TAK-101 Nanoparticles Induce Gluten-Specific Tolerance in Celiac Disease: A Randomized, Double-Blind, Placebo-Controlled Study

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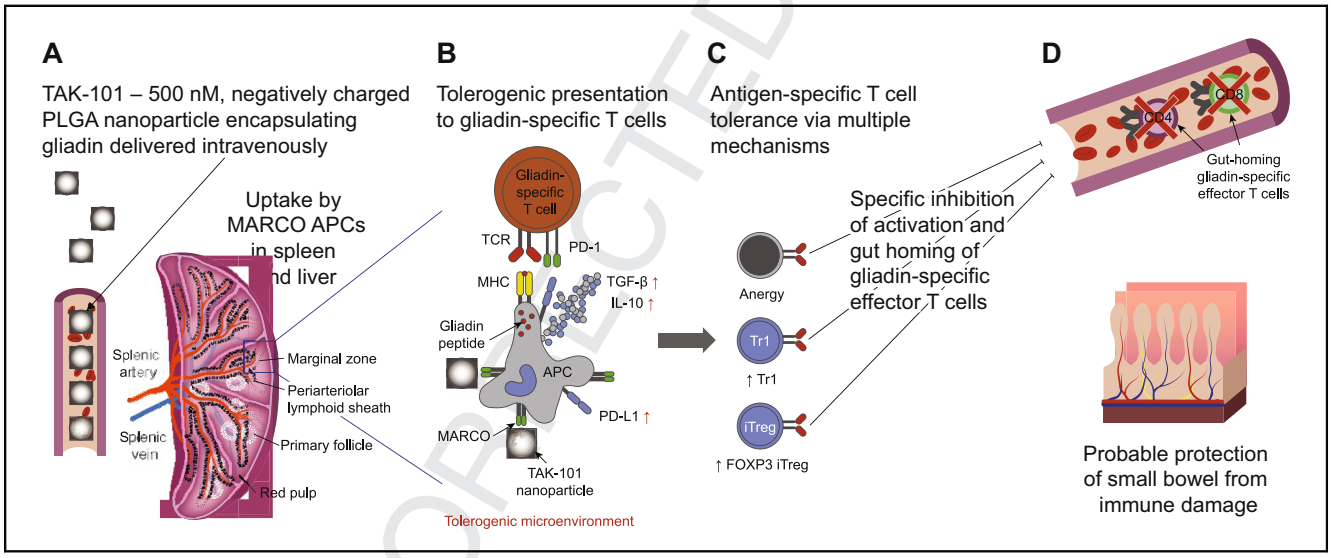

BACKGROUND & AIMS: In celiac disease (CeD), gluten induces immune activation, leading to enteropathy. TAK-101, gluten protein (gliadin) encapsulated in negatively charged poly(DL-lactide-*co*-glycolic acid) nanoparticles, is designed to induce gluten-specific tolerance. **METHODS:** TAK-101 was evaluated in phase 1 dose escalation safety and phase 2a double-blind, randomized, placebo-controlled studies. Primary endpoints included pharmacokinetics, safety, and tolerability of TAK-101 (phase 1) and change from baseline in circulating gliadin-specific interferon-γ–producing cells at day 6 of gluten challenge, in patients with CeD (phase 2a). Secondary endpoints in the phase 2a study included changes from baseline in enteropathy (villus height to crypt depth ratio [Vh:Cd]), and frequency of intestinal intraepithelial lymphocytes and peripheral gut-homing T cells. **RESULTS:** In phase 2a, 33 randomized patients completed the 14-day gluten challenge. TAK-101 induced an 88% reduction in change from baseline in interferon-γ spot-forming units vs placebo (2.01 vs 17.58, $P = .006$). Vh:Cd deteriorated in the placebo group (−0.63, $P = .002$), but not in the TAK-101 group (−0.18, $P = .110$), although the intergroup change from baseline was not significant ($P = .08$). Intraepithelial lymphocytes numbers remained equal. TAK-101 reduced changes in circulating $\alpha 4\beta 7^{+}CD4^{+}$ (0.26 vs 1.05, $P = .032$), $\alpha E\beta 7^{+}CD8^{+}$ (0.69 vs 3.64, $P = .003$), and $\gamma\delta$ (0.15 vs 1.59, $P = .010$) effector memory T cells. TAK-101 (up to 8 mg/kg) induced no clinically meaningful changes in vital signs or routine clinical laboratory evaluations. No serious adverse events occurred. **CONCLUSIONS:** TAK-101 was well tolerated and prevented gluten-induced immune activation in CeD. The findings from the present clinical trial suggest that antigen-specific tolerance was induced and represent a novel approach translatable to other immune-mediated diseases. ClinicalTrials.gov identifiers: NCT03486990 and NCT03738475.

Keywords: Gliadin; Antigen-specific Immune Tolerance.

Celiac disease (CeD) is an immune-mediated disorder triggered by gluten ingestion in individuals expressing human leukocyte antigen (HLA)-DQ2 or HLA-DQ8, resulting in damage to the small intestine.[1] CeD is one of the most common autoimmune disorders, affecting more than 1% of the global population.[2]

Ingestion of prolamin proteins, primarily gliadin, results in an abnormal T-cell response in patients with CeD. After gluten exposure, gliadin is processed and presented to $CD4^+$ T cells, initiating an inflammatory reaction characterized by $CD4^+$ T cell production of interferon (IFN)-γ and the activation of $CD8^+$ T cells and $\gamma\delta$ T cells that express the gut-homing or retention integrins $\alpha4\beta7$ or $\alpha E\beta7$.[3–5] The activated $CD8^+$ T cells increase in the small intestine epithelium with gluten exposure and are thought to mediate intestinal damage.[1,6–8]

Manifestations of CeD are diverse, and include gastrointestinal symptoms and extraintestinal disorders, including anemia, osteoporosis, neurologic disease, and dermatitis herpetiformis.[9] No medications are currently approved for the treatment of CeD, and the only available management strategy is a gluten-free diet (GFD). Diet modification alone is inadequate to achieve clinical and histologic remission in many patients owing to heterogeneous sensitivity to gluten and frequent inadvertent gluten exposure from widespread contamination of food.[10–12] In addition, the requirement for lifelong adherence to a strict GFD places a high burden on both patients and caregivers. There is therefore a critical unmet need for effective nondietary therapies for CeD.

Induction of gliadin-specific immune tolerance is a promising therapeutic solution for CeD that targets mechanisms initiating disease pathology instead of mitigating the effects of gluten exposure. Tolerogenic inhibition of specific immune responses is a highly sought-after therapeutic goal for immune-mediated diseases, including autoimmunity, allergy, and transplant rejection. Negatively charged poly(DL-lactide-*co*-glycolide) (PLGA)-antigen (Ag) nanoparticles have been developed to deliver specific antigens that induce tolerogenic inhibition via a non-inflammatory process. In rodents, PLGA-Ag nanoparticle-induced tolerance to model antigen is dependent on particle uptake via the macrophage receptor with collagenous structure (MARCO) scavenger receptor by tolerogenic antigen-presenting cells (APCs) in the splenic marginal zone and liver.[12,13] These APCs lead to anergy within Ag-specific effector T cells and activate populations of Ag-specific regulatory T cells.[13–16] This approach has been shown to be effective in mouse experimental autoimmune encephalomyelitis, murine type 1 diabetes induced by adoptive transfer of activated diabetogenic epitope-specific $CD4^+$ or $CD8^+$ transgenic T cells, and a mouse model of CeD induced by transfer of gliadin-specific T cells.[15,17–22] TAK-101 (formerly TIMP-GLIA), is composed of gliadin encapsulated in PLGA-Ag nanoparticles. We posited that TAK-101 may induce immune tolerance sufficient for the treatment of CeD.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Celiac disease is a well-characterized immune-mediated disorder incited by gliadin, a component of gluten. TAK-101, gliadin encapsulated in nanoparticles, is under development as an antigen-specific immune tolerance therapy for celiac disease.

NEW FINDINGS

TAK-101 was well tolerated in patients with celiac disease undergoing oral gluten challenge, and prevented immune activation and small intestinal mucosal injury, with no evidence of systemic immune suppression.

LIMITATIONS

These small phase 1 and 2 studies were of limited duration. Longer-term effects of TAK-101 on celiac disease symptoms were not fully evaluated.

IMPACT

TAK-101 prevented gluten-induced immune activation in CeD. This is the first trial demonstrating antigen-specific immune tolerance in autoimmune disease, which represent a novel approach translatable to other immune-mediated diseases.

Here, we present data for the safety, tolerability, pharmacokinetics (PK), and efficacy of TAK-101 from the induction of gliadin-specific T-cell tolerance in patients with biopsy-confirmed CeD in phase 1 and phase 2a studies.

Methods

Trial Design

These studies complied with the ethical principles of Good Clinical Practice in accordance with the World Medical Association Declaration of Helsinki: ethical principle for medical research involving human subjects.[23] Written informed consent was obtained from all participants, and all study procedures were performed under institutional review board approval. All authors had access to the study data and reviewed and approved the final manuscript.

Patients were aged 18 to 75 years (phase 1) or 18 to 70 years (phase 2a), were HLA-DQ2 or HLA-DQ8 positive with biopsy-confirmed CeD, and maintained a GFD for $\geq$6 months before screening, with quiescent CeD symptoms and negative serum

[*] **Authors share co-first authorship.**

Abbreviations used in this paper: **AE, adverse event; Ag, antigen; APC, antigen-presenting cell; CeD, celiac disease; CyTOF, time-of-flight mass cytometry; ELISpot, enzyme-linked immunospot; GFD, gluten-free diet; HLA, human leukocyte antigen; IEL, intraepithelial lymphocyte; IFN, interferon; IL, interleukin; MARCO, macrophage receptor with collagenous structure; PBMC, peripheral blood mononuclear cell; PK, pharmacokinetics; PLGA, poly(DL-lactide-*co*-glycolide); SFU, spot-forming unit; Th, T helper cell; Treg, regulatory T cell; Vh:Cd, villus height to crypt depth ratio.**

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antitissue transglutaminase 2 immunoglobin A. Patients were required to have no known gluten exposure for at least 10 days before screening and to be willing to maintain a GFD during the study.

Phase 1 study (NCT03486990). A first-in-human safety study was conducted from January 2018 to May 2019 (see Supplementary Material): TAK-101 was evaluated in patients with CeD in a 2-part trial consisting of a single ascending dose cohort (Part A) and a repeated ascending dose cohort (Part B; 2 doses: day 1 and 8). TAK-101 (0.1–8 mg/kg up to a maximum of 650 mg) was administered by a 30-minute intravenous infusion in accordance with the dose escalation shown in Figure 1*A*.

Phase 2a Study (NCT03738475). A randomized, double-blind, proof-of-concept study was conducted from January 2019 to July 2019 (see Supplementary Material). Patients with CeD were randomized in a 1:1 ratio (via iMedNet electronic case report forms on day 1) to pretreatment with 2 intravenous doses of placebo (normal saline) or TAK-101 (8 mg/kg, up to a maximum of 650 mg) on days 1 and 8, via a 30-minute intravenous infusion. All patients subsequently underwent a 14-day oral gluten challenge (12 g/d of gluten for 3 days followed by 6 g/d for 11 days beginning 7 days after the second infusion of TAK-101 or placebo) (Figure 1*B*). Investigators, patients, and all study staff with direct patient contact were blinded to treatment assignment. A designated unblinded pharmacist (or otherwise qualified personnel) at each site prepared each dose and had no contact with the patients and minimal contact with other site study personnel. The study protocol is registered and accessible at ClinicalTrials.gov (NCT03738475).

Study Drug

Details of TAK-101 nanoparticle synthesis and characterization are provided in the Supplementary Materials.

Endpoint Measures

Phase 1 study. The primary endpoint of the phase 1 study was an evaluation of the safety and tolerability of TAK-101 administered intravenously in patients with CeD. Secondary endpoints were the PK characteristics of TAK-101 (measured as plasma nanoparticle-free gliadin concentrations) and establishment of the dose with the best safety and tolerability profile for the phase 2a study.

Phase 2a study. The primary endpoint of the phase 2a study was change from baseline in T-cell–mediated, gluten-stimulated IFN-γ production in peripheral blood mononuclear cells (PBMCs) on day 6 of gluten challenge in patients treated with TAK-101 compared with placebo, determined using an enzyme-linked immunospot (ELISpot) assay to measure the number of gliadin-specific IFN-γ spot-forming units (SFUs).

Secondary endpoints included the following: reduction in damage to the small intestinal mucosa, measured as the change from baseline in villus height to crypt depth ratio (Vh:Cd) and change in intraepithelial lymphocyte (IEL) density using quantitative histologic assessment of duodenal biopsies and the proportion of participants who have a $\geq$0.4 decrease in Vh:Cd; the change in percentage of activated $CD4^+$, $CD8^+$, and $\gamma\delta^+$ effector memory T cells in the blood expressing either the $\alpha4\beta7$ gut-homing or $\alpha E\beta7$ gut-retention integrins, measured using time-of-flight mass cytometry (CyTOF); and gliadin-specific ex vivo T-cell proliferation and cytokine secretion after oral gluten challenge. Measurement of the proportion of other immune cell phenotypes was also conducted using CyTOF, after gluten challenge in patients treated with TAK-101 or placebo. Further secondary endpoints included PK (serum concentration of gliadin) and safety (adverse events [AEs], serious adverse events [SAEs], vital signs, changes in serum deamidated gliadin peptide immunoglobulin G levels, serum complement and cytokine levels, and hematology and serum chemistry).

ELISpot Assay

ELISpot assays for gliadin-specific T-cell–mediated IFN-γ production in PBMCs were performed to measure the increase in the amount of IFN-γ producing T cells after 6 days of gluten challenge.[24] PBMCs were collected before gluten challenge and 6 days after the start of gluten challenge. Briefly, PBMCs were isolated from heparinized whole blood using Ficoll-Paque density gradient separation (Lymphoprep; Stemcell Technologies Inc., Vancouver, Canada) and cryopreserved. Before analysis, cells were rested overnight then resuspended in complete RPMI 1640 medium containing 10% heat-inactivated human AB serum and plated at 500,000 cells per well. Cells were unstimulated (negative control), stimulated with an anti-CD3 monoclonal antibody (100,000 cells per well, positive control) or with gliadin epitope mix (12.5 μg/mL of each peptide [deamidated α-gliadin (QLQPFPQPELPYPQPQS) and deamidated ω-gliadin (PFPQPEQPFPW) peptides]) using the IFN-γ ELISpot^{pro} (Mabtech, Naka Strand, Sweden; Cat# 3420-2APW) and performed according to the manufacturer's protocol.[25–28] Peptides were acquired from JPT Peptide Technologies (Acton, Massachusetts, >95% purity). A total of 6 replicates were performed for negative controls and peptide-stimulated cells, and triplicates were completed for positive controls. SFUs were counted using an automated ELISpot reader (AID Multispot; Autoimmun Diagnostika GmbH, Strassberg, Germany). Normalized SFU values were calculated as the average SFU per million cells from unstimulated wells subtracted from the SFU per million cells for each stimulation condition and then averaged.

Vh:Cd and IEL Analysis (Histology)

Quantitative histologic methods were used to determine changes from baseline in Vh, Cd, and IEL density in duodenal biopsies taken during screening and at the end of the 14-day oral gluten challenge in patients receiving TAK-101 or placebo in the phase 2a study.[29] Histology samples were assessed centrally (4–6 biopsies per patient per endoscopy). Biopsies were reviewed by the central pathologist.

Biopsies obtained by endoscopy were taken from the distalmost part of the second part of the duodenum, or the third part of the duodenum, before and at the end of gluten challenge. Each biopsy was taken from a fold if possible. One biopsy was taken per pass and immediately placed into 10% neutral buffered formalin; it was then embedded in paraffin and oriented for Vh:Cd evaluation.[29] Biopsies were stained with hematoxylin and eosin, and evaluated independently by a senior, experienced, gastrointestinal pathologist blinded to patient identity and study visit. Recuts were performed as necessary to

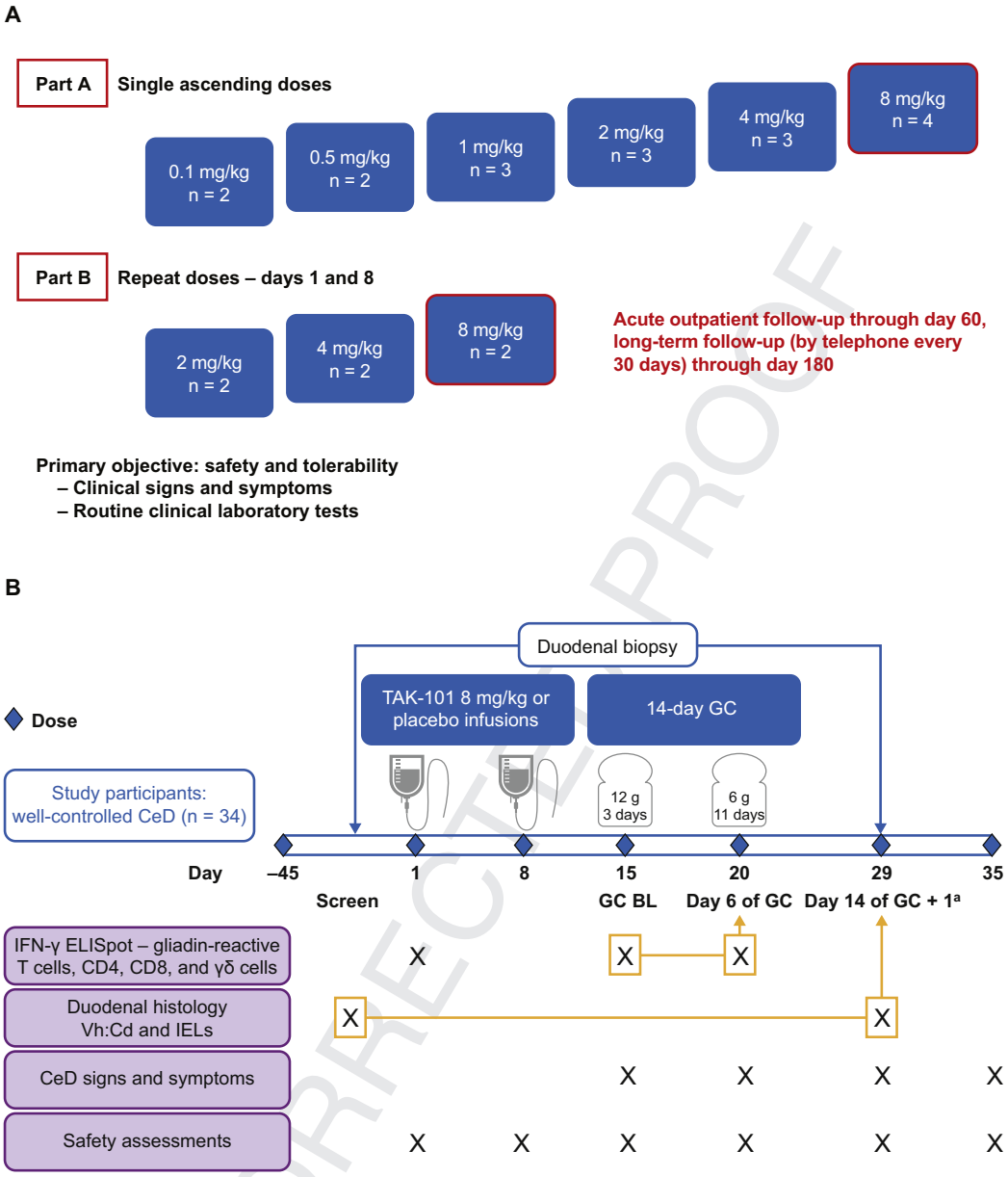

Figure 1. Phase 1 and phase 2a study designs. (*A*) Phase 1 safety study (n = 23): Part A (single ascending intravenous doses of TAK-101, n = 17) followed by Part B (2 ascending intravenous doses administered on days 1 and 8, n = 6) assessed the safety, tolerability, and pharmacokinetics of TAK-101 and established the dose to be used in the phase 2a study. (*B*) Phase 2a proof-of-concept study (n = 34) in patients infused with placebo or TAK-101, 8 mg/kg, on days 1 and 8. All patients underwent a 14-day oral GC beginning on day 15 consisting of 12 g/d for the first 3 days and 6 g/d for the following 11 days. BL, baseline; GC, gluten challenge. [a]Day 29 was equivalent to 1 day after the 14-day gluten challenge.

secure optimal orientation (defined as sections of mucosa where the entire villus and adjacent full depth of the crypt ending on the muscularis mucosa could be seen). On the optimally oriented sections, Vh:Cd was determined by measuring the mean height and mean depth of adjacent villi/proliferative crypt zones at ×100 magnification. Vh:Cds derived from at least 3 optimally oriented individual villus crypt units, derived from 4 to 6 biopsies from a single endoscopy, were averaged to produce a representative Vh:Cd for each endoscopy time point.[30] Villous lymphocyte infiltration was determined as the average number of IELs per 100 enterocytes. The IEL count was performed at ×400 magnification on the anti-CD3 immunostained slides and 100 enterocytes were counted twice.

Statistical Analysis

Descriptive statistics were used in the phase 1 study. For the phase 2a study, mean changes from baseline in the number of IFN-γ SFUs, Vh:Cd, and IEL density within and between treatment groups were compared using a Wilcoxon signed rank test and a Wilcoxon rank sum test, respectively.

Table 1. Summary of AEs and Treatment-Emergent AEs in the Phase 1 Study

Part A: single ascending dose							
	TAK-101 dose						
	0.1 mg/kg (n = 2)	0.5 mg/kg (n = 2)	1 mg/kg (n = 3)	2 mg/kg (n = 3)	4 mg/kg (n = 3)	8 mg/kg (n = 4)	All doses (n = 17)
Patients with at least 1 treatment-emergent AE, n (%)							
AE	1 (50.0)	2 (100.0)	3 (100.0)	3 (100.0)	2 (66.7)	3 (75.0)	14 (82.4)
Grade 3 (severe) AE	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.9)
Drug-related AE[a]	1 (50.0)	0 (0.0)	2 (66.7)	2 (66.7)	2 (66.7)	3 (75.0)	10 (58.8)
AE leading to withdrawal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (5.9)
Treatment-emergent AEs, n							
All AEs	4	3	10	15	7	16	55
Drug-related AEs[b]	1	0	4	4	6	13	28

Part B: repeated ascending doses				
	TAK-101 dose[b]			
	2 mg/kg (n = 2)	4 mg/kg (n = 2)	8 mg/kg (n = 2)	All doses (n = 6)
Patients with at least 1 treatment-emergent AE, n (%)				
AE	1 (50.0)	1 (50.0)	2 (100.0)	4 (66.7)
Grade 3 (severe) AE	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Drug-related AE[a]	0 (0.0)	1 (50.0)	1 (50.0)	2 (33.3)
AE leading to withdrawal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Treatment-emergent AEs, n				
All AEs	3	5	5	13
Drug-related AEs[a]	0	3	3	6

AE, adverse event.
[a]Drug-related AEs are those that the investigator assessed as being possibly or probably related to the study treatment.
[b]Patients received a single intravenous administration of TAK-101 on days 1 and 8.

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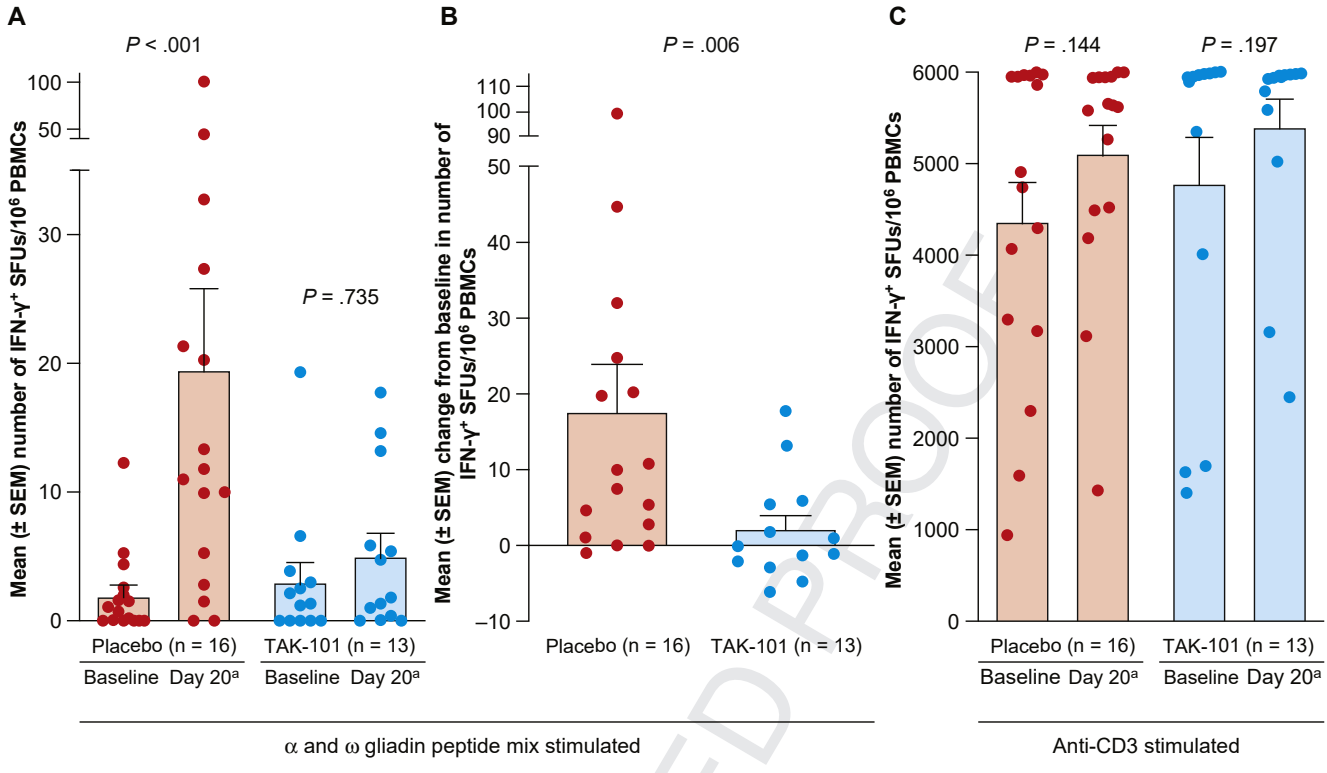

Figure 2. TAK-101 reduces gluten-specific activated T cells in peripheral blood in response to oral gluten challenge. (*A*) Number of IFN-γ+ SFUs after ex vivo stimulation of PBMCs with an HLA-DQ2-restricted deamidated α- and ω-gliadin peptide mix. (*B*) Change from baseline in the number of IFN-γ+ SFUs after ex vivo stimulation of PBMCs with a gliadin peptide mix. (*C*) Number of IFN-γ+ SFUs after ex vivo stimulation of PBMCs with anti-CD3. Induction of IFN-γ+ T cells in the peripheral blood of patients receiving placebo or TAK-101 after oral gluten challenge was examined by ELISpot assay. Patients received placebo (n = 16) or TAK-101 (n = 13) at days 1 and 8, followed by a 14-day oral gluten challenge beginning on day 15. Values for individual patients are shown as *circles*, and *bars* represent mean ± SEM. *P* values for (*A*) and (*C*) were calculated using the Wilcoxon signed rank test for the mean change from baseline within each treatment group. The *P* values for (*B*) were calculated using the Wilcoxon rank sum test for the mean change from baseline between treatment groups. [a]Day 20 was equivalent to day 6 of gluten challenge.

Methodologic details for the measurement of cell numbers by CyTOF, gliadin-specific ex vivo T-cell proliferation and cytokine secretion, PK, safety and tolerability, and full details of all statistical analyses are provided in the Supplementary Materials.

Results

Patient Disposition

Phase 1 study. Twenty-three adults with CeD were enrolled (Supplementary Figure 1). In the single ascending dose cohort (Part A, n = 17), 2 patients were enrolled for each of the first 2 TAK-101 dose levels (0.1 and 0.5 mg/kg) followed by 3 to 4 patients for each of the subsequent TAK-101 dose levels (1, 2, 4, and 8 mg/kg). In the repeated ascending dose cohort (Part B, n = 6), 2 patients were enrolled for each of the repeat doses (2, 4, or 8 mg/kg) administered 1 week apart. All patients completed the study.

Phase 2a study. Thirty-four patients with CeD were enrolled (TAK-101, n = 16; placebo, n = 18), and 33 patients (97.1%) completed the study (Supplementary Figure 2). One patient in the TAK-101 group discontinued participation owing to noncompliance with gluten consumption. Five patients (2 receiving TAK-101 and 3 receiving placebo) discontinued gluten challenge before 14 days (because of inability to tolerate the gluten) but completed all other study procedures as scheduled.

Demographic and baseline characteristics of patients in both studies are presented in Supplementary Table 1.

Endpoints

Phase 1 study: TAK-101 safety outcomes. TAK-101 was well tolerated at doses up to 8 mg/kg after single and repeated intravenous administrations 7 days apart. Mild-to-moderate flushing, headache, back pain, and fatigue were the most commonly reported AEs. One patient who received a single dose of TAK-101 1 mg/kg was reported to have an AE of nonserious "colitis." There was no documentation of any testing carried out for this AE and the patient was treated with antidiarrheals only. Symptoms resolved within 8 days and the AE was considered unrelated to the study drug. Two patients in the 8 mg/kg single-dose group experienced moderate infusion-related reactions, one of whom discontinued treatment. No patients discontinued treatment owing to

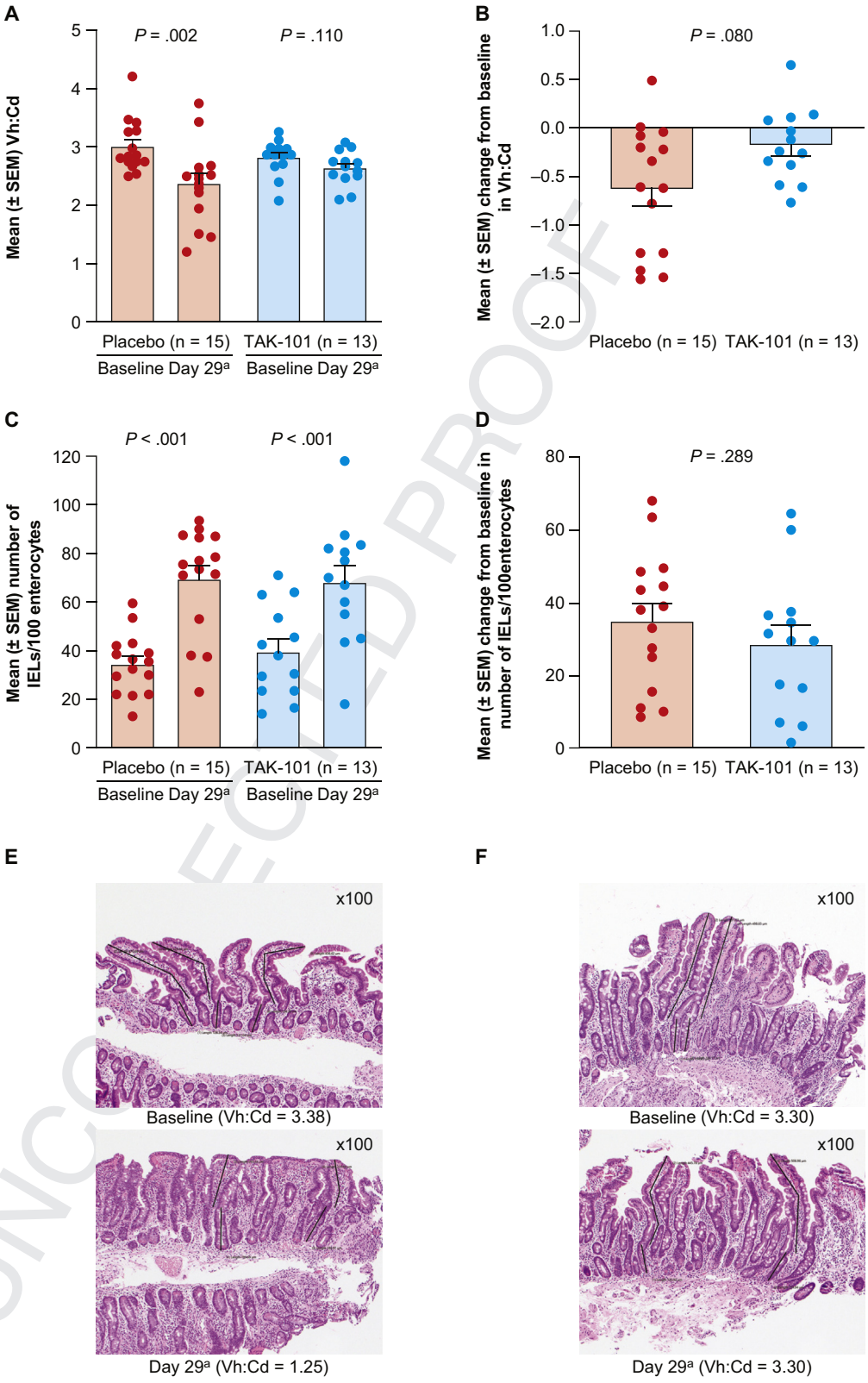

Figure 3. TAK-101 pretreatment is associated with reduced gluten challenge–induced Vh:Cd deterioration. (*A*) Vh:Cd. (*B*) Change from baseline in Vh:Cd at day 29. (*C*) Number of IELs/100 enterocytes. (*D*) Change from baseline in number of IELs/100 enterocytes at day 29. (*E*) Representative baseline and post gluten challenge biopsies from a single patient treated with placebo, showing partial villous atrophy on day 29 compared with baseline or (*F*) treated with TAK-101, showing normal villous architecture without villous atrophy on day 29. Values for individual patients are shown as *circles*, and *bars* represent mean ± SEM. The *P* values for (*A*) and (*C*) were calculated using the Wilcoxon signed rank test for the mean change from baseline within each treatment group. The *P* values for (*B*) and (*D*) were calculated using the Wilcoxon rank sum test for the mean change from baseline between treatment groups (*F*). [a]Day 29 was equivalent to 1 day after the 14-day gluten challenge.

AEs in the repeated dose group. No deaths or other SAEs were reported during the study. A summary of treatment-emergent AEs is provided in Table 1. No clinically meaningful changes from baseline were observed in hematology, coagulation, or serum chemistry parameters, vital signs, or results of physical examinations.

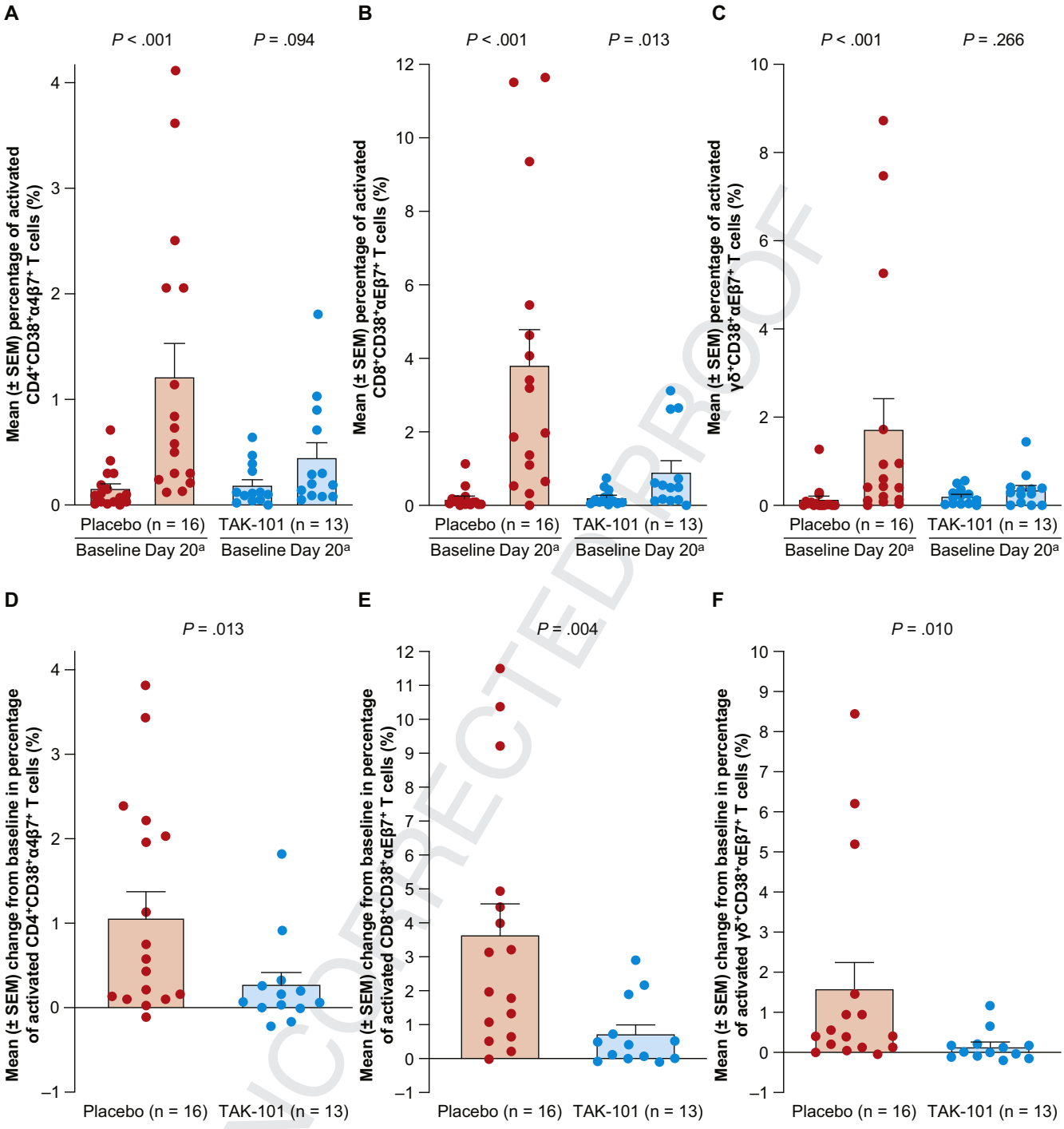

Figure 4. TAK-101 prevents the induction of activated T cells bearing gut-homing/retention integrins in response to oral gluten challenge. Percentage of activated (*A*) $CD4^+CD38^+\alpha4\beta7^+$ T cells, (*B*) $CD8^+CD38^+\alpha E\beta7^+$ T cells, and (*C*) $\gamma\delta^+CD38^+\alpha E\beta7^+$ T cells. Change from baseline in percentage of activated (*D*) $CD4^+CD38^+\alpha4\beta7^+$ T cells, (*E*) $CD8^+CD38^+\alpha E\beta7^+$ T cells, and (*F*) $\gamma\delta^+CD38^+\alpha E\beta7^+$ T cells. Percentages of activated $CD4^+$, $CD8^+$, and $\gamma\delta^+$ T cells bearing gut-homing/retention integrins ($\alpha4\beta7$ or $\alpha E\beta7$) in the peripheral blood of patients administered placebo or TAK-101, after 6 days of gluten challenge, were determined by CyTOF. Values for individual patients are shown as *circles*, and *bars* represent mean ± SEM. The *P* values for (*A–C*) were calculated using the Wilcoxon signed rank test for the mean change from baseline within each treatment group. The *P* values for (*D–F*) were calculated using the Wilcoxon rank sum test for the mean change from baseline between treatment groups. [a]Day 20 was equivalent to day 6 of gluten challenge.

Phase 1 study: TAK-101 PK outcomes. Gliadin exposure peaked at the end of each TAK-101 infusion (highest mean ± SD maximum drug serum concentration was 938 ± 26.9 ng/mL, observed in the 8 mg/kg repeat dose cohort) then rapidly declined over the next 4 hours (mean terminal elimination half-life 2.00–4.85 hours across

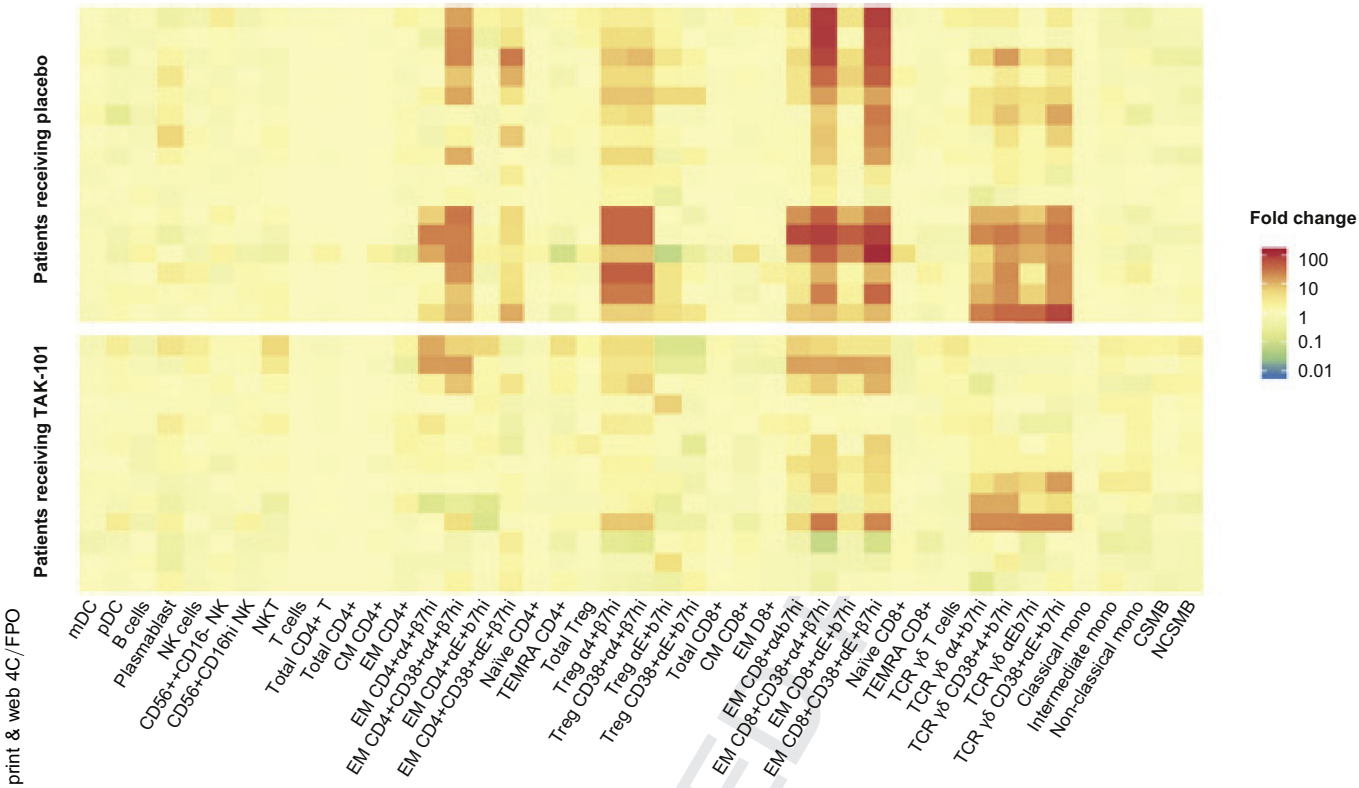

Figure 5. Fold-change from baseline in number of immune cell types in peripheral blood in individual patients treated with TAK-101 (n = 13) or placebo (n = 16) post gluten challenge, determined via CyTOF analysis. CM, central memory (T cells); CSMB, class-switched memory B cells; EM, effector memory (T cells); mDC, myeloid dendritic cells; NCSMB, non–class-switched memory B cells; NK, natural killer cells; NKT, natural killer T cells; pDC, plasmacytoid dendritic cells; TCR, T-cell receptor; TEMRA, terminally differentiated effector memory cells reexpressing CD45RA.

all dose levels in the single and repeated dose cohorts) (Supplementary Table 2). Plasma gliadin concentrations increased with rising doses of TAK-101, and similar PK parameters were observed after single and repeated TAK-101 doses. No accumulation of TAK-101 was observed from day 1 to day 8 in the repeated dose cohort. A maximum feasible dose of 8 mg/kg was determined for TAK-101 administration in the phase 2a study.

Phase 2a study: TAK-101 efficacy outcomes. In the placebo group, ex vivo gliadin peptide stimulation of PBMCs after 6 days of oral gluten challenge resulted in an approximately 10-fold increase over baseline in mean IFN-γ^+ SFUs/10^6 PBMCs (baseline mean IFN-γ+ SFUs, 1.98; day 20 mean IFN-γ+ SFUs, 19.56; $P = < .001$, Figure 2*A*). In the TAK-101 group, the gluten challenge–induced gliadin-dependent T-cell response was reduced by nearly 90% (baseline mean IFN-γ+ SFUs, 3.08; day 20 mean IFN-γ+ SFUs, 5.09; $P = .735$; Figure 2*A*). The mean change from baseline was 17.58 SFUs for placebo vs 2.01 SFUs for TAK-101 ($P = .006$; Figure 2*B*). In contrast, the number of IFN-γ+ SFUs was equivalent in the 2 treatment groups on stimulation of PBMCs with anti-CD3 (Figure 2*C*), indicating that TAK-101 acts in an antigen-specific manner.

Vh:Cd decreased from baseline in the placebo group after oral gluten challenge (mean change from baseline to day 29, −0.63; $P = .002$), consistent with gluten-induced mucosal inflammation and villous atrophy. Vh:Cd was not significantly decreased in the TAK-101 treatment group (mean change from baseline to day 29, −0.18; $P = .110$; Figure 3*A*). However, comparison of the mean change between the 2 groups (Figure 3*B*) did not reach statistical significance ($P = .080$) (see Supplementary Figure 3 for change in Vh:Cd in individual patients). A decrease in Vh:Cd of at least 0.4 was measured in 8 (53.3%) of 15 patients in the placebo group and 3 (23.1%) of 13 patients treated with TAK-101, but this difference between the groups was not significant ($P = .1367$).

The density of IELs increased in response to gluten challenge in patients given either placebo or TAK-101 ($P < .001$, Figure 3*C*), with no differences in change from baseline found between the 2 treatment groups ($P = .289$; Figure 3*D*).

Representative histopathologic sections from placebo- and TAK-101–treated patients are shown in Figure 3*E* and Figure 3*F*, respectively. These photomicrographs show flattening of the villi and reduction in crypt depth in a patient with CeD from the placebo treatment group, in response to the 14-day oral gluten challenge (Figure 3*E*), compared with a patient in the TAK-101 treatment group (Figure 3*F*).

The proportion of activated (CD38$^+$) CD4$^+$, CD8$^+$, and $\gamma\delta^+$ effector memory T cells was increased 6 days after oral

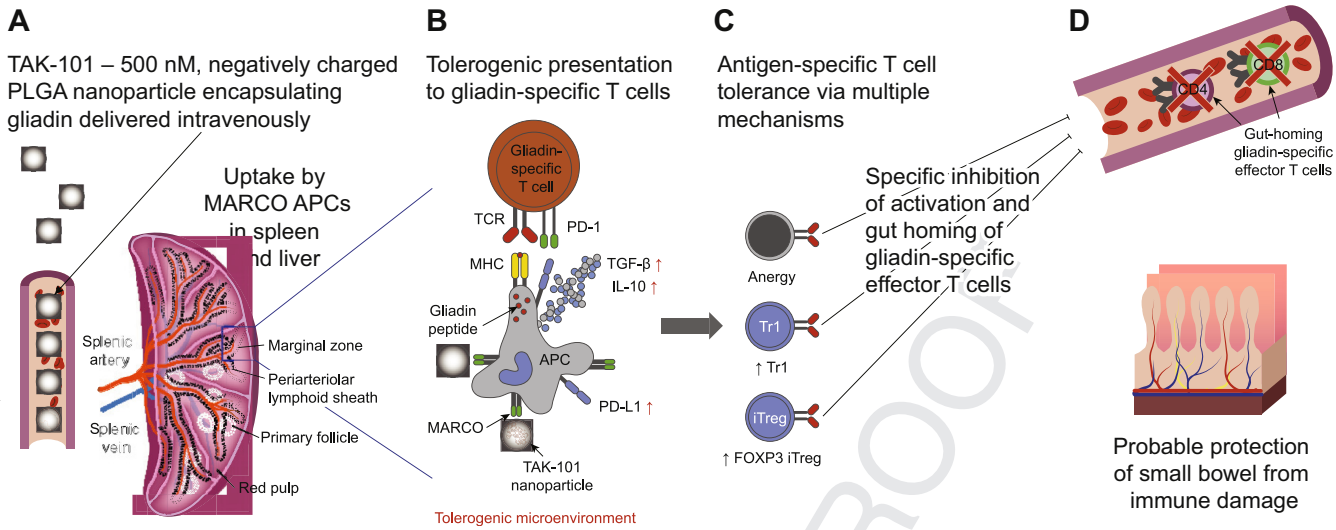

Figure 6. Proposed mechanism of action of TAK-101 based on preclinical animal model and clinical studies. (*A*) Intravenously administered gliadin-encapsulating PLGA nanoparticles are taken up by tolerogenic APCs in the liver and splenic marginal zone expressing the MARCO scavenger receptor. (*B*) PLGA particle uptake by APCs induces the upregulation of PD-L1, the release of TGF-β and IL-10, and the processing and presentation of gliadin T-cell epitopes to gliadin epitope-specific T cells. (*C*) Tolerance is induced and maintained by multiple mechanisms, including T-cell anergy, and the activation of both induced FOXP3$^+$ Tregs (iT-regs) and IL10-producing Tr1 cells. (*D*) Effective tolerance induction results in the inhibition of activation of and trafficking of gliadin-specific IFN-γ–producing T helper 1 effector cells to the small bowel, protecting the gut from immune-mediated damage. FOXP3, forkhead box P3; IL, MHC, major histocompatibility complex; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PLGA, poly(DL-lactide-*co*-glycolic acid); TCR, T-cell receptor; TGF, transforming growth factor; Tr1, type 1 regulatory T cell.

gluten challenge in the peripheral blood of patients who received placebo, but not in patients treated with TAK-101 ($P = .002$, $P < .001$, and $P < 0.001$, respectively) (Figure 4*A*–*C*). Gluten-induced increases from baseline in the proportion of CD4$^+$CD38$^+\alpha4\beta7^+$ (Figure 4*D*), CD8$^+$CD38$^+\alpha E\beta7^+$ (Figure 4*E*), and $\gamma\delta^+$CD38$^+\alpha E\beta7^+$ (Figure 4*F*). T cells were also diminished in patients given TAK-101 compared with those given placebo ($P = .013$, $P = .004$, and $P = .010$, respectively). The relative fold-change in percentages of other immune cell phenotypes, after gluten challenge in individual patients, in the placebo and TAK-101 treatment cohorts, is shown as a heat map in Figure 5. In addition to the cell types that have been previously described as sensitive to gluten challenge,[5] we also noted that gut-homing T cells with a reported regulatory phenotype (CD4$^+$, CD25$^+$, CD127$^-$)[31] were also increased with gluten challenge and suppressed by TAK-101. Dendritic cells, B lineage cells, natural killer cells, monocytes, and most T-cell subtypes, other than those T cells that expressed a4b7 or aEb7, did not change with gluten challenge.

Phase 2a study: TAK-101 safety outcomes. The most common AEs reported during dosing in the phase 2a study were gluten-related gastrointestinal disorders, and included abdominal distention or pain, diarrhea, flatulence, nausea, vomiting, and abnormal gastrointestinal sounds. All AEs were mild or moderate in intensity except gastrointestinal disorders in 1 patient in the TAK-101 group and 2 patients in the placebo group, which were severe (grade 3). No patients experienced an AE with severity of grade 4 or above and no deaths or other serious AEs were reported (Supplementary Table 3).

No effect of TAK-101 was observed on ex vivo T-cell proliferation and no clinically meaningful changes in vital signs, routine clinical laboratory test results, or serum cytokine/chemokine levels occurred.

Further safety and PK results are reported in the Supplementary Materials.

Discussion

Immunologic tolerance is a state of antigen-specific nonresponsiveness to foreign or self-antigens mediated by clonal deletion, clonal anergy, and/or the activity of regulatory T-cell (Treg) subsets.[32] In conventional autoimmune diseases, self-tolerance is broken by a variety of mechanisms, including molecular mimicry and bystander activation, leading to self-tissue damage.[33] In CeD, oral tolerance to gluten is broken by unknown mechanisms, resulting in activation of gliadin-specific IFN-γ- and interleukin (IL)21-producing CD4$^+$ T cells, and ultimately activated cytotoxic CD8$^+$ T cells.[3–5] These cells trigger inflammation in the small bowel, leading to downstream activation of harmful (auto)immune responses, causing further mononuclear cell activation, villous atrophy, and crypt hyperplasia.[34–37]

Reestablishment of immunologic tolerance is a therapeutic aim for T-helper (Th) cell 1/Th17-mediated autoimmune diseases, Th2-directed antibody-mediated allergic diseases, and CD8-mediated transplant rejection. Currently, none of the attempts to induce tolerance via parenteral administration of soluble antigens, peptides, or altered peptide ligands have led to the development of an approved therapy for reestablishing immunologic tolerance in

autoimmune diseases.[32,38,39] It has been demonstrated that intravenous administration of protein/peptide antigens delivered by carboxylated PLGA (PLGA-Ag) nanoparticles is an effective method for inducing antigen-specific tolerance in mouse models of autoimmune[13–17,20–22] and allergic diseases.[40] We chose to initiate clinical testing of the PLGA-Ag tolerance-inducing platform in CeD for multiple reasons, including (1) gliadin-specific T-cell responses to specific HLA-defined peptide epitopes are the known upstream driver of the CeD disease process[1,41]; (2) Good Manufacturing Practice-grade gliadin can be produced for clinical testing; (3) well-characterized assays are available to measure gliadin-specific T-cell and antibody responses[27,28]; (4) performance of gluten challenges in a CeD clinical trial is feasible and results in reliable immune activation[42]; and (5) intestinal biopsies have been developed as the standard methodology used to evaluate intestinal damage and inflammation.[43] Direct support for the current clinical study came from findings in a mouse model of CeD induced by the transfer of activated gliadin-specific T cells to C57BL/6-*Rag*1 recipient mice.[18] In this study, intravenous infusion of gliadin-encapsulating PLGA-Ag nanoparticles inhibited the proliferation of gliadin-stimulated T cells and their secretion of IFN-γ and IL17, increased FOXP3 (forkhead box P3) expression by regulatory T cells, decreased antigliadin antibody production, and prevented weight loss and gliadin-induced gut histopathology.[18]

We evaluated the potential of TAK-101, gliadin encapsulated in PLGA-Ag nanoparticles, to induce immune tolerance in patients with biopsy-confirmed CeD. We chose to encapsulate cGMP-grade gliadin protein extract within PLGA nanoparticles instead of specific gliadin peptides that comprise known immunodominant epitopes. Using the intact gliadin protein extract ensured that all immunodominant gliadin epitopes were encapsulated at equimolar concentrations. Furthermore, although there are immunodominant peptides, especially in HLA-DQ2.5 individuals, there is known to be a range of immunoreactive peptides that differ between individuals. Our goal is to develop a therapy that has the potential to work for all patients with CeD, regardless of HLA type. For this reason, we chose to induce tolerance to gliadin protein extract containing a broad range of epitopes, as prior animal model data suggest that this would be effective at the protein loads achievable.[20,21] The presence of deamidated gliadin was confirmed by mass spectrometry to ensure sufficient activity of the gliadin extract.

In our phase 1 and 2a studies, intravenous administration of up to 2 doses of TAK-101 8 mg/kg in patients with CeD on a GFD was well tolerated with an acceptable safety profile. An AE of nonserious "colitis" (with no confirmation by colonoscopy recorded) was reported for 1 patient who received a single dose of TAK-101 1 mg/kg, which began on day 20 post therapy and resolved on day 28. This AE was not observed with higher doses of TAK-101 and it seems unlikely that the event was due to study drug administration or immunosuppression (no antibiotics were administered). No accumulation of TAK-101 was observed from day 1 to day 8 in the phase 1 repeated dose cohort.

In the phase 2a study, the primary efficacy endpoint of reduction in the number of circulating gliadin-specific IFN-γ spot-forming T cells in response to oral gluten challenge, after treatment with TAK-101, was met. It is notable that the degree of downregulation of the gluten challenge–induced gliadin-specific T-cell response was antigen specific with no apparent effect on the overall T-cell responses to mitogenic anti-CD3 T-cell stimulation in patients treated with TAK-101. Furthermore, pretreatment with TAK-101 led to a reduction in the proportion of circulating activated ($CD38^+$) $CD4^+$, $CD8^+$, and $\gamma\delta^+$ T cells bearing gut-homing/retention integrins ($\alpha4\beta7$ or $\alpha E\beta7$) characteristic of CeD-induced intestinal inflammation, while not affecting other PBMC cell populations within the blood (Figure 5). The reliability of reduced IFN-γ–producing gliadin-specific cells as a marker for protection has not been confirmed, and further studies are required to fully evaluate the effect of TAK-101 pretreatment on symptoms in patients with CeD.[44] Future studies will also include IL2 measurements after gluten exposure, because secretion of this cytokine in the hours after single-dose gluten challenge has been shown to be a marker of immune response to gluten in humans.[44]

TAK-101 pretreatment was also associated with a reduction in gluten challenge–induced small intestinal mucosa deterioration (as measured by Vh:Cd). Although there was a significant deterioration in Vh:Cd in the placebo group, a significant change in Vh:Cd was not observed with TAK-101 pretreatment. However, the difference in change of Vh:Cd from baseline between the 2 groups did not reach statistical significance, likely owing to the reduced power of this comparison, which is a ratio of a ratio, in comparison with the analysis of change from baseline within each group. Despite the observed reduction in enteropathy with TAK-101 pretreatment, an equivalent increase in the density of IELs after gluten challenge was observed in both placebo- and TAK-101–treated patients, which is interesting considering the reduced number of gut-homing activated $CD8^+$ T cells seen in the peripheral blood. One hypothesis is that the increase in IEL density in the TAK-101 group may reflect an increased ratio of functional regulatory to effector T-cell subsets in patients receiving TAK-101 vs placebo, in light of the reduced enteropathy in those treated with TAK-101 (regulatory to effector T-cell ratios will be determined in both blood and biopsy samples in future studies). This phenomenon has been observed in animal models including an increased Treg to effector T-cell ratio in the pancreas of nonobese diabetic mice protected from type 1 diabetes by tolerization with PLGA-Ag nanoparticles encapsulating a diabetogenic pancreatic cell autoepitope.[17]

Contrary to the current results demonstrating the successful induction of gluten-specific tolerance in patients with celiac disease (using the intravenous infusion of PLGA nanoparticles encapsulating intact gliadin), a recently terminated trial showed that the intradermal injection of soluble gliadin peptides (Nexvax[44]) did not appear to successfully induce a clinically relevant degree of tolerance. There are 2 major differences between the approach to tolerance induction using Nexvax and the approach used in our study: (1) the use of soluble gliadin peptides vs the

delivery of full-length gliadin encapsulated in pro-tolerogenic PLGA nanoparticles, and (2) intradermal vs intravenous routes of antigen delivery. Our approach allows for the presentation of all possible gliadin CD4 and CD8 epitopes after uptake and processing of the nanoparticles by tolerogenic APCs. Furthermore, peripheral vaccination is expected to induce a Th1-cell response, whereas the environment of the spleen and liver is known to be immunosuppressive when antigens or even plain nanoparticles are engulfed by splenic/liver (myeloid) APCs or liver sinusoidal endothelial cells. Hence, intradermal or subcutaneous delivery of gliadin peptides is expected to target immunogenic APCs in the skin and draining lymph nodes, whereas intravenous delivery is expected to target tolerogenic APCs in the spleen and liver. We speculate that if intradermal or subcutaneous injected peptides are found in the blood, the presentation by immunogenic APCs tips the balance toward activation rather than regulation.

A priori, there is no reason why an appropriate mixture of immunodominant gliadin peptides should not induce tolerance, as we have shown that PLGA-Ag nanoparticles encapsulating a cocktail of 4 encephalitogenic myelin peptide epitopes could induce tolerance for the prevention and treatment of disease in separate groups of mice in which experimental allergic encephalomyelitis was induced by each of the individual myelin peptides or by the peptide mixture (manuscript in preparation). In addition, our previous trial in patients with early relapsing–remitting multiple sclerosis, infused intravenously with autologous apoptotic PBMCs coupled with a cocktail of 7 myelin peptides, showed successful induction of tolerance in T cells specific for 4 of the 7 autoepitopes.[45] Given these data, it is likely that the major reason for our ability to successfully induce gliadin-specific tolerance is that intravenous infusion of gliadin-encapsulating carboxylated PLGA nanoparticles effectively delivers the antigen to MARCO-expressing "pro-tolerogenic" APCs in the splenic marginal zone and spleen. These same APCs have evolved to clear apoptotic debris from the hematopoietic system to aid in maintaining self-tolerance.[13] In contrast, intradermal injection of soluble peptides leads to antigen uptake and presentation by "proimmunogenic" APCs in the dermis and draining lymph nodes that have evolved to activate T cells for protection against infectious agents.

We hypothesize that the apparent efficacy of TAK-101 in patients with CeD can be explained by preclinical studies elucidating the mechanisms by which PLGA-Ag nanoparticles induce tolerance.[13,15,16,46] Although we cannot directly assess the tissue immune effects of therapy in humans, results from previous animal model studies suggest that after intravenous infusion, TAK-101 may be taken up predominantly by pro-tolerogenic APCs in the splenic marginal zone and liver expressing the MARCO scavenger receptor that binds polyanionic surfaces. This may lead to processing and HLA-DQ2–restricted presentation of dominant gliadin epitopes by host tolerogenic APCs, upregulation of programmed death-ligand 1 co-inhibitory molecules, and release of IL10 and transforming growth factor β. This could provoke anergy induction in gliadin-specific T cells and activation of gliadin-specific Tregs, which are critical for PLGA-Ag–tolerance induction and maintenance by inhibiting T-cell activation and controlling T-cell trafficking (Figure 6). In contrast, it is believed that intradermal injection of soluble peptides leads to presentation by APCs in the draining lymph nodes, which express high levels of costimulation molecules and are pro-immunogenic and inefficient at inducing anergy and Treg activation.[17]

PLGA-Ag nanoparticles are believed to target APCs responsible for the daily scavenger receptor-dependent uptake and disposal of vast numbers of hematopoietic cells while maintaining self-tolerance.[47] Thus, we propose that carboxylated PLGA-Ag nanoparticles serve as surrogates for apoptotic debris, triggering synergistic tolerance mechanisms that evolved to deal with disposal after normal apoptotic cell death, while avoiding immune activation. The major advantage of this tolerance system is that by varying the antigen(s) encapsulated within the "universal" carboxylated PLGA nanoparticle, it is theoretically possible to treat any immune-mediated disease when the targeted (auto) antigens are known.

This phase 2a trial had limitations, including the small number of patients tested. Although the inclusion criteria covered both HLA-DQ2 and HLA-DQ2/DQ8 individuals (which allowed for measurement of IFN-γ production via ELISpot assay after stimulation with the 5 HLA-DQ2 restricted epitopes), there were no HLA-DQ8$^+$ individuals in the treatment group, owing to the low frequency of this genotype. Larger studies are therefore required to confirm our results in a wider spectrum of celiac-permissible HLA types. Some individuals discontinued gluten consumption earlier than planned. However, even with these limitations, the preliminary evidence indicates TAK-101 may reduce small bowel enteropathy. We were unable to assess whether the protective effects of TAK-101 translated into a reduction in symptoms, as this study design, with weekly patient-reported outcomes, did not allow adequate granularity to assess symptom differences between groups. Furthermore, as gluten challenge is associated with a strong nocebo effect and study enrollment may select for individuals with less severe symptoms on gluten exposure, gluten challenge studies are felt to be suboptimal for assessment of symptoms. The effect of treatment on symptoms in CeD will be rigorously assessed in future studies. The gluten challenge dose used in future studies will likely also be reduced to be in line with real-world gluten consumption levels. The high gluten doses used this current study were necessary for reliable ELISpot responses (our chosen primary endpoint based on the mechanism of action of TAK-101). In addition, the durability of TAK-101–induced tolerance in humans is unknown at this time. This raises important questions about TAK-101 therapy, such as whether there is a requirement for repeated doses to maintain tolerance, the number and frequency of doses required, and the ability of patients with CeD to resume ingestion of gluten-containing foods, which may reinforce TAK-101–induced activation of gliadin-specific regulatory T cells. Finally, TAK-101–induced tolerance targets T cells, and it is not known how long it takes for preexisting antigliadin and antitissue transglutaminase

antibodies to abate in the absence of T-cell help, which may be required for optimal clinical effect. These questions will be explored in larger subsequent clinical trials.

In conclusion, TAK-101 demonstrated a favorable safety profile and efficacy in patients with CeD through inhibition of T-cell activation and possible reduction in the deterioration of Vh:Cd following gluten challenge. These findings support further clinical development of this novel immunotherapy for CeD and other antigen-specific immune diseases.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2021.03.014.

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The data sets, including the redacted study protocol, redacted statistical analysis plan, and individual participants' data supporting the results reported in this article, will be made available within 3 months from initial request, to researchers who provide a methodologically sound proposal. The data will be provided after de-identification, in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization.

CRediT Authorship Contributions
Ciarán P. Kelly, MD (Conceptualization: Equal; Data curation: Equal; Formal analysis: Equal; Writing – review & editing: Equal).

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Daniel R. Getts, MD (Conceptualization: Equal; Formal analysis: Equal; Methodology: Equal; Writing – review & editing: Equal).

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Joseph R. Podojil, PhD (Conceptualization: Equal; Formal analysis: Equal; Methodology: Equal; Writing – review & editing: Equal).

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Conflict of interest
These authors disclose the following: Ciarán P. Kelly reports receiving advisory fees from Anokion, COUR Pharmaceuticals Development Co, Glutenostics, Immunogenx, Innovate, Janssen, Merck & Co, Milky Way, and Takeda Pharmaceuticals International Co; holding stock options in COUR Pharmaceuticals Development Co. and Glutenostics; and being a principal investigator on research grants from Aptalis, Merck & Co, and the National Institutes of Health (NIH). Joseph A. Murray reports receiving scientific advisory fees from Chugai Pharma, Janssen, Amgen, Bioniz, Intrexon, Dr Schar, and Inova Diagnostics; being an investigator on research grants from NIH, Immunogenix, Allakos, Takeda Pharmaceuticals International Co, Kanyos, COUR Pharmaceuticals Development Co, Innovate, Provention Bio, and ImmunosanT; and receiving royalties from Torax Medical and Evelo. Daniel A. Leffler reports being a full-time employee of and owning stock options in Takeda Pharmaceuticals International Co. Daniel R. Getts reports

being a co-founder and owning stock in COUR Pharmaceuticals Development Co, being a co-founder and Chief Executive Officer of Myeloid Therapeutics, and receiving advisory fees from Takeda Pharmaceuticals International Co. Glennda Smithson reports being a full-time employee of and owning stock in Takeda Pharmaceuticals International Co. M. Roy First reports receiving consulting fees from COUR Pharmaceuticals Development Co. Amy Morris was a full-time employee of COUR Pharmaceuticals Development Co. at the time of this research. Michael Boyne, Adam Elhofy, and Joseph R. Podojil report being full-time employees of and holding stock options in COUR Pharmaceuticals Development Co. Stephen D. Miller reports being a co-founder, a member of the Scientific Advisory Board, and a grantee of and holds stock options in COUR Pharmaceutical Development Co.; being a paid consultant for COUR Pharmaceuticals Development Co. and Takeda Pharmaceuticals International Co; being a paid consultant and member of the Scientific Advisory Board of NextCure Inc; being a paid consultant for Kite Pharmaceuticals; and being a paid consultant for and member of the Scientific Advisory Board of Myeloid Therapeutics. The remaining authors disclose no conflicts.

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Supplementary Material

List of Study Investigators and Sites

NCT03486990: A phase 1 first-in-human, 2-part, multicenter dose-escalation and repeat-dose study of the safety, tolerability, and pharmacokinetics of timp-glia in subjects with celiac disease

Date of first patient, first visit: 23 January 2018. Date of last patient, last visit: 24 May 2019.

Investigator	Site name
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Mark Matson, MD	Prism Clinical Research Saint Paul, Minnesota
Joseph A. Murray, MD	Mayo Gastroenterology Research Unit Rochester, Minnesota

NCT03738475: ***A randomized, double-blind, placebo-controlled study of the safety, pharmacodynamics, efficacy, and pharmacokinetics of TIMP-GLIA in subjects with well-controlled celiac disease undergoing oral gluten challenge (phase 2a)***

Date of first patient, first visit: 04 January 2019. Date of last patient, last visit: 22 July 2019

Investigator	Site name
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The above table lists only those investigators/sites who enrolled patients.

Supplementary Methods

TAK-101 Nanoparticle Synthesis and Characterization

Poly(DL-lactide-*co*-glycolic acid) (PLGA) solution was mixed with custom manufactured cGMP-grade gliadin protein extract (Sigma-Aldrich, St. Louis, Missouri) to generate a water-in-oil emulsion. This was mixed with surfactants to form an oil-in-water secondary emulsion. The solvent was removed by evaporation, yielding PLGA nanoparticles encapsulating gliadin, which were washed, filtered, and concentrated via tangential flow filtration. TAK-101 was free from surface protein as measured by flow cytometry. TAK-101 was supplied as a lyophilized powder containing approximately 100 mg of PLGA nanoparticles per vial (batch no. B17060055), with an average particle diameter of 489 nm and a zeta potential of −45 mV. The protein content was 8.4 μg of deamidated and native gliadin per milligram of PLGA. Release of encapsulated protein on TAK-101 reconstitution was <5%. The lyophilized product was sterile (US Pharmacopeia [USP] 71 sterility test) and below compendial limits for endotoxin (USP 85 bacterial endotoxins test) for a parenteral product.

Time-of-Flight Mass Cytometry Staining and Data Acquisition

To gain a better understanding of the effect of treatment with TAK-101 on diverse immune cell subtypes, time-of-flight mass cytometry (CyTOF) was performed on peripheral blood mononuclear cells (PBMCs) from whole blood at baseline, at pretreatment with TAK-101, and on day 6 after the start of gluten challenge. PBMCs were isolated from heparinized whole blood using Ficoll-Paque (Lymphoprep; Stemcell Technologies Inc., Vancouver, Canada) density gradient separation. CyTOF staining and data acquisition were performed on PBMCs as previously described.[1] The targets and labeled antibodies used for staining are listed in Supplementary Table 4.

Pharmacokinetics

TAK-101 pharmacokinetics (PK) were evaluated by determining the concentration of nanoparticle-free gliadin in plasma samples from patients enrolled in the phase 1 and phase 2a studies. Serial blood samples were collected before dosing, at the end of infusion, and periodically up to 144 hours post dose. In the phase 2a study, PK samples were collected before dosing, at the end of infusion, and 2 hours after the end of infusion on day 8. PK samples were analyzed using a validated enzyme-linked immunosorbent assay (ELISA) to quantify free gliadin in dipotassium ethylenediaminetetraacetic acid (K2EDTA) plasma.[2] The lower limit of quantification of this assay was 40 ng/mL. Individual concentration data were summarized descriptively by treatment group and collection time points. PK parameters were derived using noncompartmental analysis based on concentration data obtained in the phase 1 study.

Gliadin-specific T-cell Proliferation and Cytokine Secretion

In the phase 2a study, gliadin-specific T-cell proliferation and cytokine secretion were determined by ELISA. Blood samples were collected predose on day 1, before gluten consumption on day 15, and after gluten consumption on day 20. Cytokine and chemokine measurements included interferon (IFN)-γ; interleukin (IL)1-β, IL2, IL4, IL6, IL8, IL10, IL12p70, and IL13; and tumor necrosis factor-α (TNF-α).

Safety Measurements

In the phase 1 and 2 studies, safety measurements included assessment of frequency and severity of adverse events (AEs), physical examinations, measurement of vital signs and standard serum chemistry (including glucose, calcium, albumin, total protein, carbon dioxide/bicarbonate, chloride, potassium, sodium, total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, creatine kinase). Hematological assessments included hemoglobin, red blood cell count, white blood cell count, and differential platelet count.

Statistical Analysis

Descriptive statistics were used in the phase 1 study. For the phase 2a study, the sample size for the primary efficacy endpoint was calculated using a 2-sided .05 significance level, a statistical power of ∼70%, and an assumed increase in the mean number of IFN-γ SFUs of 75 (standard deviation [SD], 100) in the placebo group and 5 (SD, 10) in the TAK-101 group. For the primary efficacy endpoint, mean changes from baseline in the number of IFN-γ SFUs within and between treatment groups were compared using a Wilcoxon signed rank test and a Wilcoxon rank sum test, respectively. Similarly, mean changes from baseline in Vh:Cd and IEL density within and between treatment groups were compared using a Wilcoxon signed rank test and a Wilcoxon rank sum test, respectively.

Supplementary Results

Safety and Tolerability of TAK-101

In the phase 2a study, 1 patient from the TAK-101 group experienced 1 severe (grade 3) AE of diarrhea 7 days before the gluten challenge, which was considered likely to be related to TAK-101. This individual was unable to tolerate the gluten challenge and only completed 1 day of the trial. The AE resolved and no action was taken. Two patients from the placebo group experienced a total of 8 severe (grade 3) AEs; 1 participant reported 6 events (diarrhea, abdominal pain, vomiting, dizziness, nausea, and headache), which were considered unlikely to be related to placebo and occurred on the first day of the gluten challenge, and 1 participant reported 2 events (ongoing oral ulcers and intermittent angioedema), which were considered unlikely to be related to placebo and occurred on the day before the gluten challenge. The AEs were not resolved, and no action

was taken. A summary of AEs in the phase 2a study is provided in Supplementary Table 3.

To assess the safety and tolerability of TAK-101 further, levels of complement activation were measured after TAK-101 administration. With repeated dosing of TAK-101 (phase 1, part B), transient mean increases were observed in levels of C3a and SC5b-9 15 and 30 minutes after the start of the infusions: mean (SD) C3a concentration, 16.1 (12.7) ng/mL predose and 309.2 (368.8) ng/mL 30 minutes post second dose; mean (SD) SC5b-9 concentration, 114.0 (56.6) ng/mL predose and 1037.5 (1147.6) ng/mL 30 minutes post second dose in the 8 mg/kg TAK-101 cohort. Mean levels of C3a and SC5b-9 had returned toward preinfusion values by 24 hours: mean (SD) C3a concentration, 9.7 (1.9) ng/mL; mean (SD) SC5b-9 concentration, 125.5 (36.1) ng/mL 24 hours post second dose in the 8 mg/kg TAK-101 cohort. No significant changes were observed in C5a levels.

PK of TAK-101

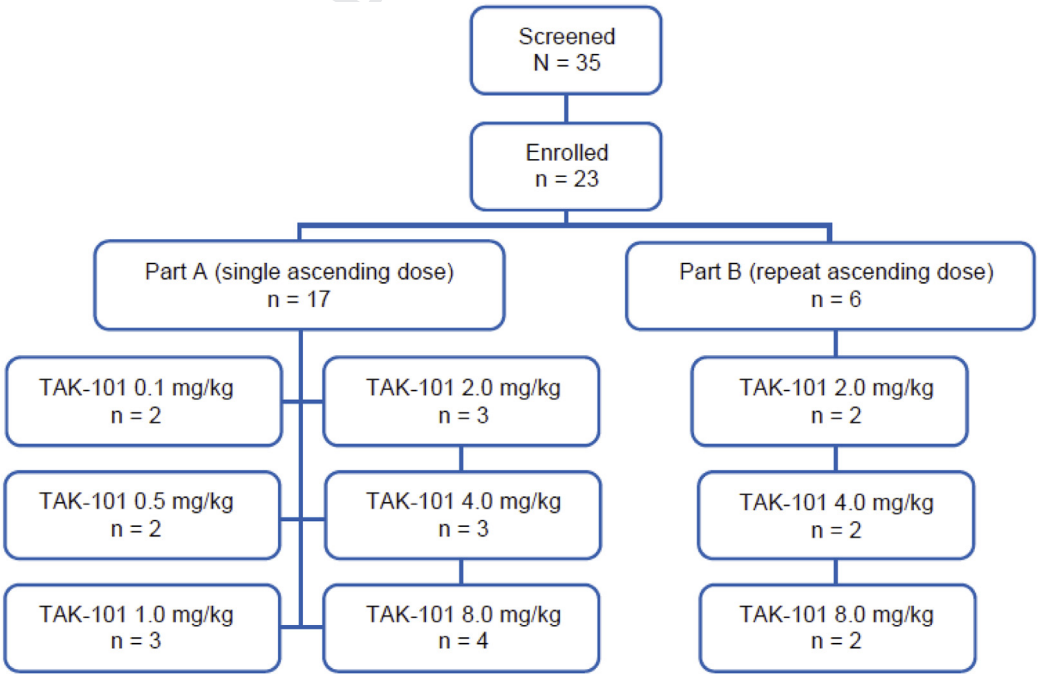
Peak concentrations (C_{max}) of gliadin were observed at the end of infusion, then levels rapidly decreased (Supplementary Table 3). At 24 hours post dose, most concentrations were below the limit of quantification. In the phase 1 study, the mean half-life ($t_{1/2}$) of TAK-101 ranged from 2.0 to 4.9 hours over the evaluated dose range and dose regimens. There was no accumulation between repeated doses on days 1 and 8 in phase 1, part B. Overall, gliadin exposure increased in an approximately dose-proportional manner over the range 0.5 to 8.0 mg/kg (as indicated by C_{max} and the area under the concentration–time curve [AUC]), although dose proportionality was not calculated owing to limited sample sizes. No dose-limiting toxicity was observed in part A or B of the phase 1 study. Owing to moderate infusion-related reactions observed in 2 of the 4 patients receiving TAK-101 8 mg/kg in part A of phase 1 (which resolved within 15–30 minutes), the subsequent infusion duration was extended, from a fixed rate over 2 hours to a progressively increasing rate over 2.5 hours, leading to a prolonged exposure compared with other dose levels. Comparable gliadin concentrations at the same time points were observed between the phase 1, part B and phase 2a studies for the 8 mg/kg dose.

Pharmacodynamics of TAK-101 (Phase 2a)

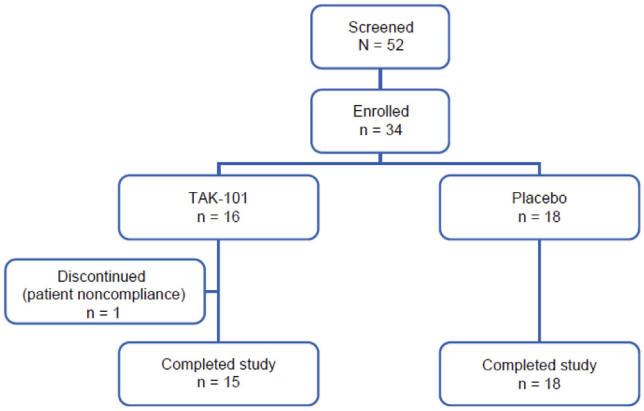
Expected changes were seen in multiple immune cell populations in the placebo group, including $\alpha 4\beta 7^+$, $CD4^+$, and $CD38^+$ T cells, $\alpha E\beta 7^+$, $CD4^+$, $CD8^+$, and $CD38^+$ T cells. Fold change across all cell types was reduced or prevented with TAK-101 treatment.

Supplementary References

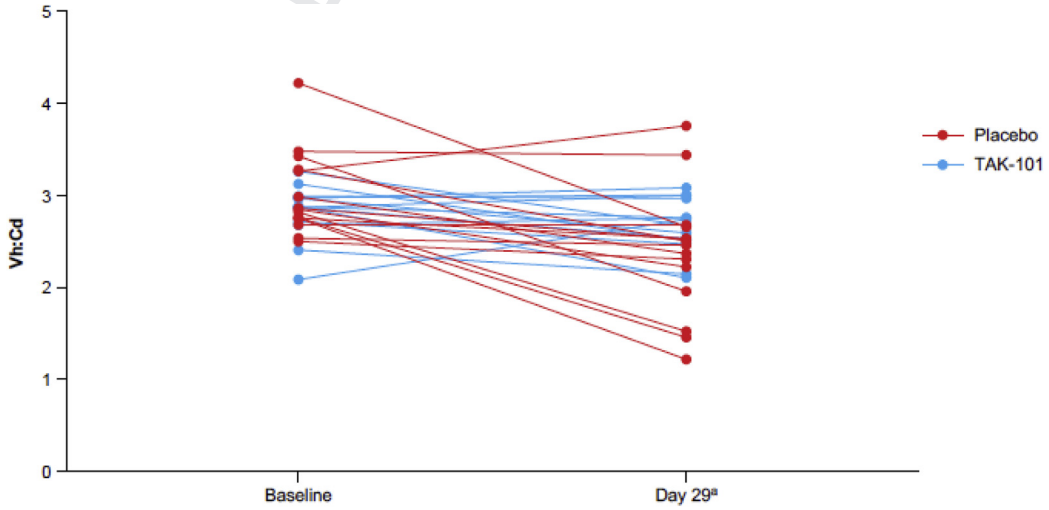
1. Bandura DR, Baranov VI, Ornatsky OI, et al. Mass cytometry: technique for real time single cell multitarget immunoassay based on inductively coupled plasma time-of-flight mass spectrometry. Anal Chem 2009; 81:6813–6822.
2. Takeda data on file, MA, USA. CSR Protocol TGLIA-5.001, Appendix 16.1.13.1 (assay validation report for gliadin ELISA); 2019. Q2



Supplementary Figure 1. Patient disposition in the phase 1 trial (NCT03486990).



Supplementary Figure 2. Patient disposition in the phase 2a trial (NCT03738475).



Supplementary Figure 3. Change from baseline in Vh:Cd at day 29 in individual patients treated with TAK-101 or placebo. Histologic sections from gut biopsies were examined by a blinded observer to determine the effects of placebo and TAK-101 on villous atrophy in response to a 14-day oral gluten challenge as described in the Methods. [a]Day 29 was equivalent to 1 day after the 14-day gluten challenge.

Supplementary Table 1. Demographic and Baseline Characteristics of Patients in Phase 1 and Phase 2a Studies

	Phase 1		Phase 2a		
Variable	Part A (SAD) All doses (n = 17)	Part B (RAD) All doses (n = 6)	TAK-101 (n = 16)	Placebo (n = 18)	Total (n = 34)
Age, *y*					
Mean (SD)	39.5 (14.6)	42.8 (11.7)	44.6 (13.5)	42.2 (16.7)	43.3 (15.1)
Median (min–max)	39.0 (19–68)	41.0 (31–59)	47.0 (21–70)	42.0 (18–67)	46.0 (18–70)
Sex, n (%)					
Men	4 (23.5)	1 (16.7)	3 (18.8)	3 (16.7)	6 (17.6)
Women	13 (76.5)	5 (83.3)	13 (81.3)	15 (83.3)	28 (82.4)
Ethnicity, n (%)					
Hispanic or Latino	1 (5.9)	0 (0.0)	1 (6.3)	0 (0.0)	1 (2.9)
Not Hispanic or Latino	16 (94.1)	6 (100.0)	15 (93.8)	18 (100.0)	33 (97.1)
Race, n (%)					
White	16 (94.1)	6 (100.0)	16 (100.0)	18 (100.0)	34 (100.0)
Body mass index, kg/m^2					
Mean (SD)	28.6 (5.3)	25.9 (3.2)	28.2 (4.8)	27.1 (4.8)	27.6 (4.8)
Median (min–max)	27.0 (20.4–37.8)	25.0 (22.5–31.0)	28.1 (17.9–38.1)	27.5 (19.4–34.6)	27.9 (17.9–38.1)
HLA type, n (%)					
HLA-DQ8	2 (11.8)	0 (0.0)	0 (0.0)	2 (11.1)[a]	2 (5.9)[a]
HLA-DQ2	14 (82.4)	6 (100.0)	16 (100.0)[a]	17 (100.0)[a,b]	33 (100.0)[a,b]
Disease duration, years					
Mean (SD)	7.2 (6.9)	11.7 (3.6)	9.15 (5.0)	9.69 (5.8)	9.44 (5.4)
Median (min–max)	6.0 (1–30)	11.5 (7–16)	9.3 (2.5–21.1)	8.5 (1.3–20.2)	9.0 (1.3–21.1)
Gluten-free diet duration, mo					
Mean	82.8 (81.3)	154.2 (47.3)	106.1 (56.9)	126.7 (72.3)	117.0 (65.4)
Median (min–max)	73.0 (14–362)	165.5 81–201	111.1 (28.3–253.6)	113.4 (13.0–268.0)	111.1 (13.0–268.0)

HLA, human leukocyte antigen; min–max, minimum–maximum; RAD, repeat ascending dose study; SAD, single ascending dose study; SD, standard deviation.
[a]HLA-DQ2 and HLA-DQ8 data not reported for 1 patient in the placebo group (phase 2a).
[b]Includes both heterozygous and homozygous patients.

Supplementary Table 2. Plasma Gliadin Pharmacokinetic Parameters in Patients With Celiac Disease After Single or Repeated Intravenous Infusions of TAK-101 in the Phase 1 Study

Part A: single ascending dose

Parameter	TAK-101 dose[a] 0.5 mg/kg (n = 2)	1 mg/kg (n = 3)	2 mg/kg (n = 3)	4 mg/kg (n = 3)	8 mg/kg (n = 3)[b]
C_{max} (ng/mL)	96.7 (42.9)	220 (6.7)	461 (76.8)	859 (185)	NA[c]
T_{max}[d]	0.54 (0.50–0.58)	0.50 (0.50–0.58)	0.50 (0.50–0.58)	0.50 (0.50–0.58)	NA[c]
AUC_{last} (ng/mL)	173 (177)	505 (63)	1990 (1190)	2960 (616)	5760 (5420)
AUC_{inf} (ng/mL)	NC[e]	605 (21)[f]	3160 (827)[f]	3220 (710)	8890 (3970)[f]
$t_{1/2}$	NC[e]	2.00 (0.50)	4.85 (3.19)	3.08 (0.25)	4.36 (2.32)[f]
CL (L/hour)	NC[e]	137 (17.7)[f]	64.6 (4.8)[f]	83.3 (8.5)	91.1 (24.0)[f]
V_{ss} (L)	NC[e]	345 (72.1)[f]	592 (238)[f]	364 (24.6)	405 (22.6)[f]
C_{max}/dose (ng/ml/mg)	2.00 (0.71)	2.81 (0.401)	2.74 (0.535)	3.22 (0.307)	NA[c]
AUC_{last}/dose (ng/mL/mg)	3.46 (3.39)	6.48 (1.42)	10.6 (4.06)	11.1 (1.04)	7.43 (6.20)
AUC_{inf}/dose (ng/mL/mg)	NC[e]	7.41 (0.97)[f]	15.5 (1.13)[f]	12.1 (1.31)	11.4 (3.01)[f]

Part B: repeated ascending doses

Parameter	TAK-101 dose[g] 2 mg/kg (n = 2)	4 mg/kg (n = 2)	8 mg/kg (n = 2)
C_{max} (ng/mL)			
Day 1	252 (17.0)	536 (119)	938 (26.9)
Day 8	NC[e]	416 (108)	738 (85.6)
T_{max}[d]			
Day 1	2.86 (2.85–2.87)	3.21 (2.82–3.60)	2.94 (2.83–3.05)
Day 8	NC[e]	3.08 (2.82–3.33)	2.90 (2.80–3.00)
AUC_{last} (ng/mL)			
Day 1	1020 (571)	3000 (884)	4600 (438)
Day 8	NC[e]	1690 (1310)	3070 (445)
AUC_{inf} (ng/mL)			
Day 1	NC[e]	3300 (997)	5090 (467)
Day 8	NC[e]	NC[e]	3270 (488)
$t^{1}/_{2}$			
Day 1	NC[e]	4.60 (2.59)	3.03 (0.01)
Day 8	NC[e]	NC[e]	2.51 (0.07)
CL (L/hour)			
Day 1	NC[e]	76.7 (9.6)	123 (19.8)
Day 8	NC[e]	NC[e]	192 (16.3)
V_{ss} (L)			
Day 1	NC[e]	493 (120)	573 (103)
Day 8	NC[e]	NC[e]	781 (24.7)
C_{max}/dose (ng/mL/mg)			
Day 1	1.54 (0.007)	2.15 (0.085)	1.52 (0.148)
Day 8	NC[e]	1.64 (0.177)	1.19 (0.064)
AUC_{last}/dose (ng/mL/mg)			

Supplementary Table 2. Continued

Part B: repeated ascending doses			
	TAK-101 dose[g]		
Parameter	2 mg/kg (n = 2)	4 mg/kg (n = 2)	8 mg/kg (n = 2)
Day 1	6.32 (3.90)	12.0 (1.34)	7.45 (1.20)
Day 8	NC[e]	6.39 (4.22)	4.92 (0.38)
AUC_{inf} /dose (ng/mL/mg)			
Day 1	NC[e]	13.2 (1.63)	8.24 (1.30)
Day 8	NC[e]	NC[e]	5.24 (0.44)

NOTE. Data are mean (SD) unless otherwise specified.
AUC_{inf}, area under the concentration–time curve from time zero extrapolated to infinite time; AUC_{inf}/dose, AUC_{inf} corrected for dose; AUC_{last}, area under the concentration–time curve from time zero to time of the last measurable concentration; AUC_{last}/dose, AUC_{last} corrected for dose; C_{max}, maximum drug concentration; C_{max}/dose, C_{max} corrected for dose; CL, total body clearance; NA, not applicable; NC, not calculated; SD, standard deviation; $t_{1/2}$, terminal elimination half-life; T_{max}, time of maximum drug concentration; V_{ss}, steady-state volume of distribution.
[a]Infusion durations were 0.5 hours for patients in the 0.5, 1, 2, and 4 mg/kg dose groups. The infusion durations for the 3 patients in the 8 mg/kg dose group were 0.5, 2.0, and 2.62 hours, respectively.
[b]For the 8 mg/kg dose group, values for the patient who received less than 15% of the scheduled dose are not included in the pharmacokinetic parameter summary statistics.
[c]NA: the duration of infusion varied by more than 25% for the patients in the 8 mg/kg group.
[d]Median (minimum–maximum).
[e]NC: parameter was not estimable for more than 1 patient.
[f]n = 2.
[g]The average duration of infusion was 2.9 hours across dose groups (range: 2.7–3.2 hours).

Supplementary Table 3. Summary of AEs in the Phase 2a Study

Category	Placebo (n = 18)	TAK-101 (n = 16)
Number of patients with an AE	18 (100.0)	16 (100.0)
Number of patients with grade 3 (severe) AE	2 (11.1)	1 (6.3)
Number of patients with drug-related AE	6 (33.3)	12 (75.0)
Number of fatal events (deaths)	0 (0.0)	0 (0.0)
Number of patients with serious AEs	0 (0.0)	0 (0.0)
Number of patients with AE leading to withdrawal	0 (0.0)	0 (0.0)

NOTE. All data are n (%).
AE, adverse event

Supplementary Table 4. Labeled Antibodies Used for Time-of-Flight Mass Cytometry Staining

Target	Clone	Mass label	Fluidigm catalog number
CD8a	RPA-T8	141Pr	Custom
CD19	HIB19	142Nd	3142001B
CD123 (IL-3R)	6H6	143Nd	3143014B
CD38	HIT2	144Nd	3144014B
CD4	RPA-T4	145Nd	3145001B
IgD	IA6-2	146Nd	3146005B
CD11c	Bu15	147Sm	3147008B
CD274 (PD-L1)	29E.2A3	148Nd	3148017B
CD25 (IL-2R)	2A3	149Sm	3149010B
CD138	DL-101	150Nd	3150012B
$\gamma\delta$TCR	11F2	152Sm	3152008
CD303 (BDCA2)	201A	153Eu	3153007B
CD3	UCHT1	154Sm	3154003B
CD56 (NCAM)	B159	155Gd	3155008B
Integrin α4	9F10	156Gd	Custom
CD194 (CCR4)	L291H4	158Gd	3158032A
CD197 (CCR7)	G043H7	159Tb	3159003A
CD14	RMO52	160Gd	3160006B
CD152 (CTLA-4)	14D3	161Dy	3161004B
Integrin β7	FIB504	163Dy	Custom
Integrin αE	Ber-ACT8	164Dy	Custom
CD16	3G8	165Ho	3165001B
CD27	L128	167Er	3167006B
CD199 (CCR9)	L053E8	168Er	3168011A
CD304 (neuropilin-1)	12C2	169Tm	3169018B
CD45RA	HI100	170Er	3170010B
CD20	2H7	171Yb	3171012B
CD1c	L161	172Yb	Custom
CD141	1A4	173Yb	3173002B
HLA-DR	L243	174Yb	3174001B
CD279 (PD-1)	EH12.2H7	175Lu	3175008B
CD127 (IL-7Rα)	A019D5	176Yb	3176004B
CD45	HI30	89Y	3089003B

BDCA, blood dendritic cell antigen; CTLA, cytotoxic T-lymphocyte-associated protein; HLA-DR, human leukocyte antigen – DR isotype; Ig, immunoglobulin; IL, interleukin; NCAM, neural cell adhesion molecule; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TCR, T-cell receptor