OPEN

Novel Orally Administered Recombinant Anti-TNF Alpha Fusion Protein for the Treatment of Ulcerative Colitis

Results From a Phase 2a Clinical Trial

Einat Almon, PhD, Yoseph Shaaltiel, PhD,* Wisam Sbeit, MD,† Alex Fich, MD,‡ Doron Schwartz, MD,‡ Mattitiahu Waterman, MD,§ Mali Szlaifer, RN, MA,* Hadar Reuveni, MSc,* Bat-chen Amit-Cohen, PhD,* Sari Alon, MSc,* Raul Chertkoff, MD,* Alona Paz, MD,* and Yaron Ilan, MD‖*

Background and Objective: OPRX-106 is an orally administered BY2 plant cell-expressing recombinant TNF fusion protein (TNFR). Oral administration of OPRX-106 was shown to be safe and effective in inducing favorable anti-inflammatory immune modulation in humans. The current study was aimed at determining the safety and efficacy of OPRX-106 in patients with ulcerative colitis (UC).

Methods: Twenty-five patients with active mild-to-moderate UC were enrolled in an open-label trial. Patients were randomized to receive 2 or 8 mg of OPRX-106 administered orally once daily, for 8 weeks. Patients were monitored for safety and efficacy including clinical response or clinical remission, based on the Mayo score. The histopathological improvement in Geboes score, calprotectin level and hs-CRP, and exploratory immune parameters by means of fluorescence-activated cell sorting and cytokine levels were monitored.

Results: Oral administration of OPRX-106 was found to be safe and well tolerated without absorption into the circulation. Out of 24 patients, 18 completed the trial. The analysis of the patients completing treatment demonstrated clinical efficacy as measured by clinical response or remission in 67% and 28%, respectively. Reduction in calprotectin levels and improved Geboes score were noted in the majority of the treated patients. The beneficial clinical effect was associated with an increase in a CD4+CD25+FoxP3 subset of suppressor lymphocytes and a reduction in interleukin 6 and interferon gamma serum levels.

Conclusions: Oral administration of the nonabsorbable OPRX-106 is safe and effective in mild-to-moderate UC, and not associated with immune suppression, while inducing favorable anti-inflammatory immune modulation.

Key Words: ulcerative colitis, anti-TNF, treatment

(*J Clin Gastroenterol* 2021;55:134–140)

Received for publication May 13, 2019; accepted December 23, 2019.

From the *Protalix, Carmiel; †Western Galilee Hospital, Nahariya; ‡Soroka Medical Center, Beer Sheva; §Rambam Medical Center, Haifa; and ‖Hadassah Medical Center, Jerusalem, Israel.

Supported by Protalix Biotherapeutics.

Y.I. is a consultant to Protalix Biotherapeutics. E.A., Y.S., S.A., R.C., and B.-c.A.-C. are employees of Protalix Biotherapeutics. The remaining authors declare that they have nothing to disclose.

Address correspondence to: Yaron Ilan, MD, Gastroenterology and Liver Units, Department of Medicine, Hebrew University-Hadassah Medical Center, Ein-Kerem, P.O. Box 1200, Jerusalem IL91120, Israel (e-mail: Ilan@hadassah.org.il).

DOI: 10.1097/MCG.0000000000001314

OPRX-106 consists of lyophilized Nicotiana tabacum (BY2) tobacco plant cells expressing the recombinant TNFR2-Fc fusion protein (rTNFR2-Fc), cultivated in a bioreactor system ProCellEx. The rTNFR2-Fc consists of the soluble form of the human TNF2 receptor fused to the Fc fragment of a human IgG1 antibody domain which imparts it a longer serum half-life. Plant cell wall which contains cellulose, serves as a natural protective agent against the gastric environment. The amino acid sequence of rTNFR-Fc is similar to the sequence of the approved anti-TNFR agent etanercept.[1,2]

OPRX-106 has been evaluated to be an effective anti-tumor necrosis factor alpha (TNFα) therapy. It is also being explored as a means for exerting a beneficial immune response through local biological effects in the gut, with no systemic absorption and with better safety relative to currently approved anti-TNFα proteins.

Oral immune therapy is based on the concept of oral administration of nonabsorbable compounds which target the gut immune system to redirect the systemic immune system toward an anti-inflammatory direction, without immunosuppression.[3,4]

Preclinical studies showed that oral administration of OPRX-106 alleviated immune-mediated liver injury in a concanavalin model. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were decreased and were comparable with those in mice which had received high-dose steroids. The beneficial effect was also observed as a marked decrease in hepatic necrosis.[1] In the 2,4,6-trinitrobenzene sulfonic acid (TNBS) colitis model, oral administration of OPRX-106 alleviated weight loss and improved bowel histology. A reduction in I-IkB-alpha phosphorylation in treated mice was also observed. These effects were associated with an alteration in the distribution of CD4+CD25+FOXP3+ cells regulatory T cells (Tregs).[1] Similarly, OPRX-106 localized to the duodenum in dextran sulfate sodium (DSS)-induced colitis and reduced the severity of colitis, while inhibiting macrophage recruitment to the inflammation site. It also reduced serum TNFα, promoted IL-10 serum levels, and altered the functional spleen Tregs. In the high-fat diet model of nonalcoholic steatohepatitis, oral administration of OPRX-106 changed the distribution of CD4+CD25+FoxP3+ cells between the liver and spleen with an increase in the intrasplenic-to-intrahepatic CD4+CD25+FoxP3+ Tregs ratio, and a decrease in the intrasplenic-to-intrahepatic CD8+CD25+FoxP3+ lymphocyte ratio. An increase in intrahepatic natural killer T (NKT) cells and a reduction in the intrasplenic-to-intrahepatic NKT ratio was observed. Assessment of the CD4:CD8 ratios showed the sequestration of CD8+

lymphocytes in the liver. These effects were associated with a reduction in serum triglyceride levels, a decrease in the AST levels, serum glucose levels, and HOMA-IR score. A reduction in hepatic triglycerides content was observed in the high dose-treated mice.[2]

The safety and the exploratory immune modulatory effects of orally administered OPRX-106 were shown in a phase I study in humans. Three different doses (2, 8, or 16 mg/d) of OPRX-106 were orally administered for 5 consecutive days in 14 healthy volunteers. Treatment was found to be safe and well tolerated. The pharmacokinetic (PK) study showed that OPRX-106 is not absorbed into circulation. No effect on white blood cells (WBC) and lymphocyte counts was noted. A dose-dependent effect was observed on systemic lymphocytes. The oral administration of all 3 dosages was associated with an increase in CD4+CD25+ and CD8+CD25+ subset of suppressor lymphocytes. An increase in CD4+CD25+FoxP3 Tregs was noted in the group treated with 8 mg. Also, NKT regulatory cells, CD3+CD69+, and CD4+CD62 lymphocyte subsets increased with treatment. No change in serum TNFα was observed.[5]

The present study was aimed at determining the safety PK and exploratory efficacy of orally administered human TNFR fusion protein expressed in plant cells, in patients with mild to moderate ulcerative colitis (UC).

METHODS

Study Design and Product

The clinical trial was a phase 2a, open-label, randomized study, which examined the safety, PK, and exploratory efficacy of OPRX-106 in patients with UC. The subjects were enrolled in 8 centers in Israel, Serbia, and Bulgaria. OPRX-106 consisted of lyophilized, genetically modified plant cells expressing the human tumor necrosis factor receptor-Fc fusion protein (TNFR-Fc), a dimeric soluble fusion protein of the TNF-2 receptor protein and the Fc fragment of IgG1. The product was manufactured by the sponsor Protalix Ltd. (Carmiel, Israel). The trial was registered at ClinicalTrials.gov, NCT02768974.

Study Population

Eligible subjects were adult males or females who were 18 to 70 years of age. Patients had to be diagnosed with UC according to the following European guidelines for a minimum of 3 months: medical history, physical examination, laboratory tests [anemia, increased levels of C-reactive protein (CRP), fecal samples for blood], historical histopathological evidence from flexible sigmoidoscopy or colonoscopy showing active mild-to-moderate UC, and a high level of calprotectin in stool (>100 μg/mg). Exclusion criteria included a history of colonic or rectal surgery other than hemorrhoidal surgery or appendectomy, patients receiving total parenteral nutrition or severe UC evidenced by the following signs of toxicity: heart rate >90 beats/min at rest and a temperature >38.0°C. Other exclusion criteria included ulcerative proctitis with disease limited to <15 cm from the anal verge, use of >4.8 g of 5-aminosalicylic acid (5-ASA) or equivalent, corticosteroid or 5-ASA enemas, foams, or suppositories within 2 weeks before screening or at any time during the study, use of anti-inflammatory drugs (cromones, xanthines, leukotriene antagonists) or natural remedies (probiotics, omega-3 fatty acids) within 4 weeks before screening or at any time during the study, use of oral or parenteral antibiotics within 2 weeks before the screening or at any time during the study, use of chronic nonsteroidal anti-inflammatory (NSAID) therapy, use of immune suppressive agents including anti-TNF agents, azathioprine, mercaptopurine (6MP), or methotrexate 12 weeks before screening or at any time during the study and use of steroids 12 weeks before screening or at any time during the study.

Study Design

Twenty-five UC patients were enrolled for this study. However, 1 subject withdrew consent before study drug administration. Therefore, 24 UC patients of 18 years and above were finally enrolled in the study including 11 males and 13 females with a mean age of 42.63 ± 13.74 years (range, 23 to 73 y), who received 2 or 8 mg of OPRX-106 at least once daily, for 8 weeks. On day 1 and on week 8, the subjects were monitored for 6 hours succeeding OPRX-106 administration for PK sampling. Physical examination and blood samples for testing liver and kidney functions and complete blood count (CBC) were performed at screening and on weeks 1, 2, 4, 6, 8, and 10 (follow-up visit). A follow-up lower endoscopy was performed on week 8. Adverse events (AEs) and concomitant medications were evaluated at each study visit.

Pharmacokinetics Study

Blood samples were tested for the presence of OPRX-106 levels at baseline visit and on week 8 before study drug administration and at 1, 2, 4, and 6 hours after study drug administration.

Flow Cytometry Analysis of Peripheral Blood Lymphocytes

The immune modulatory effects of OPRX-106 on lymphocyte subsets were evaluated by flow cytometry analysis on day 1, week 4, and week 8. The following protocol was followed for the flow cytometry analysis. Cells were suspended in 100 μL fluorescence-activated cell sorting (FACS) buffer (2% bovine serum albumin, in phosphate-buffered saline) and incubated with the following surface antihuman antibodies: antihuman CD4-APC, antihuman CD8-Pacific Blue (PB), antihuman CD16-APC-Alexa Fluor 700 (APC-AF700), antihuman CD25-FITC, antihuman CD45-Krome Orange (KO), antihuman CD56-APC-Alexa Fluor 750 (APC-AF700), and antihuman CD62L-FITC (Beckman Coulter) for 15 minutes. Tubes stained for intracellular staining with Foxp3 antibody (anti-FoxP3-PE, Beckman Coulter) were fixed and permeabilized followed by incubation for 1 hour. Cells were washed with FACS buffer and analyzed. Cell phenotyping was performed by Navios 10 colors 3 Lasers (Beckman Coulter) and analyzed by Kaluza software. The FACS analysis was performed at AML-American Medical Laboratories, Tel Aviv, Israel. Only the live cells were counted, and background fluorescence from non–antibody-treated lymphocytes and isotype control was subtracted.

Serum Cytokines

To determine the potential effects of orally administrated OPRX-106 on cytokine levels, serum levels of TNFα, interferon gamma (IFN-γ), IL-6, IL-10, and IL-12 (p70) were determined by highly sensitive enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturer's instructions. TNFα, IL-6, IL-10, and IL-12 (p70) were quantified by ELISA using Quantikine (R&D Systems, Catalogue number: HSTA00E, HS600B, HS120, and HS120, respectively), and IFN-γ was quantified using HS (eBioscience, Catalogue

number: BMS228HS). The analysis was performed at American Medical Laboratories (AML), Herzliya Pituach, Israel.

Efficacy and Safety Assessments

Study endpoints included safety, PK, and exploratory efficacy parameters. Clinical response (improvement) at baseline versus week 8 was defined by the following criteria: decrease in the Mayo score of at least 3 points, decrease in the subscore for rectal bleeding of at least 1 point, a rectal bleeding subscore of 0 or 1, clinical remission at week 8 which is defined as clinically symptom-free, Mayo Score ≤ 2 with no individual subscore exceeding 1 point after treatment, histopathological improvement in Geboes histologic grading from baseline to week 8, improvement in high sensitivity C-reactive protein (hs-CRP) levels from baseline to week 8, improvement in fecal calprotectin levels from baseline to week 8, and changes in systemic immune modulation parameters from baseline to week 8. Mayo responsiveness and remission were defined as a decrease in the total Mayo score; reductions in Geboes grading, CRP, and calprotectin were exploratory secondary endpoints improvements of any degree between baseline and week 8 were calculated as response. Endoscopic scoring was performed at each site by the investigator at the start and end of the study. Mucosal healing was an exploratory secondary endpoint and any improvement from baseline to week 8 was considered a response. Histology was evaluated by a central reader blinded pathologist. AEs were evaluated at each study visit. These were defined as any unfavorable and unintended signs, symptoms or disease that seemed during the study period whether considered related to the study drug, including accidental injuries, reasons for any change in medications, reasons for admission to hospital or reasons for surgical procedures and any laboratory abnormality assessed as clinically significant by the investigator. The AEs were monitored from the start of the treatment until 2 weeks after the final visit dose.

Statistical Analysis

Quantitative data were summarized with mean and standard error, SD, median, minimum, and maximum values for both actual and change from baseline results. Qualitative data were summarized to show frequency and percentage within each category. Data manipulation, tabulation of descriptive statistics, calculation of inferential statistics, and graphical representations were performed using SAS (v9.3 or higher) for Windows.

RESULTS

Patients

Twenty-five patients with mild-to-moderate UC were enrolled in the trial and 24 received the study drug (1 patient withdrew consent before receiving the first dose of the study drug). Out of 24 patients, 22 (92%) were classified as having moderate UC based on a Mayo score >6. Table 1 summarizes the patient characteristics in each of the 2 groups.

Pharmacokinetics

The variable OPRX-106 concentrations C_{max} and T_{max} indicated no or minimal absorption of OPRX-106 into the blood circulation. Out of 24 subjects, 9 had no detectable OPRX-106 level at any point in time, 15 subjects had at least 1 sample with detectable but insignificant levels of OPRX-106 ranging from 46 to 112 pg/mL.

TABLE 1. Patients Characteristics

Parameter	2 mg/d (n = 13)	8 mg/d (n = 11)
Mean age (y) ± SD (range)	42.62 ± 10.41 (28-63)	42.64 ± 17.43 (23-73)
Male:Female	6:7	5:6
Ethnicity		
Caucasian	13	11
Mean baseline values (± SD)	2 mg/d	8 mg/d
Mayo score*	7.69 ± 1.11	6.82 ± 1.83
Mayo Endoscopic subscore	2.2 ± 0.4	2.0 ± 0.9
Geboes score	12.00 ± 4.76	11.00 ± 6.60

Two subjects discontinued due to UC exacerbation, 1 subject discontinued due to lack of response, 1 subject discontinued due to usage of antibiotics treatment.

*Two subjects discontinued due to lack of response.

UC indicates ulcerative colitis.

Antidrug Antibodies

A total of 80 serum samples from 24 subjects were tested (in duplicates) for the presence of anti-OPRX-106 antibodies with no evidence for anti-OPRX-106 antibodies being observed.

Safety

Oral PRX-106 was well tolerated and no serious AEs were reported. None of the patients discontinued the therapy due to AEs. The dropout rate was consistent with other trials in similar populations. A total of 40 AEs were reported in 15 (63%) patients with 95% (38) of the AEs being mild to moderate and 5% (2) being severe AEs (nausea defined as possibly related). Of these events, 40% (16/40) were reported as treatment-related. Other events recorded were headache (4), increased creatine phosphokinase (CPK) (2) and 1 complaint of each of the following events: dysphagia, nausea, chills, fatigue, peripheral edema, increased appetite, dizziness, pruritus, hypertension, and eosinophilia. 60% (24/40) of AEs were reported as not related. No difference was noted between doses (2 or 8 mg). Table 2 summarizes the AEs.

Figure 1 shows the beneficial effect of OPRX-106 as seen by an improvement in the Mayo score. A total of 12 patients (67%) achieved a clinical response, and 5 achieved clinical remission (28%). No significant differences were noted between the 2 dosage groups.

Figure 1 shows the beneficial effect of OPRX-106 as seen by an improvement in mucosal healing. A total of 11 patients (61%) showed improvement in their endoscopy score, defined as a decrease in endoscopy subscore at week 8. Six patients achieved mucosal healing (33%). No significant differences were noted between the 2 dosage groups.

Improved Geboes score was noted in 11/18 (61%) patients. A reduction in calprotectin levels was demonstrated in 13/18 (72%) patients, and a reduction in hs-CRP serum levels were observed in 14/18 (78%) patients, further supporting a systemic anti-inflammatory effect of the drug.

Table 3 shows that the response to therapy was associated with a trend for a decrease in IFNγ, TNFα, and interleukin 6 (IL-6) serum levels of pro-inflammatory cytokines. Panel A shows the percentage of patients with decreased IL-6 levels arranged according to decreasing Geboes score. Panel B shows the percentage of patients with reduced IFNγ and IL-6 classified by clinical remission and response. The data show a

TABLE 2. Adverse Events

MedDRA System Class	Preferred Term	N (Events)
Blood and lymphatic system disorders	Anemia	2
	Eosinophilia	1
	Hypochromic anemia	1
Eye disorders	Dry eye	1
Gastrointestinal disorders	Abdominal pain	1
	Anal fissure	1
	Colitis ulcerative	7
	Constipation	1
	Diarrhea	1
	Dysphagia	2
	Hemorrhoids	1
	Nausea	1
	Vomiting	1
General disorders and administration site conditions	Chills	1
	Fatigue	1
	Influenza like illness	1
	Edema peripheral	1
	Pyrexia	1
Infections and infestations	Nasopharyngitis	1
	Pharyngitis	1
	Upper respiratory tract infection	1
Investigations	Blood creatine phosphokinase increased	2
Metabolism and nutrition disorders	Increased appetite	1
Nervous system disorders	Dizziness	1
	Headache	4
Psychiatric disorders	Sleep disorder	1
Skin and subcutaneous tissue disorders	Pruritus	1
Vascular disorders	Hypertension	1

trend for a correlation between the clinical effect of OPRX-106 and the systemic anti-inflammatory effect. No relationship was seen between the clinical effect and the effect on reduction of serum TNFα levels.

A similar trend was demonstrated with an increase in the CD3+CD4+CD25+Foxp3+ Tregs in patients that achieved clinical response/remission (Table 4). Taken together, the effect on the systemic immune system was correlated with the clinical and laboratory response to orally administered nonabsorbable OPRX-106.

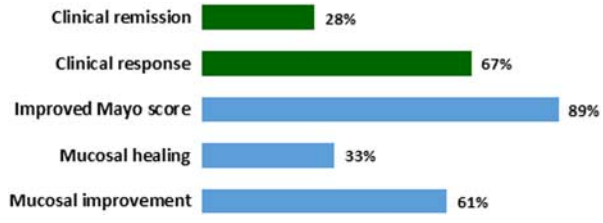

FIGURE 1. Following 8 weeks of treatment with OPRX-106, 28% of the patients achieved clinical remission and 67% of the patients achieved clinical response; 89% of the patients had any improvement in their Mayo score. 33% of the patients achieved mucosal healing and 61% of the patients had mucosal improvement. full color online

TABLE 3. Effect of OPRX-106 on Cytokine Levels

No. Patients With Decreased Levels of Cytokine at Week 8 Compared With Baseline

	Clinical Response or Remission (Based on Mayo Score)		
Cytokine	**All (N = 18)**	**Yes (n = 12)**	**No (n = 6)**
IL-6	7	6	1
TNFα	11	7	4
IFNγ	10	7	3
	Decreased Geboes score		
	All (N = 18)	Yes (n = 11)	No (n = 7)
IL-6	7	5	2
TNFα	11	6	5
IFNγ	10	6	4

IFNγ indicates interferon gamma; IL-6, interleukin 6; TNFα, tumor necrosis factor alpha.

DISCUSSION

The results of the study show that oral administration of OPRX-106 was safe, well tolerated and clinically effective in patients with mild-to-moderate UC. OPRX-106 was minimally absorbed systemically as shown in the PK analysis, and from the lack of antidrug antibodies in all treated patients. OPRX-106 had no major side effects. No immune suppression was noted as shown by lack of bone marrow suppression or alterations in subsets of lymphocytes. Although not being systemically absorbed and suppressing the immune system, OPRX-106 further exerted a beneficial anti-inflammatory effect on the systemic immune system. This was manifested by a decrease in serum levels of the pro-inflammatory cytokines IFNγ and IL-6, which correlated with the clinical response. Similarly, an increase in the CD3+CD4+CD25+Foxp3+ subset of lymphocytes correlated with clinical response as well. The beneficial effect on the systemic immune system had a favorable clinical impact in disease parameters. The data show that 67% patients achieved clinical improvement of which 28% achieved clinical remission. Reduction in calprotectin and hs-CRP levels and improved Geboes score were noted in majority of the tested patients.

Biological agents that target TNFα improved the therapeutic approach to inflammatory diseases.[6,7] Although the parenteral administration of recombinant anti-TNF proteins reduces disease activity, a significant number of patients do not respond favorably to these compounds.[8] Primary response failure and subsequent response failure, where an initial response is followed by subsequent relapses, have been described.[8–10] Drug immunogenicity and variability in bioavailability also contribute to treatment

TABLE 4. Effect of OPRX-106 of Regulatory T Cells

No. Patients With Increased CD3+ CD4+ CD25+ FoxP3 +Population at Week 8 Compared With Baseline

Achieving Clinical Response or Remission (n = 11)	Others* (n = 6)
6	3

*Patients who did not achieve clinical response/remission in the study.

failures.[8,11] Parenteral administration of TNF antagonists bears the risk of opportunistic and nonopportunistic infections, vaccinations, neurological complications, hepatotoxicity, hematological side effects, malignancies, infusion reactions, and autoimmunity. The use of these agents in the elderly, young, fertile, or pregnant and lactating women, patients with heart failure, or with acute infections are some of the concerns related to this treatment.[12,13]

Etanercept is a recombinant, dimeric, soluble TNFR fusion protein that blocks only soluble TNF but not the membrane-bound TNF.[14] Parenterally, it is used in rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, psoriasis, and ankylosing spondylitis.[6,15] It is not useful in the treatment of Crohn's disease (CD) or UC.[14,16] Development of inflammatory bowel disease (IBD) should be suspected in patients receiving etanercept, who develop gastrointestinal symptoms.[17] Unmasking of gastrointestinal disorders during etanercept treatment suggests differences between etanercept and other anti-TNFα antibodies in molecular structures, TNF neutralizing effects and PK.[18–20] In rheumatoid arthritis, etanercept down-regulates inflammation via the inhibition of soluble TNF. Anti-TNF antibodies alleviate CD activity as a result of antibody-induced monocytes and T cell apoptosis in the gut.[21] In contrast, etanercept, blocks soluble TNF but not membrane-bound TNF and does not induce T-cell apoptosis.[22] Inflammation in IBD patients depends on signaling via membrane-bound TNF.[23] Although both infliximab and etanercept can neutralize TNFα, only Infliximab bound to activated lymphocytes and lamina propria mononuclear cells from patients with CD induce apoptosis of lamina propria T cells.[24] Etanercept is also distinct from infliximab in modulating pro-inflammatory genes in activated human leukocytes.[25] The p75 receptor structure of the etanercept has a lower binding affinity for the extracellular part of transmembrane TNF.[24]

Loss of response (LOR) to anti-TNF therapy is common among patients with IBD and presents a challenge to physicians in the management of symptoms.[26] The incidence of LOR among adult IBD patients undergoing anti-TNF based therapy is 36%,[27,28] and some studies suggest that up to 50% of the patients with UC or CD will experience LOR to infliximab after an initial response to the drug.[29] There was no difference in LOR over time between patients treated with different combination regimens or different anti-TNF agents.[30] The presence of a perianal lesion, onset at younger age, and involvement of colon are relative risk factors of LOR.[27] However, the development of neutralizing antibodies is a major cause. Antiadalimumab antibodies arise earlier than previously acknowledged and their impact may be more pronounced for LOR.[31] Detection of neutralizing antibody activity correlates with clinical LOR, and with prediction of subsequent LOR.[32] In almost half of IBD patients developing antiadalimumab antibodies and LOR, known immunogenicity of adalimumab was reversed by the addition of immunomodulatory therapy.[33] Dose intensification, switch to another anti-TNF, and addition of an immunomodulator to reverse immunogenicity were suggested as means for overcoming LOR.[34]

The oral administration of nonabsorbable OPRX-106 may overcome the LOR in patients with IBD better than the parenteral administration of etanercept as well other anti-TNF-based formulations, reflecting its potential to induce a systemic beneficial immune modulatory effect by targeting the gut immune system. Oral administration of OPRX-106 also overcomes some of the side effects encountered by parenteral administration, including the long-term effects of immune suppression. OPRX-106 might exert a local impact on the gut-associated lymphoid tissue, which does not exist when parentally administered. As this drug is not absorbed when orally administered, postabsorption signaling from the gut exerts an additive or synergistic effect on the gut-associated lymphoid tissue.[4] A similar effect has been described for other oral immunotherapy-based compounds.[35–40] Oral immune therapy uses the inherent ability of the gut immune system to promote Tregs and different subsets of lymphocytes,[41,42] in association with alterations of dendritic cells, functioning at the level of the mesenteric lymph nodes and gut-associated lymphoid tissue to exert potent anti-inflammatory effects.[43,44] OPRX-106 may have a lower likelihood of LOR and improve compliance due to oral therapy and better safety. Potentiating the effects of oral immune therapy at the gut immune system requires an adjuvant impact to activate crosstalk of dendritic cells with other cells of the gut-associated lymphoid tissue.[4,45–47] Cellulose present in the plant cell wall of OPRX-106 is biologically active at the gut level and exerts a potent immune adjuvant effect.[1] Preclinical studies showed an adjuvant immune effect of the plant cell wall. Indeed, oral administration of BY2(−) plant cells exerted a partial immune modulatory influence, although to a lesser degree than that exerted by cells expressing the TNFR protein. The data further supported the previously described notion that the oral administration of immune modulatory agents highly benefits from the coadministration of an adjuvant.[45,48]

The oral administration of OPRX-106 promoted CD4 +CD25+FoxP3+ Tregs, thereby reducing the serum levels of pro-inflammatory cytokines. Dysregulation of Tregs is a significant risk factor in conferring human autoimmune diseases.[49] This subset of Tregs maintains tolerance to self and foreign antigens.[50] These cells are essential for the prevention of autoimmunity, making their promotion an attractive therapeutic target.[51,52] Tregs were lower in TNFα transgenic mice, and antagonizing TNFα restored their suppressor activity.[53] The beneficial effect of anti-TNFα therapy in rheumatoid arthritis is associated with the promotion of Tregs.[54] Parenteral administration of anti-TNF antibodies induces a newly differentiated population of Tregs, which compensates for the defective natural Tregs in various immune-mediated diseases.[55–58] The present study further supports a beneficial effect of the nonabsorbable nonimmunosuppressant OPRX-106 on the systemic immune system.

In conclusion, oral administration of plant cells expressing recombinant anti-TNF fusion protein shows a beneficial biological activity in UC patients. An orally delivered nonabsorbable OPRX-106 was safe, well tolerated, and did not have any side effects associated with general immune suppression. The oral delivery of therapeutic proteins has been a long-term goal with relatively limited success. Lack of systemic absorption, absence of a change in systemic WBC, and the systemic immune modulation documented by alteration of different subsets of lymphocytes, support the notion that oral OPRX-106 may overcome some of the obstacles encountered by parenteral administration of currently used anti-TNF agents. It may provide an answer to the long-term immune suppression encountered in patients with chronic disorders who use these agents for prolonged periods of time, in addition to LOR due to neutralizing antibodies. OPRX-106 may provide oral, safe, and effective anti-TNFα-based therapy for IBD. Although the current study was an open-labeled uncontrolled study, the data support the further development of OPRX-106 in controlled studies in patients with IBD and other immune-mediated disorders.

ACKNOWLEDGMENTS

The authors thank the investigators and their patients for their participation in the study.

REFERENCES

1. Ilan Y, Gingis-Velitski S, Ben Ya'aco A, et al. A plant cell-expressed recombinant anti-TNF fusion protein is biologically active in the gut and alleviates immune-mediated hepatitis and colitis. *Immunobiology*. 2017;222:544–551.
2. Ilan Y, Ben Ya'acov A, Shabbat Y, et al. Oral administration of a non-absorbable plant cell-expressed recombinant anti-TNF fusion protein induces immunomodulatory effects and alleviates nonalcoholic steatohepatitis. *World J Gastroenterol*. 2016;22: 8760–8769.
3. Ilan Y. Review article: novel methods for the treatment of non-alcoholic steatohepatitis - targeting the gut immune system to decrease the systemic inflammatory response without immune suppression. *Aliment Pharmacol Ther*. 2016;44:1168–1182.
4. Ilan Y. Oral immune therapy: targeting the systemic immune system via the gut immune system for the treatment of inflammatory bowel disease. *Clin Transl Immunology*. 2016;5:e60.
5. Almon E, Khoury T, Drori A, et al. An oral administration of a recombinant anti-TNF fusion protein is biologically active in the gut promoting regulatory T cells: results of a phase I clinical trial using a novel oral anti-TNF alpha-based therapy. *J Immunol Methods*. 2017;446:21–29.
6. Wiedmann MW, Mossner J, Baerwald C, et al. TNF alpha inhibition as treatment modality for certain rheumatologic and gastrointestinal diseases. *Endocr Metab Immune Disord Drug Targets*. 2009;9:295–314.
7. Mao EJ, Hazlewood GS, Kaplan GG, et al. Systematic review with meta-analysis: comparative efficacy of immunosuppressants and biologics for reducing hospitalisation and surgery in Crohn's disease and ulcerative colitis. *Aliment Pharmacol Ther*. 2017;45:3–13.
8. Bendtzen K, Ainsworth M, Steenholdt C, et al. Individual medicine in inflammatory bowel disease: monitoring bioavailability, pharmacokinetics and immunogenicity of anti-tumour necrosis factor-alpha antibodies. *Scand J Gastroenterol*. 2009;44: 774–781.
9. Mitrev N, Vande Casteele N, Seow CH, et al. Review article: consensus statements on therapeutic drug monitoring of anti-tumour necrosis factor therapy in inflammatory bowel diseases. *Aliment Pharmacol Ther*. 2017;46:1037–1053.
10. Pariente B, Laharie D. Review article: why, when and how to de-escalate therapy in inflammatory bowel diseases. *Aliment Pharmacol Ther*. 2014;40:338–353.
11. Requena P, Gonzalez R, Lopez-Posadas R, et al. The intestinal antiinflammatory agent glycomacropeptide has immunomodulatory actions on rat splenocytes. *Biochem Pharmacol*. 2010;79: 1797–1804.
12. Stallmach A, Hagel S, Bruns T. Adverse effects of biologics used for treating IBD. *Best Pract Res Clin Gastroenterol*. 2010;24: 167–182.
13. Miehsler W, Novacek G, Wenzl H, et al. A decade of infliximab: the Austrian evidence based consensus on the safe use of infliximab in inflammatory bowel disease. *J Crohns Colitis*. 2010;4:221–256.
14. Rahimi R, Nikfar S, Abdollahi M. Do anti-tumor necrosis factors induce response and remission in patients with acute refractory Crohn's disease? A systematic meta-analysis of controlled clinical trials. *Biomed Pharmacother*. 2007;61:75–80.
15. Hoy SM, Scott LJ. Etanercept: a review of its use in the management of ankylosing spondylitis and psoriatic arthritis. *Drugs*. 2007;67:2609–2633.
16. Sandborn WJ, Hanauer SB, Katz S, et al. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. *Gastroenterology*. 2001;121:1088–1094.
17. O'Toole A, Lucci M, Korzenik J. Inflammatory bowel disease provoked by etanercept: report of 443 possible cases combined from an IBD referral center and the FDA. *Dig Dis Sci*. 2016;61:1772–1774.
18. Haraoui B, Krelenbaum M. Emergence of Crohn's disease during treatment with the anti-tumor necrosis factor agent etanercept for ankylosing spondylitis: possible mechanisms of action. *Semin Arthritis Rheum*. 2009;39:176–181.
19. Ahmad K, Rogers S. Development of Crohn disease in a patient on etanercept for psoriasis. *Br J Dermatol*. 2007;157:396.
20. Dubeau MF, Iacucci M, Beck PL, et al. Drug-induced inflammatory bowel disease and IBD-like conditions. *Inflamm Bowel Dis*. 2013;19:445–456.
21. ten Hove T, van Montfrans C, Peppelenbosch MP, et al. Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. *Gut*. 2002;50:206–211.
22. Di Sabatino A, Ciccocioppo R, Cinque B, et al. Defective mucosal T cell death is sustainably reverted by infliximab in a caspase dependent pathway in Crohn's disease. *Gut*. 2004;53: 70–77.
23. Mudter J, Neurath MF. Apoptosis of T cells and the control of inflammatory bowel disease: therapeutic implications. *Gut*. 2007;56:293–303.
24. Van den Brande JM, Braat H, van den Brink GR, et al. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. *Gastroenterology*. 2003;124:1774–1785.
25. Haider AS, Cardinale IR, Whynot JA, et al. Effects of etanercept are distinct from infliximab in modulating proinflammatory genes in activated human leukocytes. *J Investig Dermatol Symp Proc*. 2007;12:9–15.
26. Yanai H, Hanauer SB. Assessing response and loss of response to biological therapies in IBD. *Am J Gastroenterol*. 2011;106: 685–698.
27. Zhang QW, Shen J, Zheng Q, et al. Loss of response to scheduled infliximab therapy for Crohn's disease in adults: a systematic review and meta-analysis. *J Dig Dis*. 2019;20:65–72.
28. Qiu Y, Chen BL, Mao R, et al. Systematic review with meta-analysis: loss of response and requirement of anti-TNFalpha dose intensification in Crohn's disease. *J Gastroenterol*. 2017;52: 535–554.
29. Yokoyama Y, Kamikozuru K, Watanabe K, et al. Inflammatory bowel disease patients experiencing a loss of response to infliximab regain long-term response after undergoing granulocyte/monocyte apheresis: a case series. *Cytokine*. 2018;103: 25–28.
30. Varma P, Rajadurai AS, Holt DQ, et al. immunomodulator use does not prevent first loss of response to anti-TNF Therapy in inflammatory bowel disease: long term outcomes in a real-world cohort. *Intern Med J*. 2019;49:753–760.
31. Ungar B, Engel T, Yablecovitch D, et al. Prospective observational evaluation of time-dependency of adalimumab immunogenicity and drug concentrations: the POETIC Study. *Am J Gastroenterol*. 2018;113:890–898.
32. Weisshof R, Ungar B, Blatt A, et al. Anti-infliximab antibodies with neutralizing capacity in patients with inflammatory bowel disease: distinct clinical implications revealed by a novel assay. *Inflamm Bowel Dis*. 2016;22:1655–1661.
33. Ungar B, Kopylov U, Engel T, et al. Addition of an immunomodulator can reverse antibody formation and loss of response in patients treated with adalimumab. *Aliment Pharmacol Ther*. 2017;45:276–282.
34. Ben-Horin S. Loss of response to anti-tumor necrosis factors: what is the next step? *Dig Dis*. 2014;32:384–388.
35. Lalazar G, Mizrahi M, Turgeman I, et al. Oral administration of OKT3 MAb to patients with NASH, promotes regulatory T-cell Induction, and alleviates insulin resistance: results of a phase IIa blinded placebo-controlled trial. *J Clin Immunol*. 2015;35:399–407.
36. Israeli E, Goldin E, Fishman S, et al. Oral administration of non-absorbable delayed release 6-mercaptopurine is locally active in the gut, exerts a systemic immune effect and alleviates Crohn's disease with low rate of side effects: results of double blind phase II clinical trial. *Clin Exp Immunol*. 2015;181:362–372.

37. Halota W, Ferenci P, Kozielewicz D, et al. Oral anti-CD3 immunotherapy for HCV-nonresponders is safe, promotes regulatory T cells and decreases viral load and liver enzyme levels: results of a phase-2a placebo-controlled trial. *J Viral Hepat*. 2015;22:651–657.
38. Mizrahi M, Shabat Y, Ben Ya'acov A, et al. Alleviation of insulin resistance and liver damage by oral administration of Imm124-E is mediated by increased Tregs and associated with increased serum GLP-1 and adiponectin: results of a phase I/II clinical trial in NASH. *J Inflamm Res*. 2012;5:141–150.
39. Adar T, Ben Ya'acov A, Lalazar G, et al. Oral administration of immunoglobulin G-enhanced colostrum alleviates insulin resistance and liver injury and is associated with alterations in natural killer T cells. *Clin Exp Immunol*. 2012;167:252–260.
40. Israeli E, Ilan Y. Oral administration of Alequel, a mixture of autologous colon-extracted proteins for the treatment of Crohn's disease. *Therap Adv Gastroenterol*. 2010;3:23–30.
41. Mizrahi M, Ilan Y. The gut mucosa as a site for induction of regulatory T-cells. *Curr Pharm Des*. 2009;15:1191–1202.
42. Castro-Sanchez P, Martin-Villa JM. Gut immune system and oral tolerance. *Br J Nutr*. 2013;109(suppl 2):S3–S11.
43. Ilan Y. Oral immune regulation toward disease-associated antigens: results of phase I clinical trials in Crohn's disease and chronic hepatitis. *Ann N Y Acad Sci*. 2004;1029:286–298.
44. Ilan Y. Oral tolerance: can we make it work? *Hum Immunol*. 2009;70:768–776.
45. Ilan Y, Maron R, Tukpah AM, et al. Induction of regulatory T cells decreases adipose inflammation and alleviates insulin resistance in ob/ob mice. *Proc Natl Acad Sci U S A*. 2010;107:9765–9770.
46. Lalazar G, Zigmond E, Weksler-Zangen S, et al. Oral administration of beta-glucosylceramide for the treatment of insulin resistance and nonalcoholic steatohepatitis: results of a double-blind, placebo-controlled trial. *J Med Food*. 2017;20:458–464.
47. Ilan Y. Compounds of the sphingomyelin-ceramide-glycosphingolipid pathways as secondary messenger molecules: new targets for novel therapies for fatty liver disease and insulin resistance. *Am J Physiol Gastrointest Liver Physiol*. 2016;310:G1102–G1117.
48. Ilan Y, Zigmond E, Lalazar G, et al. Oral administration of OKT3 monoclonal antibody to human subjects induces a dose-dependent immunologic effect in T cells and dendritic cells. *J Clin Immunol*. 2010;30:167–177.
49. Suen JL, Chiang BL. CD4(+)FoxP3(+) regulatory T-cells in human systemic lupus erythematosus. *J Formos Med Assoc*. 2012;111:465–470.
50. Schmetterer KG, Neunkirchner A, Pickl WF. Naturally occurring regulatory T cells: markers, mechanisms, and manipulation. *FASEB J*. 2012;26:2253–2276.
51. Siri A, de Boysson H, Boursier G. [Regulatory T lymphocytes CD4(+): current issues]. *Med Sci (Paris)*. 2012;28:646–651.
52. Chen W, Konkel JE. Development of thymic Foxp3(+) regulatory T cells: TGF-beta matters. *Eur J Immunol*. 2015;45:958–965.
53. Biton J, Semerano L, Delavallee L, et al. Interplay between TNF and regulatory T cells in a TNF-driven murine model of arthritis. *J Immunol*. 2011;186:3899–3910.
54. Huang Z, Yang B, Shi Y, et al. Anti-TNF-alpha therapy improves Treg and suppresses Teff in patients with rheumatoid arthritis. *Cell Immunol*. 2012;279:25–29.
55. Nadkarni S, Mauri C, Ehrenstein MR. Anti-TNF-alpha therapy induces a distinct regulatory T cell population in patients with rheumatoid arthritis via TGF-beta. *J Exp Med*. 2007;204:33–39.
56. Pang L, Wang L, Suo T, et al. Tumor necrosis factor-alpha blockade leads to decreased peripheral T cell reactivity and increased dendritic cell number in peripheral blood of patients with ankylosing spondylitis. *J Rheumatol*. 2008;35:2220–2228.
57. Calleja S, Cordero-Coma M, Rodriguez E, et al. Adalimumab specifically induces CD3(+) CD4(+) CD25(high) Foxp3(+) CD127(-) T-regulatory cells and decreases vascular endothelial growth factor plasma levels in refractory immuno-mediated uveitis: a non-randomized pilot intervention study. *Eye (Lond)*. 2012;26:468–477.
58. Sugita S, Yamada Y, Kaneko S, et al. Induction of regulatory T cells by infliximab in Behcet's disease. *Invest Ophthalmol Vis Sci*. 2011;52:476–484.