Phase I, Double-blind, Randomized, Placebo-controlled, Dose-escalation Study of NI-0401 (a Fully Human Anti-CD3 Monoclonal Antibody) in Patients with Moderate to Severe Active Crohn's Disease

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Background: NI-0401 is a fully human monoclonal antibody, which binds to the CD3 subunit of the T-cell receptor, causing modulation of T-cell activity. We investigated the safety and the ability to modulate the TCR-CD3 complex of NI-0401 in patients with active Crohn's disease (CD).

Methods: A double-blind, placebo-controlled, randomized, multicenter, dose-escalating trial was conducted in CD patients age 18–70 years, a Crohn's Disease Activity Index (CDAI) of 220–450, and detectable levels of C-reactive protein. The primary outcome was safety and the ability of NI-0401 to modulate the TCR-CD3 complex on T cells. Efficacy parameters included the proportion of patients achieving remission (CDAI <150), clinical response (CDAI fall ≥ 100), and change from baseline in the CD Endoscopy Index of Severity (CDEIS).

Results: Forty patients received placebo ($n = 7$) or NI-0401 ($n = 33$) 0.05–10 mg daily for 5 days. NI-0401 doses ≤ 1 mg were well tolerated. Infusion reactions occurred at doses ≥ 2 mg. The extent and duration of TCR-CD3 modulation increased with dose. No differences between groups were observed in the proportions of patients achieving clinical remission or response. The mean CDEIS at week 6 differed significantly between the 1-mg and placebo group.

Conclusions: NI-0401 was tolerated at doses ≤ 1 mg with manageable side effects. NI-0401 induced a dose-dependent modulation of the TCR-CD3 complex. No significant improvement of CDAI was observed but 1 mg NI-0401 demonstrated an improvement in CDEIS.

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Key Words: Crohn's disease, biologic therapies, anti-CD3, phase I clinical trial, NI-0401

Crohn's disease (CD), a chronic inflammatory condition involving the gastrointestinal tract, is characterized by recurrent exacerbations and remissions. Understanding of the underlying pathogenesis, which has rapidly evolved over the last decade, has lead to the development of more specific and potent drugs for the treatment of CD. Current therapies, aimed at inducing and maintaining remission and avoiding surgery, include corticosteroids, immunosuppressives, and inhibitors of tumor necrosis factor alpha (TNF-α).[1,2] CD has been associated with activation of intestinal T lymphocytes, leading to a Th1/Th17 type of inflammatory response with secretion of interferon gamma (IFN-γ), interleukin (IL)-17, and TNF.[3] The pivotal pathophysiological role of T cells in CD has been further demonstrated by the observation that anti-CD4, anti-IL-12/23, and anti-TNF

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antibodies effectively deplete pathogenic T cells. Consequently, direct inhibition of T-cell activity via modulation of CD3 may represent a new treatment option in CD. This is supported by preliminary data on the effect of visilizumab, a humanized anti-CD3 monoclonal antibody (mAb), on moderate to severe CD. However, treatment with visilizumab is restricted by its safety profile due to cytokine release.[4] NI-0401 is a fully human anti-CD3 mAb) that has been designed to address the need for a CD3-directed antibody with an improved safety profile. NI-0401 has a mutated IgG1 Fc arm designed to reduce the binding to Fc gamma receptors in order to minimize the infusion-related reactions (IRRs) associated with cytokine release.[5]

Here we report the results of a randomized, double-blind, placebo-controlled, dose-escalation study that evaluated the safety of NI-0401 and the ability of this compound to modulate the TCR-CD3 complex on T cells in patients with moderate to severe active CD despite therapy.

MATERIALS AND METHODS

Patients

This was a randomized, prospective, placebo-controlled, double-blind, dose-escalation trial conducted at 12 hospital centers located in The Netherlands, Belgium, Poland, and the United Kingdom.

Eligible patients, males and females, were 18–70 years of age, with confirmed active CD of at least 6 months duration. Active disease was defined as a CD Activity Index (CDAI) of 220–450 with detectable CRP level ($\geq$0.1 mg/L) and endoscopically confirmed colitis, ileitis, or ileocolitis.

Patients were excluded if any of the following criteria were present: a history or suspicion of active tuberculosis, requirement for immediate surgery, surgery within 3 months prior to screening, confounding gastrointestinal conditions, a symptomatic bowel stenosis, current or recent severe infection (within 3 months of screening), or a history of cancer.

Study Design

The initial intention was to randomize patients to escalating doses of NI-0401, from 10 mg to 80 mg, or placebo on 5 consecutive days (Clinical trials gov. identifier NCT00630643 listed on www.clintrials.gov).

However, after an acute IRR observed in the first patient exposed to 10 mg, subsequent patients were infused with lower doses: 0 (placebo, $n = 7$); 0.05 mg ($n = 5$); 0.1 mg ($n = 3$); 0.5 mg ($n = 11$); 1 mg ($n = 12$), and 2 mg ($n = 1$) administered as a 2 hours intravenous (i.v.) infusion daily for 5 days. Due to the side effects in the patient receiving 2 mg, the dose was not escalated above 1 mg. Safety was evaluated by a Data Monitoring Committee (DMC) and the decision to dose escalate was based on the safety data, the extent and duration of TCR-CD3 complex modulation, and peripheral circulating T-cell count at prior to each intended dose-escalation group. The DMC requested the code to be broken for the first patient. Patients were followed for 6 months posttreatment. Patients underwent screening and baseline evaluations, including baseline colonoscopy, before randomization.

Study Drug and Randomization

NI-0401 was generated using Medarex's HuMAb Mouse technology, which is a transgenic line containing key gene sequences from human genes that code for both the heavy and light chains of human immunoglobulins. NI-0401 is a fully human monoclonal IgG1k antibody directed against the CD3 epsilon chain of the TCR-CD3 complex expressed on T cells. The heavy chain constant region of NI-0401 is mutated from leucine to alanine and glutamic acid at positions 234 and 235, respectively.[6]

The study drug was provided in vials containing 40 mg of NI-0401 or placebo with no possible distinction between the two. An adequate quantity of NI-0401 or placebo was diluted with 100 mL of NaCl and infused over 2 hours. Eligible patients were randomized in steps of higher doses to the trial via central randomization in a blinded manner. Site pharmacists received blinded study treatment kits that were labeled with a kit number. A computer-generated randomization list was produced by the study statistician and kept in a secure place with restricted access to the statistician only. Following identification of an eligible patient, sites faxed information to the study coordinating center. After confirmation of patient eligibility the study statistician provided the site with the kit number to be used for the patient. In the event of a medical emergency, sites could request identification of the study treatment assignment if necessary to ensure effective management of the event. All study personnel and patients were blinded.

Concomitant Medical Therapies

Concomitant use of oral corticosteroids ($\leq$20 g daily prednisone or equivalent) or 5-aminosalicylate (5-ASA) agents (at a stable dose for at least 2 weeks prior study day 1 [SD1] visit), thiopurines, or methotrexate (both at a stable dose for at least 8 weeks prior to SD1 visit) was permitted provided that treatment remained stable at least until week 6 of the study period. Patients who had received anti-TNF agents were also eligible to participate, but were excluded if the last dose of treatment was less than 3 months of the SD1 visit. Premedication with analgesics/antipyretics (acetaminophen or ibuprofen) was permitted but not mandatory.

Patient Schedule and Safety/Efficacy Evaluations

Patients were assessed at baseline and days 1, 2, 3, 4, 5, 8, 10, 14, 21, 28, 48, 64, 96, and 192. The primary

outcome measure was the safety of NI-0401. Adverse events (AEs) were characterized according to severity as assessed by the investigator using the WHO Toxicity Grading Criteria. T-cell receptor (TCR)-CD3 modulation was a co-primary endpoint. It was expressed as the percentage of TCR-CD3 complexes removed per cell at a given study visit relative to baseline. The expression levels of CD3 on T cells were measured by flow cytometry. Briefly, peripheral venous blood was collected by venipuncture into Vacutainer CPT tubes containing sodium citrate. Anticoagulated whole blood (50 μL) was incubated for 30 minutes at 4°C with FITC-anti-CD8, PerCP-anti-CD4, and anti-CD3 antibody conjugated with phycoerythrin (PE) at a 1:1 ratio. After incubation with FACS lysing solution for 30 minutes at room temperature, the samples were analyzed by flow cytometry. The QuantiBRITE system, a set of four calibrated beads with known levels of PE, was used for quantification of CD3 expression on $CD4^+$ and $CD8^+$ T cells. The mean CD3 fluorescence intensity were converted into number of antibodies bound per cell (ABC) as described in the manufacturer's instructions. The ABC value, used for the calculation of the TCR-CD3 modulation, corresponds to the mean of duplicate measurements. All reagents used for the quantification of CD3 expression were purchased from BD Biosciences (San Jose, CA). The pharmacodynamic effect of NI-0401 on circulating lymphocyte count was also evaluated by flow cytometry. Absolute counts of $CD4^+$ and $CD8^+$ T cells were measured using Trucount tubes and the following fluorochrome-conjugated antibodies: FITC-anti-CD8 and PerCP-anti-CD4 as described in the manufacturer's instructions (BD Biosciences). Secondary objectives included the therapeutic response to NI-0401 over time defined as either clinical remission (a CDAI score of less than 150 postbaseline) or clinical response (a CDAI reduction of at least 100 points from baseline), and the effect of NI-0401 on mucosal repair using the Crohn's Disease Endoscopy Index of Severity (CDEIS). Endoscopy was performed at the local center; visual images of the endoscopic examination were sent to a central scoring center (P. Rutgeerts, Leuven, Belgium), which provided the CDEIS scores for all patients. An endoscopy was performed on each patient during screening to confirm the presence of active disease. An endoscopic response was defined as a decrease of at least 3 points in the CDEIS (week 6 compared to baseline). Serum NI-0401 levels were measured with a Gyrolab-based assay using an mAb specific for NI-0401 idiotype. Briefly, the anti-NI-0401 anti-idiotypic mAb was biotinylated and immobilized on streptavidin-coated beads at 0.1 mg/mL into Gyrolab Bioaffy CD microlaboratories. Test serum samples were added at a one-third final dilution and the presence of NI-0401 was detected using an Alexa Fluor antihuman IgG1 antibody at 50 nM. Fluorescence intensity was measured using the Gyrolab workstation LIF. The method has not been validated yet. However, quality control samples were used to ensure some level of confidence in the drug concentrations measured. For this purpose NI-0401 was spiked at four concentrations (20, 150, 800, and 5000 ng/mL) in the predose samples of each patient tested. The lower limit of detection and quantification of the assay varied between patients ranging from 10–50 ng/mL and 100–150 ng/mL, respectively.

Plasma/serum samples were analyzed for Epstein–Barr virus (EBV) viral load, cytokines, and C-reactive protein (CRP) levels. EBV viral load was assessed by polymerase chain reaction (PCR) in plasma and the limit of detection of the assay was 500 copies EBV DNA/mL. Serum levels of IL-2, IFN-γ, TNF-α, and IL-6 were measured using a validated multiplex bead assay with lower limits of quantification of 7.25, 8.0, 10.8, and 7.1 pg/mL respectively. Levels of CRP were measured in serum using Roche Immunoturbidimetry methodology as described by the manufacturer.

Anti-drug antibody was assessed by enzyme-linked immunosorbent assay (ELISA) in plasma samples using a standard bridging assay at predose and weeks 12 and 24. Briefly, microtiter plates were passively coated with 100 μL of NI-0401 at 0.2 μg/mL. Test plasma samples were added at a 1/50 final dilution and the presence of antidrug antibodies was detected using biotinylated NI-0401 at 0.2 μg/mL. Human plasma from patients with CD was used as the matrix to validate the assay. The validation of the assay demonstrated specificity and sensitivity using an affinity-purified anti-NI-0401 rabbit polyclonal antibody that was generated as positive control. The assay sensitivity was 200 ng/mL.

Statistics

As this was the first clinical evaluation of NI-0401, there were no precise assumptions about the magnitude of the expected therapeutic effect. Data from each dose group were analyzed separately. The intention to treat (ITT) population included all randomized patients who received at least one dose of study medication. All safety data were analyzed using the ITT population only, while efficacy analysis was also performed using the per protocol (PP) population (excluding patients for which efficacy could not be assessed properly). For continuous parameters (change from baseline CDAI, CDEIS) comparisons between each active treatment group and placebo were made using analysis of variance (ANOVA) methods (SAS v. 9.1, Cary, NC) calculated using a general linear model with a Dunnett's test to control for multiplicity. Categorical parameters (proportions of patients with remission, clinical response) were compared between each active treatment group (excluding the individual patients who received 2 mg and 10 mg) and

placebo using a Pearson chi-square statistic. In order to assess true effect of NI-0401, patients receiving increased doses and/or additional CD therapies during the first 6 weeks of the study were considered nonresponders regardless of the change in the CDAI.

An independent statistical review was performed by E.W. Steyerberg, PhD (Department of Public Health, Erasmus MC, Rotterdam, The Netherlands).

Role of the Sponsor

The study was designed by the sponsor, NovImmune, in collaboration with the Initiative on Crohn's and Colitis, The Netherlands (ICC). Trial execution was supervised by the sponsors in collaboration with the investigators and the contract research organizations (Clinquest, Genexion, QED, and Monipol).

Ethical Considerations

The Institutional Review Board at each site approved the protocol. All patients gave written informed consent.

RESULTS

Patients

A total of 48 patients were screened for study participation between February 2006 and June 2007, of whom 40 patients were randomized into this trial (Fig. 1). Reasons for screen failure included a low CDAI score ($n = 5$), confounding gastrointestinal conditions (*Clostridium difficile*-positive stool culture, $n = 2$), and absence of endoscopic inflammation ($n = 1$).

The baseline characteristics of the groups were similar (Table 1). Three patients did not complete the 5-day treatment period and four others did not complete the 24 weeks of follow-up (Fig. 1). Infusion reactions were the main reason for noncompletion of the 5 days of study treatment. The initial intention was to randomize patients to escalating doses of NI-0401, starting at a dose of 10 mg daily. However, after an acute IRR observed in the first patient exposed, subsequent patients were infused with lower doses: 0 (placebo, $n = 7$); 0.05 mg ($n = 5$); 0.1 mg ($n = 3$); 0.5 mg ($n = 11$); 1 mg ($n = 12$); and 2 mg ($n = 1$) administered as a 2-hour i.v. infusion daily for 5 days. Following a severe infusion reaction in the first patient receiving a 2-mg i.v. dose, the dose escalation was halted. Patients who received less than two infusions of NI-0401 ($n = 3$), had a concomitant disease (colorectal cancer diagnosed within 2 weeks of study entry, $n = 1$), or had missing efficacy data ($n = 1$) were excluded from the ITT population (Fig. 1).

Safety

The overall incidence of AEs was higher in the NI-0401 infused groups compared to the placebo group, with the most common events reported as mild. Premedication with analgesics/antipyretics (acetaminophen or ibuprofen) was given to 65% of the patients ($n = 26$).

The first exposed patient to 10 mg NI-0401 developed, after the first infusion, IRRs characterized by mild chills, severe nausea, fever, vomiting, headache, diarrhea, and hypotension.

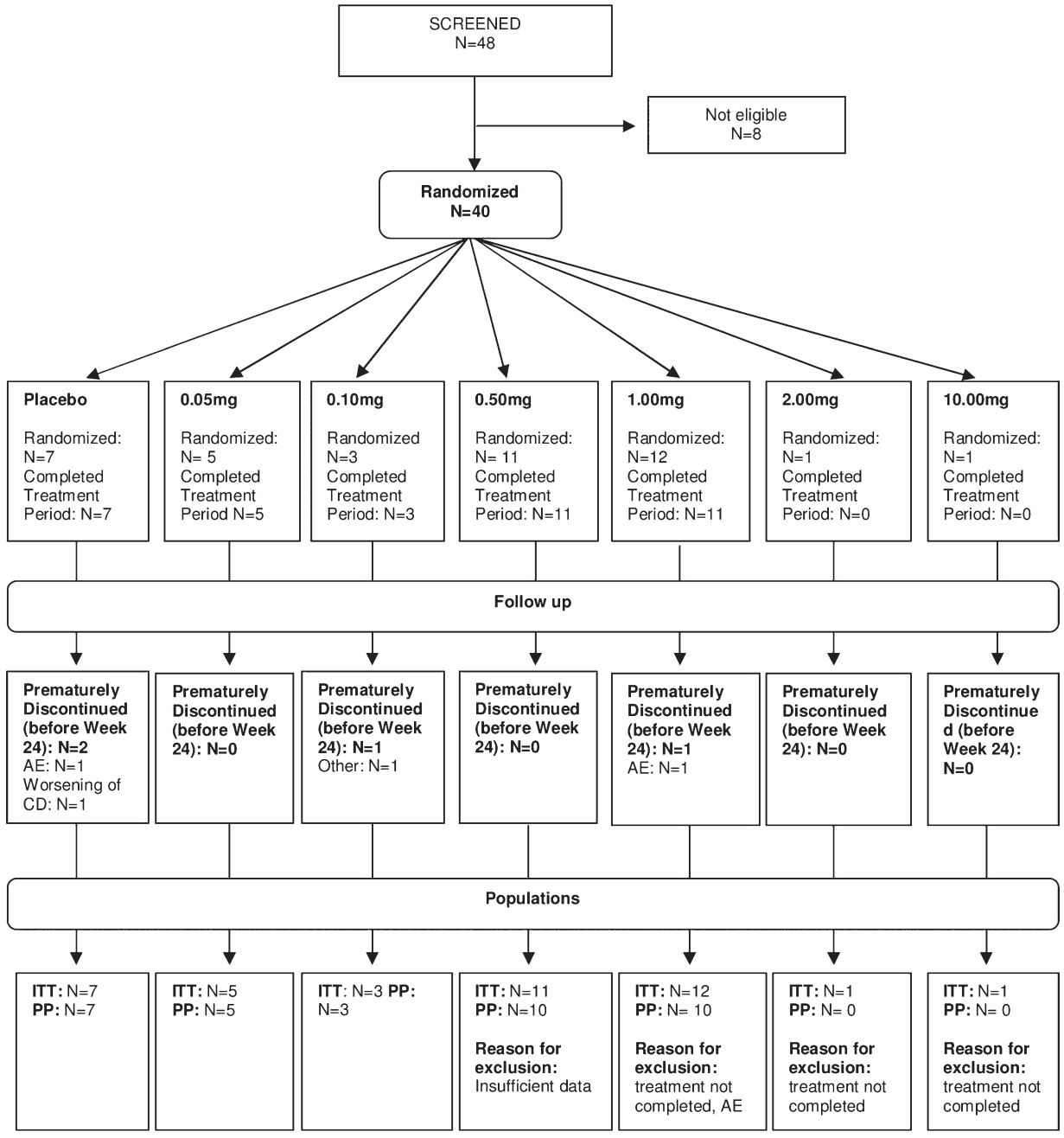
Doses up to 1 mg daily for 5 days were generally well tolerated but IRRs occurred in all patients treated with NI-0401. The most common reported IRRs were fever (80%), headache (62.5%), and nausea (50%) (Table 2). In the majority of cases these symptoms were mild (66%) or moderate (33%) in intensity and were reported following the first two infusions of the five-infusion treatment course. The severity tended to increase with increasing dose of NI-0401.

A single patient was exposed to 2 mg NI-0401 and experienced severe IRRs characterized by myalgia, fever, nausea, vomiting, and diarrhea with associated hypotension on the first infusion day. These symptoms persisted over 24 hours and although the patient recovered without any symptoms, the sponsor and DMC decided to stop the dose escalation.

Following the completion of the treatment period and during the remaining 6-month observation period, 85% of the patients reported at least one study drug-related AE. Infections were reported by 17 patients. Six were considered drug-related of moderate or severe intensity. Five were reported as moderate: one in each of the placebo and treatment groups between 0.05 and 2 mg. The reported infections were respectively: nasopharyngitis, perianal abscess, an infected ear lobe, an oral fungal infection, and herpes labialis. The only AE graded as a severe infection was an acute tonsillitis that occurred in the 10-mg patient.

Fifteen serious adverse events (SAEs) were reported, seven of which were considered unrelated to study medication (Table 3). Six of the eight related SAEs were all associated with infusion reactions. The other two were: acute tonsillitis, which was reported in the single patient exposed to the 10 mg dose, and an intestinal obstruction due to worsening of CD which occurred on study day 5 and was fully resolved on study day 7 in a patient who received treatment with 0.1 mg NI-0401. No deaths occurred.

No clinically significant laboratory abnormalities were observed at any dose level other than the expected effects on T-cell dynamics such as transient reduction of circulating T-cell count. The plasma concentrations of cytokines increased during the first 2 days of treatment with NI-0401 (data not shown) and levels tended to rise with increasing doses of NI-0401. In the majority of the patients, elevations of IL-6 were found. It was not possible



ITT: Intention To Treat
PP: Per Protocol

FIGURE 1. Study schedule. Schematic overview of the different treatment groups and number of included patients.

to establish a relationship between cytokine release and IRR symptoms in most cases.

EBV reactivation was noted in 5/11 patients in the 0.5-mg dose group at weeks 3, 4, and/or 8 and in 6/12 patients in the 1-mg dose group at weeks 2, 3, 4, 6, and/or 8 (data not shown). The maximal duration of EBV reactivation was 2 weeks in the 0.5-mg group and 4 weeks in the 1-mg group. EBV reactivation was also observed at week 3 in the patient who received one infusion of 10 mg. No frank clinical symptoms were associated with EBV

TABLE 1. Baseline Characteristics of Study Population

Characteristics	Placebo $n = 7$	0.05 mg $n = 5$	0.10 mg $n = 3$	0.50 mg $n = 11$	1.00 mg $n = 12$	2.00 mg $n = 1$	10.0 mg $n = 1$	Total $n = 40$
Male %	4 (57)	0 (0)	1 (33)	6 (55)	5 (42)	0 (0)	1 (100)	17 (43)
Mean age (SD)	34.3 (10.1)	32.8 (18.1)	34.3 (11.2)	28.8 (6.5)	33.2 (10.5)	52	27	32.5 (9.6)
Mean disease duration (years) (SD)	10.6 (9.7)	9.2 (8.2)	11.0 (3.6)	7.5 (6.4)	6.7 (7.3)	6.0	2.0	8.1 (7.2)
Disease location n (%)								
- Colon	2 (28.6)	1 (20)	0 (0)	3 (27.3)	7 (58.3)	0 (0)	0 (0)	13 (32.5)
- Ileum	0 (0)	1 (20)	1 (33.3)	1 (9.1)	1 (8.3)	1 (100)	0 (0)	5 (12.5)
- Ileocolonic	5 (71.4)	3 (60)	2 (66.7)	7 (63.6)	4 (33.3)	0 (0)	1 (100)	22 (55)
Mean CRP (mg/L) (SD)	11.8 (18.0)	34.4 (33.2)	11.7 (15.0)	21.3 (22.9)	14.0 (10.8)	4.6	5.0	17.5 (20.1)
Mean CDAI (SD)	323.1 (84.6)	296.2 (39.2)	350.7 (116.1)	321.5 (67.4)	340.9 (73.8)	266.0	315.0	325 (70.6)
No. of patients with endoscopy	6	5	3	10	11	1	1	37
Mean CDEIS (SD)	9.2 (5.9)	15.9 (12.5)	11.8 (11.1)	11.6 (8.4)	14.7 (10.2)	14.4	23.3	13.1 (9.1)
Steroids	3 (43%)	2 (40%)	1(33%)	4 (36%)	3 (25%)	—	—	13 (32%)
Immunosuppressives	5 (71%)	4 (80%)	2 (67%)	7 (64%)	7 (58%)	1 (100%)	—	26 (65%)
5 ASA agents	1 (15%)	1 (20%)	1 (33%)	4 (36%)	4 (33%)	—	—	11 (27%)
Prior anti-TNF	4 (57%)	5 (100%)	3 (100%)	7 (64%)	10 (83%)	1 (100%)	—	30 (75%)

reactivation other than tonsillitis in the single patient that received the single infusion of 10 mg NI-0401 and in all cases the virus was spontaneously cleared.

Pharmacodynamic Effects on T Cell

Figure 2 displays the mean percentage modulation of CD3 expression noted at each dose level with the exception of 2 and 10 mg. Both the magnitude and duration of CD3 modulation increased in a dose-related manner. In all groups the CD3 expression levels returned to baseline ≈1 week after the end of treatment (day 14).

A transient reduction in the total peripheral T-cell count (both $CD4^+$ and $CD8^+$ subsets) was noted following treatment with NI-0401. On average the peripheral T-cell

TABLE 2. Number of Patients Experiencing Infusion-related Reaction (IRR; Percentage) Per Treatment Group Reported in >10% of the Patients

Event	Placebo	0.05 mg	0.10 mg	0.50 mg	1.00 mg	2.00 mg	10.0 mg	All
Subject experiencing IRR	3 (42.8%)	5 (100%)	3 (100%)	11 (100%)	12 (100%)	1 (100%)	1 (100%)	36 (90%)
Cardiac disorders								
Hypotension	0 (0%)	0 (0%)	0 (0%)	4 (36.4%)	5 (41.7%)	1 (100%)	1 (100%)	11 (27.5%)
Gastrointestinal disorders								
Diarrhea	0 (0%)	0 (0%)	0 (0%)	1 (9.1%)	5 (41.7%)	1 (100%)	1 (100%)	8 (20%)
Nausea	1 (14.3%)	0 (0%)	2 (66.7%)	6 (54.5%)	9 (75%)	1 (100%)	1 (100%)	20 (50%)
Vomiting	0 (0%)	1 (20%)	2 (66.7%)	3 (27.3%)	10 (83.3%)	1 (100%)	1 (100%)	18 (45%)
General disorders and administration site conditions								
Chills	0 (0%)	0 (0%)	0 (0%)	7 (63.6%)	9 (75%)	0 (0%)	1 (100%)	17 (42.5%)
Fever	2 (28.6%)	4 (80%)	2 (66.7%)	10 (90.9%)	12 (100%)	1 (100%)	1 (100%)	32 (80%)
Nervous system disorders								
Dizziness	3 (42.9%)	2 (40%)	1 (33.3%)	2 (18.2%)	1 (8.3%)	0 (0%)	0 (0%)	9 (22.5%)
Headache	1 (14.3%)	4 (80%)	2 (66.7%)	8 (72.7%)	8 (66.7%)	1 (100%)	1 (100%)	25 (62.5%)
Musculoskeletal and connective tissue disorders								
Myalgia	1 (14.3%)	0 (0%)	0 (0%)	3 (27.3%)	2 (16.7%)	1 (100%)	0 (0%)	7 (17.5%)
Skin and subcutaneous tissue disorders								
Skin rash	1 (14.3%)	1 (20%)	1 (33.3%)	2 (18.2%)	7 (58.3%)	0 (0%)	0 (0%)	12 (30%)

TABLE 3. SAE Reported Per Dose Group

	Placebo	0.05 mg	0.10 mg	0.50 mg	1.00 mg	2.00 mg	10.0 mg	ALL
	$n = 7$	$n = 5$	$n = 3$	$n = 11$	$n = 12$	$n = 1$	$n = 1$	$n = 40$
Gastrointestinal disorders	1 (14.3%)	1 (20%)	2 (66.7%)	1 (9.1%)	2 (16.6%)	—	—	7 (17.5%)
Abdominal pain	—	—	—	1 (9.1%)	—	—	—	—
CD aggravated	1 (14.3%)	—	—	—	—	—	—	—
Colonic fistula	—	—	—	—	1 (8.3%)	—	—	—
Colon carcinoma	—	—	—	—	1 (8.3%)	—	—	—
Anorexia	—	—	1 (33.3%)	—	—	—	—	—
Intestinal obstruction	—	—	1 (33.3%)	—	—	—	—	—
Vomiting	—	1 (20%)	—	—	—	—	—	—
Immune system disorders	—	—	—	3 (63.6%)	1 (8.3%)	1 (100%)	1 (100%)	6 (15%)
Cytokine release Syndrome	—	—	—	3 (63.6%)	1 (8.3%)	1 (100%)	1 (100%)	6 (15%)
Infections and infestations	—	—	1 (33.3%)	—	—	—	1 (100%)	2 (5%)
Acute tonsillitis	—	—	—	—	—	—	1 (100%)	1 (2.5%)
Perianal abscess	—	—	1 (33.3%)	—	—	—	—	1 (2.5%)

count returned to baseline levels within 3 weeks. For five patients in the 0.5 mg ($n = 1$) and 1 mg ($n = 4$) dose groups, the $CD8^+$ T cell counts returned to baseline values by week 4 (data not shown).

Pharmacokinetics

Serum NI-0401 levels for most patients were below the level of quantification of the assay. The three patients exposed to doses greater than 20 μg/kg body weight (one patient in the 1-mg dose group and the patients in the 2-mg and 10-mg dose groups) had detectable serum levels of NI-0401. The concentration profiles in these patients were sufficient to enable calculation of area under the curve $(AUC)_{0\text{-}6h}$, which showed that exposure to NI-0401 increased in a dose-related manner (data not shown).

Immunogenicity

Plasma from each patient was assessed for an anti-drug antibody response 3 and 6 months after initiation of treatment. All patient samples tested negative for NI-0401 antibodies (data not shown).

Clinical Remission and Response

At inclusion the mean CDAI score of the 40 randomized patients was 325 (SD = 70.6) with no significant differences between study groups ($P = 0.88$, Table 1). There was no significant difference between the placebo group and the treatment groups in the frequencies of patients achieving a clinical response or clinical remission at any timepoint posttherapy (Table 4).

Endoscopic Response

Valid endoscopy recordings at both baseline and week 6 were obtained for 35 of the randomized patients. Consequently, the endoscopy analyses consisted of 35 patients in the ITT population and 31 patients in the PP population (Table 5). One patient in the 1-mg dose group with an endoscopy outcome was excluded from the ITT endoscopic analyses because this patient had inflammation in the ileum that could not be visualized and was not scored. Endoscopy scores for other parts of the gastrointestinal tract of this subject were zero at all timepoints. The week 6 mean change from baseline CDEIS was −4.7 in the NI-0401 1-mg group (ITT population) compared to an increase

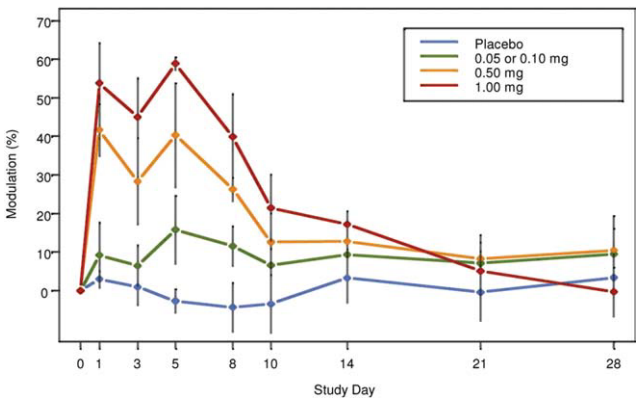


FIGURE 2. Mean CD3 modulation by dose group. Both the magnitude and duration of CD3 modulation increased in a dose-related manner (error bars on data points represent the standard error of the mean).

TABLE 4. CDAI Mean and Median Up to Week 6

	Placebo $n = 7$	0.05 mg/0.1mg $n = 8$	0.50 mg $n = 11$	1.00 mg $n = 12$	2.00 mg $n = 1$	10.0 mg $n = 1$
SD1 mean/median	323.1/348	316.6/310.5	320.4/316	340.9/327	266/266	315/315
SD2 mean/median	287.0/304	292.8/272	255.5/272	292.5/297	330/330	254/254
Week 2 mean/median	276.4/308	305.6/299.5	224.9/220	242.3/245	228/228	182/182
Week 4 mean/median	278.6/273	287/271.5	248.9/268	300.5/287	161/161	Unknown
Week 6 mean/median	231.3/210	285.3/292	253.3/259	234.4/230	174/174	246/246

TABLE 5. Mean (± SD) Difference in Endoscopic Scores Between Week 6 and Baseline

Population	Endoscopic Score	Placebo	0.05/0.10 mg	0.50 mg	1.00 mg	2.00 mg	10.0 mg
ITT	*n*	6	8	9	10	1	1
	CDEIS	1.9 ± 3.2	−1.0 ± 3.7	1.5 ± 4.8	−4.7 ± 5.3	14.5	−16.9
PP	*n*	6	8	8	9	—	—
	CDEIS	1.9 ± 3.2	−1.0 ± 3.7	1.6 ± 5.1	−5.2 ± 5.3	—	—

of 1.9 in the placebo group ($P = 0.0353$). The mean CDEIS change for the 1-mg dose group in the endoscopic PP population was −5.2 as compared to 1.9 in the placebo group ($P = 0.0163$). This demonstrates that 8/9 (89%) patients receiving 1 mg NI-0401 decreased CDEIS score between baseline and week 6, compared with only 1 patient (17%) in the placebo group. 77.8% of the patients in the 1-mg dose group (PP population) achieved an endoscopic response (Table 6).

CRP Level as an Outcome Measurement

The mean CRP at study entry was 17.5 mg/L (SD = 20.1). No statistically significant difference was seen in the mean change of CRP level between study entry and week 6.

DISCUSSION

The induction and maintenance of disease remission is a major therapeutic goal when treating patients with active CD. A majority of patients fail to maintain remission over time, notwithstanding treatment with immunosuppressives. Currently, immunosuppressive agents and anti-TNF compounds represent the only therapeutic options to maintain remission in CD. However, in about half of the patients this strategy is not effective.[7–9] Furthermore, the side effects of these drugs limit their use and may lead to noncompliance and drug discontinuation in the long run.[10] Therefore, there remains an important unmet medical need for additional therapies that may offer prolonged response.

As CD is associated with activation of T cells and production of a T-cell inflammatory response, treatments aimed at reducing T-cell activation may pose an alternative therapeutic goal for this condition. In addition, treatment with anti-CD3 monoclonal antibodies in type I diabetes has demonstrated ongoing preservation of the β-islet cell function during a follow-up of 2 years following an initial short-term course of therapy, suggesting that this mechanism of action may reinstate immune tolerance.[11,12] NI-0401 is a fully human monoclonal antibody that binds to the CD3 chain on T cells, causing internalization of the TCR-CD3 complex and downregulation of T-cell activation following antigen exposure, which in turn can be expected to reduce inflammation in affected tissues.

Although NI-0401 was designed to limit drug-induced cytokine release, its administration resulted in the production of proinflammatory cytokines, albeit at much lower levels than that observed following i.v. administration of OKT3.[13,14] Like the other non-Fc gamma receptor-

TABLE 6. Frequency and Number of Endoscopic Responders in ITT and PP Populations

Population	Placebo	0.05/0.10 mg	0.50 mg	1.00 mg	2.00 mg	10.0 mg
ITT	0.0% (0/6)	25.0% (2/8)	11.1% (1/9)	70.0% (7/10)	0.0% (0/1)	0.0% (0/1)
PP	0.0% (0/6)	25.0% (2/8)	12.5% (1/8)	77.8% (7/9)	—	—

binding anti-CD3 mAbs, NI-0401 probably acts as a partial agonist of the TCR-CD3 complex, causing transient T-cell activation in an Fc gamma receptor-independent manner. The underlying mechanisms responsible for the residual cytokine release observed following i.v. treatment with NI-0401 remain unclear. Pretreatment with corticosteroids has been demonstrated to ablate the cytokine release following OKT3 administration in patients.[15] In the present study, pretreatment was allowed with acetaminophen, but not with corticosteroids.

Limited conclusions regarding the therapeutic effect of NI-0401 on CD can be made from this initial safety study. This study was not powered to assess clinical efficacy and no significant improvement of CDAI was observed. The endoscopic response was defined as a decrease of at least 3 points in the CDEIS and a simultaneous decrease in the SES-CD of at least 2 points. GETAID has proposed that a significant endoscopic improvement can be defined as a reduction in the CDEIS score of 4.5.[16]

On average, the CDEIS score was reduced by more than 4.5 points in the 1-mg dose group and using this cut-off value to define the clinical response, six patients would have been considered as endoscopic responders in the 1-mg dose group versus none in the placebo group. Although the pharmacokinetic analysis in most of the patients could not be conducted because the concentration values were very close or lower than the lower limit of quantification, it can be hypothesized that even low concentrations of NI-0401 can induce response at mucosal levels.

Clinical and nonclinical studies suggest that efficacy is associated with the level of CD3/TCR modulation. It can be hypothesized from the dose-effect response on the modulation of the T cells demonstrated in this study that higher doses of NI-0401 in association with a short course of corticosteroid premedication may enable longer-term T-cell modulation, which may increase the potential for induction and potentially maintenance of remission.

In conclusion, this is the first study exploring the safety of a fully human monoclonal anti-CD3 antibody in patients with moderate to severe CD. Although NI-0401 was designed to limit drug-induced cytokine release, its administration at the intended concentrations resulted in acute IRRs associated with cytokine release. Treatment with NI-0401 at low concentrations is safe and was able to induce CD3 modulation; however, the clinical effect was limited. Further studies are needed to elucidate the effect of NI-0401 in combination with pretreatment with corticosteroids in order to reduce infusion-related symptoms at higher concentrations.

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