leukocyte antigen (HLA-DQ2 or HLA-DQ8), tissue transglutaminase (TG2), and gluten (Schuppan & Zimmer, 2013).

Gluten is a large protein with two main fractions, glutenin and gliadin (Wieser, 2007). In celiac patients, gluten is particularly resistant to digestion by gastrointestinal enzymes, and long peptides can be found in the intestinal lumen. Gliadin is presented to CD4+ T cells, provoking an inflammatory response with an exacerbated IFN-y (Interferon-gamma) secretion by activated T-cells, leading to intestinal damage with the subsequent symptoms and complications: diarrhea, malabsorption, gastrointestinal malignancies, and other autoimmune diseases (Han et al., 2015; Kelly et al., 2021; Ludvigsson et al., 2014). Therefore, therapeutic techniques that delete effector T cells or induce regulatory T cells may be beneficial, such as TAK-101 (Kivelä et al., 2020).

TAK-101 is a negatively charged nanoparticle encapsulating gliadin that works by interaction with macrophage receptors and tolerogenic antigen-presenting cells (APCs) in the spleen and liver to induce tolerance to this protein. These particles are taken up by APCs, which then release beta growth factor and interleukin-10. They process and present gliadin epitope-specific T cells with gliadin T-cell epitopes. T cells migrate to the small intestine to protect it from the immune system's harm. TAK-101 given intravenously was safe, tolerable, and caused an 88 percent reduction in IFN-γ spot-forming units compared to placebo in a recent phase 1/2a, double-blind, randomized, placebo-controlled trial (Kelly et al., 2021).

Administering drugs intravenously can be optimal in delivering sizable molecular weight drugs such as TAK-101; however, this route can cause excessive

pain, necrosis, and tissue sloughing. Microneedles (MN) offers a novel alternative for delivering TAK-101. MN are devices that perforate the skin to deliver molecules into the dermis and can be more patient-friendly, less painful, and less invasive than the intravenous route (Jamaledin et al., 2020; Kirkby et al., 2020). Up to this point, only hollow MN have reached the pharmaceutical market as medical devices (Cárcamo-Martínez et al., 2021).

Currently, no drug therapy reliably prevents the effects of dietary gluten or has been approved by regulators to treat CD; the only available treatment is adherence to a lifelong, strict gluten-free diet (GFD) which can affect the social, physiological, and economic life domains (Caio et al., 2019; Cappell et al., 2020; Pourhoseingholi et al., 2017).

This study aims to assess the safety of TAK-101 administered via hollow M.N.s in a Phase 1 trial and, subsequently, its efficacy in a Phase 2 trial in a population of CD patients aged 18-65 years old over 180 days for each phase; Phase 1 will be an open-label study with safety and pharmacokinetics of plasma gliadin as the primary and secondary outcome, respectively. After that, depending on its results, Phase 2 will be a randomized placebo-controlled trial evaluating TAK-101 efficacy following oral gluten challenge (OGC) assessed by changes in IFN-γ enzyme-linked immunospot (ELISpot) assay (primary outcome). Changes in CD4, CD8, γδ cells and duodenal histology, and CD signs and symptoms will be evaluated as secondary outcomes.

Materials and Methods

Trial Design

Phase 1 is an open-label, 2-part multicenter study.

Phase 2 is a double-blinded multicentered randomized placebo-controlled trial.

Considering the study design, we will use convenience sampling from multiple Clalit Health Medical Organization (HMO) centers in Jerusalem, Israel.

Randomization

In Phase 2, patients will be randomized to one of the two arms. Stratified randomization by gender will be implemented (Choung et al., 2015). We will use 1:1 ratio permuted (undisclosed) block randomization using RALLOC in STATA.

Allocation sequences will be enclosed with pharmacists. Once the consent is signed, a sequence number will be allocated to that patient.

The pharmacist or statistician will create the allocation sequence using STATA. When a patient meets the eligibility criteria, the study coordinator will explain the trial, obtain informed consent and allot a sequence number. According to the allocation of this unique sequence number, pharmacists will hand over the Hollow Microstructured Transdermal System (hMTS) to the clinical team without revealing any details. The clinical care team will not be involved in the process.

The Materials and Methods should be described with sufficient details to allow others to replicate and build on the published results. Please note that the publication of your manuscript implicates that you must make all materials, data, computer code, and protocols associated with the publication available to readers. Please disclose at the submission stage any restrictions on the availability of materials or information. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited.

Blinding

Phase 1: an open-label study.

Phase 2: participants, the clinical care team (physician, endoscopic team, pathologist, and others), and the outcome assessor will be blinded. The study coordinator (aware of randomization) will not have access to raw clinical data. Identical cartridges with or without TAK-101 will be prepared to avoid performance bias.

Emergency unblinding should occur in extreme circumstances, with a preliminary evaluation by the research team where "risk-benefit" has been evaluated and knowledge of the treatment is essential. In extraordinary plenary, the principal investigator should use the emergency unmasking system through the hotline by calling people involved to an emergency meeting and unmasking them on the spot. The investigator must report all code breaks (with reason) as they occur in the informed consent. Unmasking should be documented to prevent a lack of credibility.

Participants

Inclusion criteria for Phase 1 and 2:

1. 18 to 65 years old patients.
2. Body mass index (18.5 - 35.0 kg/m^2).
3. CD confirmed by biopsy and serology.
4. Well-controlled CD (mild or no ongoing signs or symptoms attributable to CD).
5. Current serology: IgA tTG < 2 × upper limits of normal (ULN) and IgG DGP < 3 × ULN.
6. GFD ≥ 10 days (Phase 1) and ≥ 6 months (Phase 2).
7. Signed informed consent.
8. Agree with doing the 14-days OGC.

9. Patients who received the COVID-19 vaccine at least 30 days before inclusion.

Exclusion criteria for Phase 1 and 2:

1. < 18 years old patients.
2. Has enrolled in previous trials within 12 weeks before signing the informed consent.
3. Has ever received TAK-101.
4. Inflammatory gastrointestinal disorders or autoimmune diseases other than well-controlled thyroid disease or type 1 diabetes.
5. Prior abdominal surgery in the past six months.
6. Has known or suspected refractory CD or ulcerative jejunitis.
7. An IgE-mediated reaction and/or anaphylaxis to wheat, barley, or rye that has been clinically proven.
8. In ongoing systemic treatment (immunosuppressant or corticosteroid) or 12 weeks pre-OGC.
9. Has known or suspected chronic liver disease or is hepatitis B or C or HIV positive.
10. Any active malignancy with or without ongoing treatment.
11. Advanced heart failure (NYHA class III-IV), advanced respiratory disease.
12. History of alcoholism or drug abuse within the past 2 years.
13. Any neuropsychiatric illness affecting a patient's cognition and participation in the trial.
14. Pregnant and lactating women.
15. Confirmed COVID-19 disease in the past 6 months.

Recruitment Strategy

Health centers in Israel will be chosen based on the rate of documented diagnosed CD patients' availability to meet the estimated study sample size. Based on three Israelian H.M.O.s, 44,600 CD patients were registered until June 2020, including 17,696 CD patients in Clalit (Peleg-Gabai, 2020). To recruit patients, each site will provide a list of patients who meet the eligibility criteria. Clinicians' invitation letters for patients to enroll in the trial will be sent. Public enrollment strategies will be adopted (leaflets, pamphlets, community-based celiac disease programs, and social media advertising by physicians and medical staff members).

Study visits will be carefully planned. All the medical staff must be well informed and trained. A research assistant will be available to answer each inquiry patients have by calls. Eligible patients will be contacted, and interested patients will be scheduled for further evaluations. Eligible patients will be recruited for four months for phase 1 and 6 to 7 months for Phase 2. The follow-up time is 180 days for each phase.

Adherence

PHASE 1

In part A, to assess adherence, the investigator will measure Gliadin-Specific T-cell proliferation and Cytokine Release Markers (from doses of 4 mg/day) on days 1 and 7.

For Part B, these markers will be measured (all amounts of MN-TAK-101) on days 1 and 7. Immune Complex Detection by C1q Binding will be measured on days 1, 7, 8, 14, 38, and 60. To assess the adherence of the GFD. After MN-TAK-101 administration, clinical personnel will contact participants via telemedicine, and clinic visits will be limited to increase subjects' adherence (part A: days 7 and 14; part B: days 14 and 21, 38 and 60).

PHASE 2

Subjects will do OGC from days 15 to 28. To assess adherence, the investigator will measure serum ELISpot assays for IFNy on days 22, 29-30, and 90. Additionally, to assess adherence, subjects will fill out the Celiac Symptom Index-Modified Questionnaire for several days. Clinical personnel will conduct an adherence assessment through telemedicine on days 120, 150, 180 and clinic visits on days 15, 22, 29-30, 60, and 90.

Timeline

Information about the timeline of phase 1 and phase 2 is shown in Figure 1 and Figure 2, respectively.

Interventions

PHASE 1

In Part A patients will be divided into escalating dose cohorts according to the traditional 3+3 design with rapid dose escalation on day 1; subsequently, in part B another group of patients will be divided into repeated ascending dose cohorts (on days 1 and 8) following the accelerated titration 2+2 design (Figure 3).

PHASE 2

Patients will be randomized between MN-TAK-101 and MN-Placebo (normal saline delivered via MN). Following Kelly et al. (2021), TAK-101 can be administered on days 1 and 8; however, the dose and

administration time will be established depending on Phase 1 results. Patients will do a 14-day O.G.C. to assess the efficacy of MN-TAK-101 (Figure 4).

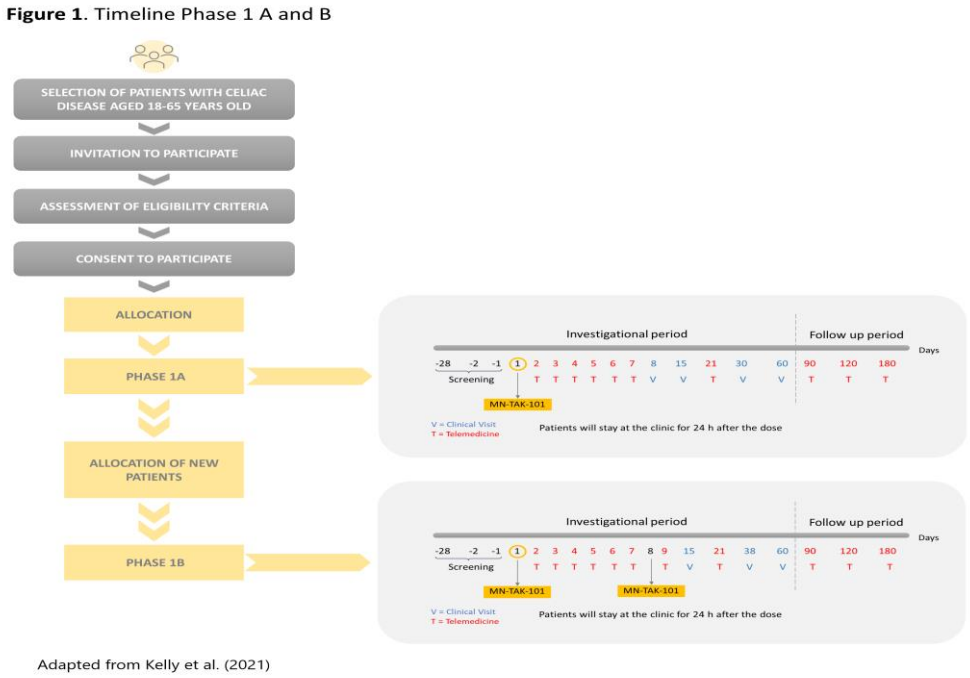


Figure 1. Timeline Phase 1 A and B

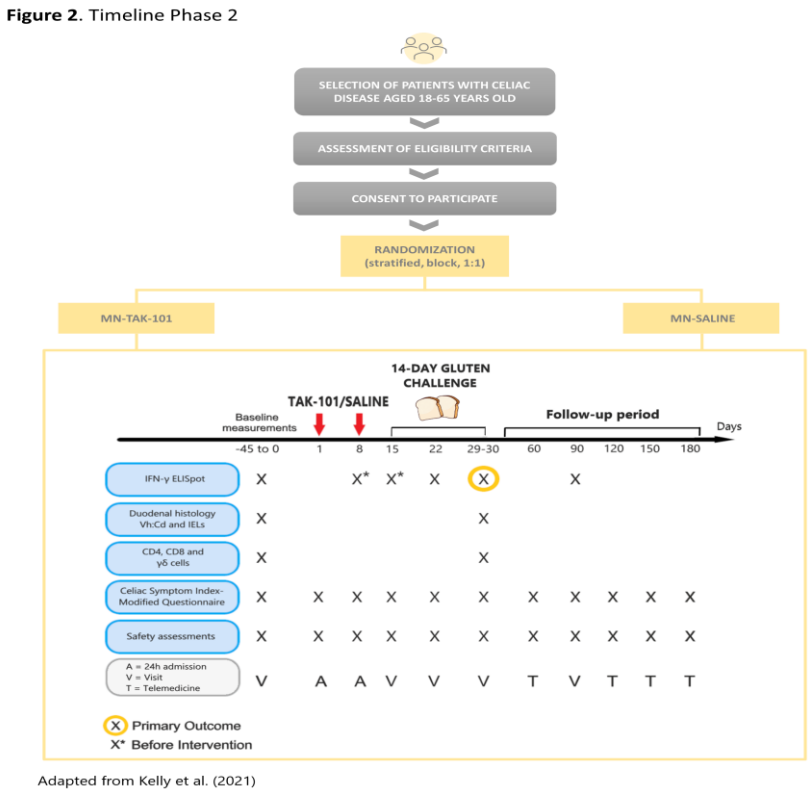
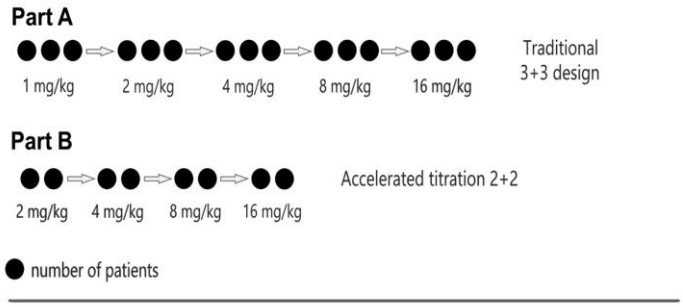


Figure 2. Timeline Phase 2

Figure 3. Dose escalation



Note

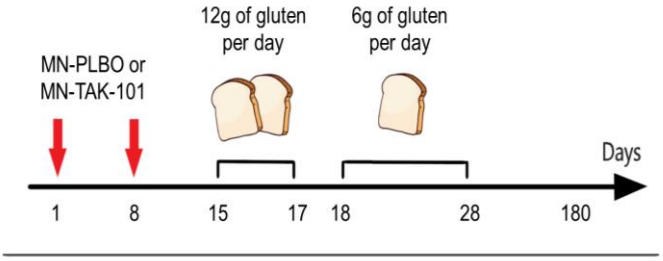
Before dosing the next subject of the same cohort, it will be required to wait for at least 168 hours.

Patients will fast approximately 2 hours before the beginning of the delivery of MN-TAK-101 and 1 hour after its end, except for water. After receiving the dose, they will stay at the clinic for 24 hours for safety control (major toxicities may emerge during this period).

Adapted from Kelly et. al (2021)

Figure 3. Dose escalation

Figure 4. Gluten challenge



Note

14-day OCG (from day 15 to day 28) to assess efficacy of MN-TAK-101:

- From day 15 to 17: consumption of 12 g of gluten per day
- From day 18 to 28: consumption of 6 g of gluten per day

Except for the OGC, the subjects must follow a gluten-free diet for the entire period of the study.

The gluten used for the OGC will be the Vital Wheat Gluten (75.2% Protein) powder by Stybel Flour Mills supplied in individual foil packets (approximately 6.37g of gluten in approximately 8.5g of powder per packet). The packets will be stored in a dry environment.

The packets will be distributed at the clinic on the first day of the OGC, and patients will be instructed on gluten consumption. They will also receive a phone number which they can call if they have any doubts.

Adapted from Kelly et al. (2021)

Figure 4. Gluten challenge

Investigational product and medical device

COUR Pharmaceuticals Development Company, Inc. will provide TAK-101.

TAK-101 is a first-in-class, non-immunosuppressive drug made comprised of gliadin extract in a negatively charged polymer matrix of poly-lactic-co-glycolic acid (-35mV to -50mV) (PLGA). Per mg of PLGA particles, there are approximately 10 µg of refined gliadin. TAK-101 comes as a lyophilized powder in a 20-mL glass vial containing approximately 1 mg refined gliadin and 100 mg PLGA particles (Kelly et al., 2021).

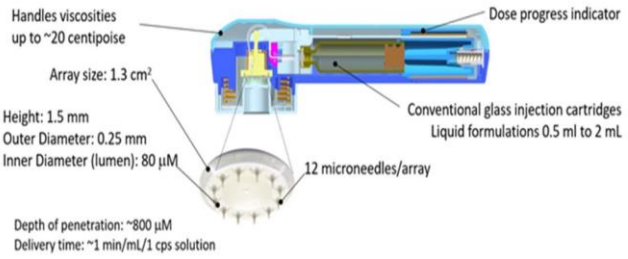
To be administered, TAK-101 will be reconstituted in sterile water (2.5 mL) and then diluted in 0.9% Sodium Chloride Injection USP. (normal saline). Rubber siliconized vials with 20 mm single-vent gray stoppers and aluminum, 20 mm, a flip-off seal will contain the obtained suspension. It will be stored in a light-protected and temperature-monitored (2°C to 8°C) environment (Kelly et al., 2021).

The dose of TAK-101 will be weight-adjusted on day 1.

The clinical team will administer the intervention via the hMTS with a force of 0.004-0.16 N (required force to penetrate the stratum corneum) (Burton et. al, 2011).

Animal studies showed that hMTS can deliver different molecules with pharmacokinetic profiles and relative bioavailabilities similar to subcutaneous injections (Burton et al. 2011). It will be manufactured and provided by 3M - Kindeva® (Figure 5).

Figure 5. Hollow Microstructured Transdermal System



Note

It is a 1 cm2 polymeric disk with 12 hollow microneedles with a sterile flow path connected to a conventional glass cartridge. A spring powers the liquid formulation into the needles (1500µm long), allowing the penetration of the stratum corneum and the epidermis by directly reaching the dermis. They can deliver up to 2mL with a delivery time that goes from 5 to 20 minutes (Burton et Al., 2011)

After filling the cartridge with TAK-101, the device has to be inserted into the thigh (the recommended area for administration). Due to the limited range of volume of hTMS (up to 2.0 mL), multiple administrations will be necessary. To shorten the time, more hMTS can be placed at once in the recommended area. Once hMTS has delivered its dose, it has to be completely substituted by a new one.

Provided by Kindeva - 3M

Figure 5. Hollow Microstructured Transdermal System

Premature discontinuation from the Investigational Product or the study is described in Table 1.

Premature Discontinuation from Study Product*	**Premature Discontinuation from Study**
Safety (AEs or clinically significant laboratory abnormalities). The subject will be followed clinically until the resolution or stabilization of the event.	Withdrawal of the consent to continue the study for reasons other than an AE.
Pregnancy	Lost to follow-up.
Severe COVID-19 infection.	Non-compliance or unwillingness to follow the procedures in this protocol.
Withdrawal of the consent to continue the study for reasons other than an AE.	Investigator's decision.
Non-compliance or unwillingness to follow the procedures in this protocol.	
Investigator's decision.	

*discontinued subjects will be followed for safety
Table 1. Premature discontinuation

Outcomes		**Screening**	**Investigational Period**							**Follow-up Period**		
		–28 to –1	**1**	**2 to 7**	**8**	**14**	**21**	**30**	**60**	**90**	**120**	**180**
Primary outcome	Adverse Events (TEAEs) and Serious Adverse Events (SAEs)	●	●	●	●	●	●	●	●	●	●	●
	Physical Examination	●			●	●		●	●			
	ECG Findings	●	●		●	●		●	●			
	SaO2 levels measured by pulse oxymetry		●									
	Vital Signs	●	●		●	●		●	●			
	Hematology, Serum Chemistry, Coagulation and Urinalysis	●	●		●	●		●	●			
	Gliadin-Specific T-cell Proliferation and Cytokine Release Markers (from doses ≥ 4.0 mg/kg)		●		●	●						
	Laboratory Abnormalities	●	●	●	●	●	●	●	●			
Secondary	Pharmacokinetic parameters: - Cmax - Clast - Tmax - Tlast - AUCinf - AUClast		●		●							

■ Clinic residency (24 hours) ■ Telephone and video calls

Table 2. Outcomes of Phase 1 Part A

TEAES and SAEs: collected from the signing of the informed consent through the final follow-up. Treatment-emergent AEs will be differentiated at the time of first dosing. Data collected during scheduled in-clinic or telephone visits and any spontaneously reported information through the AE reporting period will be documented in the subject's record. Grade 3 or higher TEAEs and drug-related AEs: AE grades will be evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Event (NCI CTCAE), version 5.0. (Grade 1 scaled as Mild; Grade 2 scaled as Moderate; Grade 3 scaled as severe or medically significant but not immediately life-threatening; Grade 4 is scaled as life-threatening consequences; Grade 5 is scaled as death related to AE). Drug-related adverse events should be considered as those adverse events which are assessed as possibly or probably related to the study treatment by the investigator.

Physical Examination Findings: The Physical Examination at Screening and day 1 (pre-dose) is a Full Physical Exam, including an assessment of general appearance, eyes, ears, nose, throat, neck (including thyroid), lymph nodes, chest (lungs)/respiratory system, cardiovascular system, abdomen (liver, spleen), skin, extremities, musculoskeletal, neurological system (including mental status) and any other assessment not categorized but deemed necessary by the investigator. The Physical Examination performed during the other days is an Abbreviated Physical Exam, including an assessment of general appearance, skin, chest (lungs)/respiratory system, cardiovascular system, abdomen (liver, spleen).Additional assessments/full Physical Examination may be conducted as clinically indicated at any time.

ECG Findings: All ECGs should be performed after the subject has been supine for at least 5 minutes. At screening (from day -28 until day -1), ECG will be implemented in triplicate measurements, 1 minute apart and within 5 minutes (pre-drug baseline). On day 1 it will be implemented as a single measurement (predose, 1h, 4h, 12h, 24h). On days 7, 14, 30, 60 it will be performed only if prior ECG was abnormal in a clinically significant way.

SaO2 levels measured by Pulse Oxymetry: It will be measured from approximately 1 hour prior to the start of study drug infusion (0 hours) through 4 hours post dose and at 1h, 2h, 4h, 8h, 12, 24h.

Vital Signs: Vital signs except body temperature should be performed after the subject has been supine for at least 5 minutes. Vital signs include body temperature (oral and/or tympanic measurement), blood pressure (SBP and DBP), pulse (beats per minute) and respiratory rate (breaths per minute). At day 1 they will be assessed at predose, every 15 minutes during infusion, 2h, 4h, 8h, 12h, 24h. During the other days they will be measured once.

Hematology, Serum Chemistry, Coagulation, and Urinalysis: Hematology: red blood cells (RBC), reticulocytes (Retic), hemoglobin (HGB), hematocrit (Hct), mean corpuscular volume (MCV), platelets (PLT), white blood cells (WBC), WBC differential (absolute, relative %), neutrophils (Neutro), monocytes (Mono), eosinophils (Eos), basophils (Baso) and lymphocytes (Lymph). Serum Chemistry: alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin (indirect), bilirubin (total (TBL)), blood urea nitrogen (BUN), BUN/creatinine ratio, calcium (Ca), carbon dioxide (CO2) or bicarbonate, chloride (Cl), cholesterol (total), creatinine (Cr), creatine kinase (CK), glucose, γ-Glutamyl transferase (GGT), globulin, calculated albumin/globulin ratio, lactate dehydrogenase (LDH), magnesium, phosphorus/Inorganic phosphate, potassium, sodium, triglycerides, total protein, uric acid, calculated creatinine clearance, human chorionic gonadotropin (hCG) and follicle-stimulating hormone (FSH) if menopause is suspected. Coagulation: prothrombin time (PT), partial thromboplastin time (PTT), international normalized ratio (INR) and additional thrombotic/vascular markers (a) such as D-dimer, vWF, ICAM-1, fibrinogen, prothrombin Fragment 1,2 and P-selectin. Urinalysis: dipstick (bilirubin, blood, glucose, ketones, leukocytes, nitrate (Nitrite), protein, urobilinogen, and pH), color, specific gravity, turbidity, urinary protein to creatinine ratio (UPCR) by random [spot] direct measurement, microscopy - if abnormalities observed on parameters above (casts, crystals, epithelial cells, leucocytes, erythrocytes, bacteria).

Gliadin-Specific T-cell Proliferation and Cytokine Release Markers (from doses ≥ 4.0 mg/kg): From day 1 pre-dose up to 144 hours post-dose on day 14.

Secondary outcomes: Assessment at day 1 predose, at multiple time points during the dose (30 min, 35 min and end of infusion) and at multiple time points post-dose (1h, 4h, 12h, 24h, 144h).

Adapted from Kelly et al. (2021)

Outcomes		Screening	Investigational Period									Follow-up Period		
		–28 to –1	1	2	3 to 7	8	9	15	21	38	60	90	120	180
Primary outcome	Adverse Events (TEAEs) and Serious Adverse Events (SAEs)	●	●	●	●	●	●	●	●	●	●	●	●	●
	Physical Examination	●	●			●		●		●	●			
	ECG Findings	●	●			●		●		●	●			
	SaO2 levels measured by pulse oxymetry		●			●								
	Vital Signs	●	●			●		●		●	●			
	Hematology, Serum Chemistry, Coagulation and Urinalysis	●	●	●		●	●	●		●	●			
	Gliadin-Specific T-cell Proliferation and Cytokine Release Markers		●			●								
	Laboratory Abnormalities	●	●	●	●	●	●	●	●	●	●			
	Immune Complex Detection by C1q Binding		●			●		●		●	●			
	C3a and SC5B-9 Levels		●	●										
Secondary	Pharmacokinetic parameters: - Cmax - Clast - Tmax - Tlast - AUCinf - AUClast		●			●		●						

Table 3. Outcomes of Phase 1 Part B

Outcomes

Since this study replicates the study by Kelly et. al (2021), the outcomes will be similar (Table 2, Table 3, and Figure 6).

PHASE 1

Primary Outcome

Safety and tolerability of MN-TAK-101 at different doses in subjects with CD. The assessments will include:

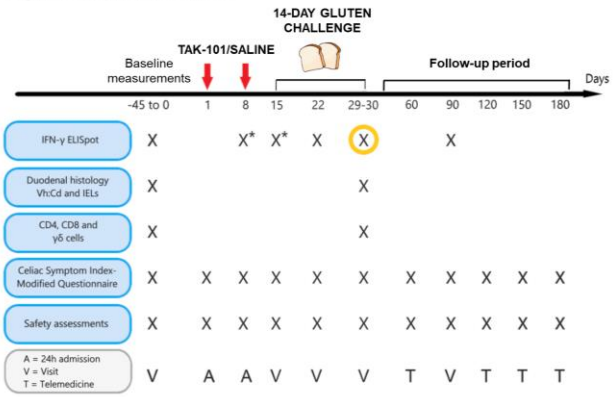
1. The number of people who experienced one or more treatment-emergent adverse events (TEAEs) or significant adverse events (SAEs).
2. The number of participants who experienced TEAEs of grade 3 or above, as well as drug-related adverse events (DRAEs).
3. The number of people who had clinically meaningful physical examination results.
4. The number of people who had clinically meaningful electrocardiogram (ECG) results.
5. By pulse oximetry, the number of participants having a clinically significant change in arterial oxygen saturation levels from baseline.
6. The number of participants who had a clinically significant change in vital sign values from baseline.
7. The number of people who had a clinically significant change in hematology, serum chemistry, coagulation, or urinalysis from baseline.
8. Participants with a clinically significant change in gliadin-specific T-cell proliferation and cytokine release indicators from baseline (from doses equal to or greater than [4.0 mg/kg] in part A; for all doses in part B).
9. The number of people who had clinically significant laboratory abnormalities was counted.
10. Immune complex detection by C1q binding (only Part B).

11. C3a and SC5B-9 levels (only part B).

Secondary Outcomes

The secondary outcomes are the measurements of TAK-101 based on the plasma nanoparticle-free gliadin concentration (Kelly et al., 2021). They will be used to determine the Pharmacokinetics parameters, which will be the following: (I) Maximal observed concentration (Cmax); (II) Last measurable concentration (Clast); (III) Time of maximal observed concentration (Tmax); (IV) Time of last measurable concentration (Tlast); (V) Area under the curve from time zero and extrapolated to infinity (AUCinf); (VI) The area under the concentration-time curve from time zero to time of the last measurable concentration (AUClast).

Figure 6. Outcomes in Phase 2



Note

IFN-γ SFUs in PBMCs using ELISpot assay: at baseline and at day 29-30 [primary outcome]; at baseline and at days 8 (pre-dose), 15 (prior to OGC), 22 and 90 [secondary outcome].
There is a positive response when the average SFU in wells with a given peptide is at least twice that of the average SFU in the no-peptide control wells.

Vh:Cd using duodenal histology: at baseline and at day 29-30.
In CD patients, Villi are often shortened, and Crypts are often elongated. Hence, a decreased Vh:Cd ratio shows a worsening disease.

IELs using duodenal histology: at baseline and at day 29-30.
The increase of intraepithelial lymphocytes is associated with CD.

CD4, CD8 and γδ cells: at baseline and at day 29-30.
Phenotype (unique cell population) for CD8 cell is EM CD8 > aE+b7hi > aE+b7hiCD38+, for CD4 is EM Th > a4+b7hi > a4+b7hiCD38+ and for Gamma Delta T-cells is TCRgd T cells > aE+b7hi > aE+b7hiCD38+.

CSI-M: at baseline and at days 1, 8, 15, 22, 29-30, 60, 90, 120, 150, 180.
It will be used to assess symptoms before, during, and after OGC. At baseline and at days 1, 8, 15 22, 29-30 and 90 patients will fill the questionnaire at the clinic since they will be there for other assessments; at days 60, 120, 150 and 180 the questionnaire will be filled through telephone calls.
The CSI-M is a clinically oriented easily administered questionnaire with 6 items. It is derived from a subset of questions from the CSI questionnaire, including diarrhea, nausea, rumbling in stomach, stomach felt bloated, diarrhea, and low energy level abdominal pain domains. Each question is assessed on a scale of 1 to 5 (none of the time, a little of the time, some of the time, most of the time and all of the time, respectively). Higher CSI scores are correlated with more severe CD symptoms.

Safety assessments:

- At Least 1 TEAE and SAE: from the first dose of the study drug up to day 180
- Vital Signs: from the first dose of the study drug up to day 180
- Hematology, Serum Chemistry Laboratory Values: from the first dose of the study drug up to day 180
- Deamidated Gliadin Peptide Immunoglobulin G (DGP-IgG) Antibodies: from baseline to days 8, 15, 22, 29-30 and 90
- Serum Complement Levels of C3a and SC5B-9: from baseline (pre-dose value on day 1) to days 2, 8 and 15
- Serum Complement Levels of C5a: from baseline (pre-dose value on day 1) to days 2, 8 and 15
- Serum Complements Levels of C1q Binding: from baseline (pre-dose value on day 1) to days 15, 22, 29-30 and 90
- Serum Cytokines (IFN-γ, IL 1-β, IL-2, IL-4, IL-6, IL-8 , IL-10, IL-12p70, and TNF-Alpha): from baseline (1 pre-dose) to days 2, 8, 15 and 90

Baseline is defined as the last sample collected before the first dose of study medication (Screening Period) on day 1.

Adapted from Kelly et. al (2021)

Figure 6. Outcomes in Phase 2
PHASE 2

Primary Outcome

Change of IFN-γ Spot Forming Units (S.F.U.s) in peripheral blood mononuclear cells (PBMCs) from baseline to day 29-30 in CD patients managed with either MN-TAK-101 or MN-PLBO and who attended a 14-day O.G.C. The number of IFN-γ S.F.U.s will be assessed using the gliadin-specific ELISpot assay.

The final sample collected before the first dosage of study medication (Screening Period) on day 1 is referred to as the baseline.

Secondary Outcomes

1. Changes of IFN-γ SFUs in PBMCs using ELISpot assay.
2. Changes in the ratio of villus height to crypt depth (Vh:Cd) and changes in the number of intestinal intraepithelial lymphocytes (IELs) using duodenal histology.
3. Changes in CD4, CD8, and γδ cells frequency.
4. Changes in the Celiac Symptom Index-Modified Questionnaire.
5. Safety assessments.
6. The number of people who have had at least one TEAE or SAE.
7. The number of participants who had a clinically significant change in vital signs from baseline.
8. The number of participants whose hematological or serum chemistry laboratory values had a clinically significant change from baseline.
9. Change in deamidated gliadin peptide immunoglobulin G (DGP-IgG) antibodies.
10. Change in C3a and SC5B-9 levels on serum complement
11. Change in serum complement levels of C5a.
12. Change in serum complements levels of C1q binding.
13. Change in serum cytokines (IFN-γ, IL 1-β, IL-2, IL-4, IL-6, IL-8 , IL-10, IL-12p70, and TNF-Alpha).

Data Management

Source documentation will consist of existing medical records and/or study records developed and maintained by the investigator. All data will be entered electronically using electronic case report forms (eCRFs) using an Electronic Data Capture (EDC) system provided and approved by the investigator. The data entry screens will resemble the paper forms approved by the Institutional Review Board (IRB). The type of activity that an individual user may undertake is regulated by the privileges associated with his/her user identification code and password. Modifications in the database will be documented through either the data

change system or an inquiry system. Additional errors will be detected by programs designed to detect missing data, missing forms, and missing visits. These errors will be summarized along with detailed descriptions for each specific problem in Data Query Reports, and a weekly email report will be sent (Chan et al., 2013).

All the subject's information will be password protected, secured, and exceptionally accessed by the study staff after permission from the IRB.

Data monitoring

An independent safety committee will be constituted to monitor the individual's safety undergoing treatment with different doses of MN-TAK-101. This committee will consist of 3 physicians with experience in CD. Physicians and all team members will answer questions raised by the Data Monitoring Committee (DMC) upon request and throughout DMC meetings.

During both part A and B of Phase 1, data locks can occur.

In Phase 1 part A, patients will be monitored in the clinic for at least 24 hours after dose and will be followed up on as outpatients. AEs, vital signs, ECGs, and laboratory information will be evaluated by the DMC before the next higher-level dose cohort begins. After finishing Part A and confirmation by the DMC to proceed, eligible subjects (n = 3) will receive two MN-TAK-101, on Day 1 and Day 8 (seven days apart). Each participant in Part B will be monitored in the clinic for 24 hours after each dose and undergo the same tests and follow-up provided in Part A. Through at least 168 hours after dosing, the DMC will be pooled at the following times to decide whether it is acceptable to continue dosing subjects: (I) When each dose cohort is finalized; (II) Before moving from part A to part B.

The DMC may meet ad hoc at any time to determine the acceptability of continued dosing and when emerging issues occur. The DMC will be responsible for evaluating available post-administration safety data, including but not limited to vital signs, A.E.s, ECGs, pulse oximetry, and available laboratory information.

Decisions that can be taken if the stopping criterion is not met: (I) 0 Dose Limiting Toxicity (DLT) out of 2 patients in the accelerated titration 2+2: move to the next cohort; (II) 1 DLT out of 2 patients in the accelerated titration 2+2: expand the cohort, enrolling two more patients at the same dose; (III) 0 DLT out of 3 patients in the 3+3 dose escalation: move to the next cohort; (IV) 1 DLT out of 3 patients in the 3+3 dose escalation: expand the cohort, enrolling three more patients at the same dose; (V) Two or more DLTs out of 3 patients in the 3+3 dose escalation: halt dose escalation and treat a total of 6 patients at the previous lower dose to determine the maximum tolerated dose.

Before providing the dose to the new participants, the investigator will be informed about the DMC decisions concerning dosing. The medical monitor must be notified in case of any SAE and any Common Terminology Criteria for Adverse Events (CTCAE) Within 24 hours of the designee (or investigator's) awareness of such an incident, grade 2 or greater toxicity.

Sample Size Calculation

Sample size calculation for Phase 1 is estimated between 23-41 subjects and will depend on the results of phases 1A and 1B.

Phase 2 sample size was calculated considering a 2-tailed test, 0.05 significance level, 90% statistical power, and previous literature data. TAK-101 trial (Kelly et al., 2021) reported an 88% effect size (mean change in IFN-γ spot forming units of 2.01 vs. 17.58 (TAK-101 vs. Placebo, P: 0.006) and had a 15% drop-out rate considering subjects that completed OGC in their trial. Standard deviation was calculated based on the standard error of the mean of that trial (SD=SEM x $\sqrt{N}$), being 25.6 in the placebo and 7.2 in the intervention group. Therefore, the estimated sample size for Phase 2 is 78 subjects, 39 individuals in each group.

Statistical analysis for primary and secondary outcomes

Descriptive statistics will be used for the efficacy and safety variables collected in Phase 1.

According to central tendency, Phase 2 descriptive continuous variables will be expressed as mean ± standard deviation or median ± interquartile range; and categorical variables will be described by frequency and proportion. All inferential statistical analyses will be conducted using a two-sided P-value.

Parametric tests will be preferred for primary and secondary outcomes. Non-normal distributed data identified by histograms will be converted to normal using log transformation, when feasible.

Phase 2 primary outcome comparing groups' mean INF-γ changes will be assessed using a t-test. In case of normality is not achieved, Mann-Whitney will be used.

Longitudinal secondary outcomes will be assessed with linear mixed models (INF-γ measured six times); comparison of histological changes will be addressed using a t-test or Mann-Whitney, according to the normality; and Fisher's exact test will assess categorical data association (CD symptoms and adverse events).

Missing Data

We will describe reasons for missing data and dropouts, for example, refusal to proceed to unpleasant procedures (biopsy, laboratory exams), absence of visits or exams, and adverse events. Missing data will be handled with intention-to-treat analysis using the multiple imputation method.

Discussion

The aim of this Phase 1/2a trial is to assess the safety and efficacy of TAK-101 delivered via microneedles in CD patients.

Phase 1 has an open-label ascending dose 2-part design (part A with the traditional 3+3 design with rapid dose escalation, followed by part B in which there will be a repeated dose escalation with the accelerated titration 2+2 design). According to the results of Phase 1, Phase 2 will be a randomized, double-blind, controlled trial where patients will receive two doses of either TAK-101 or a placebo delivered via hMTS. The assessment for efficacy will be done by measuring IFN-γ on days 29-30 after a 14-day OGC. Then, the study will continue until day 180 for other safety and efficacy assessments.

We believe that the strengths of this study are focused on MN devices. It is a new technology that was created to deliver big molecules like proteins such as TAK-101 and to relieve the pain and discomfort in the patients compared to other devices commonly used like hypodermic needles (Jamaledin et al., 2020; Kirkby et al., 2020). Another strength can be found in our study design for Phase 1: waiting seven days after giving the intervention to the subsequent patient - even in the same cohort - allows us to check for DLTs and prioritize safety for each patient carefully.

On the other hand, we know that the main limitation and controversy of Phase 1 is the choice of having CD patients instead of healthy individuals, but it is necessary to address Phase 2 and guarantee the volunteers' safety; an open-label study allows us to increase the knowledge of the safety of a new drug, especially, when there is no previous study that demonstrates adverse effects of TAK-101 delivered by microneedles (Day, 2007). CD does not have any specific treatment. Patients afflicted with this disease want or need to access certain promising experimental studies, but they are not eligible due to formalities in clinical trials. Therefore, an open-label trial will generate data on the intervention and provide patients with needed treatment. The choice of having celiac patients was supported by the assumption that the safety will not be sufficiently verified if not tested in individuals with a gluten immune response. Patients with biopsy-confirmed, asymptomatic celiac disease who are on a gluten-free diet made up the study population. This study's participants represent a group most likely to react successfully to a gluten challenge, which would not occur with healthy subjects (Kelly et al., 2021). Additionally, there is a concern that some proteins would leak from the nanoparticles and trigger symptoms of CD, again justifying the inclusion of CD patients to address this concern. Hence it is necessary to recruit CD patients to ensure safety.

However, we must emphasize that it is a new and innovative trial, which nobody has tried with MN and TAK-101 together. Both elements have been tested in other trials separately, and they are in experimental phases (Kelly et al., 2021). The last concern is the patients' adherence to the whole study process (for example, the gluten-free diet adherence, the phone calls, follow-up and center visits, the biopsy procedure, and blood tests).

Currently, there are no approved treatments for CD. Only in the last years, there has been the development of TAK-101, which has had promising results with intravenous delivery in a short-time period (Kelly et Al., 2021). Due to the increasing interest and use of MNs in the health field, this trial explores a novel way of delivery of TAK-101 via microneedle, and the results obtained from this future study can further improve the knowledge of CD and its treatment.

Trial Registration

This trial will be registered on clinicaltrials.gov, the mytrial (my.health.gov.il), an Israelian clinical trials registry. The protocol will be updated with the trial identifier(s) upon registration.

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