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Phase 1 Clinical Study of siRNA Targeting Carbohydrate Sulphotransferase 15 in Crohn's Disease Patients with Active Mucosal Lesions

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Abstract

Background and Aims: Carbohydrate sulphotransferase 15 [CHST15] is a specific enzyme biosynthesizing chondroitin sulphate E that binds various pathogenic mediators and is known to create local fibrotic lesions. We evaluated the safety of STNM01, a synthetic double-stranded RNA oligonucleotide directed against CHST15, in Crohn's disease [CD] patients whose mucosal lesions were refractory to conventional therapy.

Methods: This was a randomized, double-blind, placebo-controlled, concentration-escalation study of STNM01 by a single-dose endoscopic submucosal injection in 18 CD patients. Cohorts of increasing concentration of STNM01 were enrolled sequentially as 2.5 nM [$n = 3$], 25 nM [$n = 3$], and 250 nM [$n = 3$] were applied. A cohort of placebo [$n = 3$] was included in each concentration. Safety was monitored for 30 days. Pharmacokinetics was monitored for 24 h. The changes from baseline in the segmental Simple Endoscopic Score for CD [SES-CD] as well as the histological fibrosis score were evaluated.

Results: STNM01 was well tolerated and showed no drug-related adverse effects in any cohort of treated patients. There were no detectable plasma concentrations of STNM01 at all measured time points in all treatment groups. Seven of nine subjects who received STNM01 showed reduction in segmental SES-CD at Day 30, when compared with those who received placebo. Histological analyses of biopsy specimens revealed that STNM01 reduced the extent of fibrosis.

Conclusion: Local application of STNM01 is safe and well tolerated in CD patients with active mucosal lesions.

Key Words: Crohn's disease; CHST15; mucosal healing

1. Introduction

Medical treatment of Crohn's disease [CD] is based on modulation of mucosal inflammation. Symptom control alone can be largely achieved by the currently available treatment options.[1,2] However, actively inflamed mucosal lesions still remain in most patients, and are the scaffolds for progressive structural damage of the bowel wall. Therefore, treatment goals of CD are currently being conceptualized and include not only symptom control but also prevention of structural bowel damage such as intestinal strictures and impaired gastrointestinal physiology.[3–7] Especially, it is now reported that mucosal healing [MH] is the most important predictor of sustained clinical remission and resection-free survival in CD patients. However, induction of MH by the current systemic therapies is often limited and incomplete.[2,7]

The precise mechanism of persistence of active mucosal lesions is unexplored, thus the therapeutic strategy to achieve MH is not fully established. However, co-existence of ulcerations and inflammation/fibrosis in mucosal lesions is one of the main hindrances to achieve MH, as this contributes to chronic barrier dysfunction of the intestinal epithelium, which contributes to impaired mucosal repair.[2] In response to inflammation-driven bowel damage, 'fibrotic healing' occurs instead of adequate MH[8]; therefore targeting fibrotic healing is a reasonable approach to achieve adequate MH. Since a multifactorial process is involved in creating fibrotic lesions, therapeutic approaches against single mediators alone would not be sufficient to resolve fibrotic lesions.[8,9] We therefore focused on extracellular matrix [ECM] molecules that modulate the function of muliple mediators at sites of injury, and hypothesized that blockade of certain ECM molecules could normalize the mucosal architecture *in vivo*.

Carbohydrate sulphotransferase 15 [CHST15], formerly known as N-acetylgalactosamine 4-sulphate 6-O-sulphotransferase [GalNAc4S-6ST], is a type II transmembrane Golgi protein that biosynthesizes highly sulphated disaccharide units [E-units] of chondroitin sulphate [CS], which binds to various functional proteins and pathogenic microorganisms.[10–12] It was reported that the synthesis of oversulphated CS is significantly increased in the colon of patients suffering from active CD.[13] Oversulphated CS-E is reported to accelerate collagen fibril formation and to bind to type V collagen, which is increased in the submucosa of CD patients.[14–16] CS-E is also reported to bind to CD44, chemokines like MCP-1/CCL2 and SDF-1/CXCL12, and growth factors like PDGF and TGF-β, indicating its involvement in adhesion, migration and proliferation of fibroblasts.[9,12] CS-E is also reported to bind to Receptor for Advanced Glycation End-product [RAGE], further suggesting the involvement in fibrosis and tumour metastasis pathways.[17] We hypothesized that the CHST15/CS-E axis plays a vital role in creating and maintaining the mucosal fibrotic architecture in CD by providing a scaffold for fibroblasts and profibrotic mediators, and thus represents a potential therapeutic target for inflammation-driven fibroproliferative disease in the gut.

To explore the role of the CHST15/CS-E axis, we synthesized double-strand RNA oligonucleotides specifically designed to inhibit the expression of the CHST15 gene.[18] The effect of CHST15 siRNA on fibrosis was shown in rats with dilated cardiomyopathy[19] and in experimental colitis models.[20] We conducted a phase 1 clinical study in CD patients who did not achieve MH by previous treatments [non-healers]. Here we report safety and efficacy results of the phase 1 study and provide follow-up results.

2. Materials and Methods

2.1. Patients and inclusion/exclusion criteria in clinical study

Eighteen subjects were randomized. Inclusion criteria were as follows.

[i] Male/female;
[ii] CD with active endoscopic lesion[s] ranging from 1 to 11 in segmental Simple Endoscopic Score for Crohn's disease [SES-CD] [6,21]; Crohn's Disease Activity Index [CDAI] score of less than 150 [remission] or mild [151 to 219];
[iii] treated for more than 2 months before the screening tests by conventional drugs (mesalazine, corticosteroids, thiopurines, anti-tumour necrosis factor [TNF] antibodies) and experienced an insufficient mucosal response or resistance to the current conventional treatment confirmed by both the primary doctors and principal investigators;
[iv] the diameter of the narrowed mucosal lesion is 14 mm or more; and [v] between 18 and 65 years of age.

Exclusion criteria were as follows.

[i] History of serious cardiac, haematological, or pulmonary disease;
[ii] history of complete colon resection surgery for CD;
[iii] a complication of CD such as fistula, trephination, or bleeding, or intestinal adhesions to other organs;
[iv] anal stenosis or perianal abscess with fever;
[v] total parenteral nutrition;
[vi] a hepatic impairment or renal disorder;
[vii] history of malignant tumour within the past 5 years;
[viii] history of abdominal phthisis;
[ix] a complication of serious infection that requires hospitalization;
[x] treated with an anti-TNF- antibody and has one or more of the following conditions: [a] tuberculosis; [b] acute and chronic infection; [c] a repeated relapse and exacerbation of a chronic infectious disease; [d] a serious infection within the 24 weeks preceding the study drug administration; [e] a serious infection requiring hospitalization, [f] hepatitis B or C; [g] a live or attenuated vaccine within the 8 weeks preceding the study drug administration; and[h] a high risk of infection;
[xi] history of clinically serious allergic symptom;
[xii] positive for HBs antigen, HCV antibody, HIV antigen/antibody;
[xiii] alcohol or drug dependency;
[xiv] change in therapy regimen or addition of a new treatment for CD within the 14 days preceding the study drug administration;
[xv] participation or plans to participate in another clinical study during the course of this study;
[xvi] received any other investigational product within the 6 months preceding the informed consent;
[xvii] psychiatric or neurological disorder;
[xviii] incapable of or restricted to the protocol-directed examinations or procedures:
[xix] not willing and able to use a contraceptive method which the investigator considers reliable;
[xx] pregnancy or lactation for females.

2.2. Clinical study design

The overall study design is shown in Figure 1. This was a multicentre, randomized, double-blind, placebo-controlled, concentration-escalation study of STNM01 [synthesized by BioSpring, Germany] by a single-dose endoscopic submucosal injection. The mucosal lesion was identified by total colonoscopy at screening.

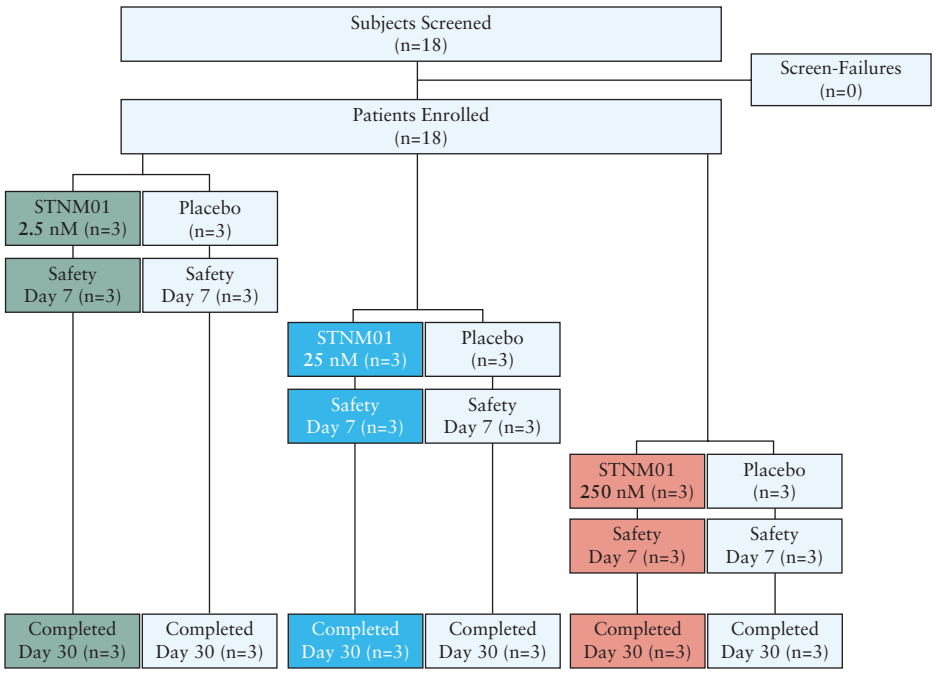

Figure 1. Study flowchart. Eighteen Crohn's disease [CD] patients were enrolled in the study. Three concentration levels of STNM01 were evaluated.

The biggest ulcer was selected [Segmental SES-CD was ≥ 3]. In the case of absence of ulcer, the lesion with the heaviest inflammation was selected [Segmental SES-CD was ≥ 1]. If the subject showed two or more lesions having the same severity, the lesion located closest to the anal verge was identified as the target lesion. STNM01 was submucosally administered to surround the periphery of the target lesion, using an endoscope. There were eight total injection sites. The volume of injection was 1 ml/site per injection, thus 8 ml/one lesion in total. The study was initiated with the STNM01 concentration of 2.5 nM in Step 1 [n = 3 + 3 placebo], followed by 25 nM in Step 2 [n = 3 + 3 placebo], and 250 nM in Step 3 [n = 3 + 3 placebo], after reviewing and confirming safety in the previous step. Dynamic allocation was used in the process. In each step, eligible subjects were admitted to the study site and received a single dose of the study drug on Day 1. Subjects were discharged after confirming that there were no safety concerns, at approximately 24 h after administration. They returned to the study site for follow-up examinations 7 and 30 days after administration. The changes from baseline in segmental SES-CD, CDAI, and histological fibrosis score at Days 7 and 30 were evaluated as efficacy endpoints [Figure 1].

2.3. Outcome measures in Phase 1 study

The primary objective of the study was to evaluate the safety of STNM01. The secondary and exploratory objectives were to assess the effect of STNM01 on endoscopy-based MH, biopsy-based fibrosis, and plasma drug concentrations. Safety data (incidence and type of adverse events [AEs];or markedly abnormal changes in laboratory values, vital signs and 12-lead electrocardiogram [ECG]) of all treated subjects were analysed according to treatment received. The incidence and the type of AEs were summarized by treatment groups. Efficacy analyses included all randomized subjects analysed by randomized treatment. The secondary endpoint was the change from baseline of the Segmental Simple Endoscopic Score for Crohn's Disease [SES-CD] at Day 30 and was evaluated by central review. Complete MH was judged as segmental SES-CD = 0, meaning no ulceration and no signs of active inflammation.[6,22] Partial MH was defined as clear endoscopic improvement but with persistent ulceration or present active inflammation.[6,22] As this study focused on one single mucosal lesion, partial MH was judged as decrease of segmental SES-CD > 1 point from baseline, indicating marked reduction of the size of the same ulcer, the percentage of the ulcerated surface, or the percentage of the affected surface. Endoscopic findings related to quality of ulcer healing were also evaluated by central review.

For histological analysis as an exploratory endpoint, two biopsies were taken from the proximal and distal sides of the edges of the target ulcerated mucosal lesion. In the case of absence of ulcers, biopsies were taken from the centre of the inflamed target lesion including scars. Biopsy specimens were fixed in 4% formalin, embedded in paraffin and were stained with Masson's trichrome [MT] methods.[23,24] The fibrosis score was estimated using MT-stained sections as follows: 0, normal; 1, pericryptic fibrosis; 2, fibrosis within mucosa; 3, fibrosis reaching to muscularis mucosa; 4, fibrosis reaching to submucosa.[24] Histological assessment for the fibrosis score was performed by three independent pathologists, blinded to clinical or treatment data. In the case of discrepancy between pathologists, the worst score was taken for assessment. The change from baseline in histological fibrosis scores at Day 30 was similarly evaluated by central review. Plasma drug concentrations at 5 min, 15 min, 30 min, 60 min, 90 min, 2 h, and 24 h after drug administration were measured by the enzyme-linked immunosorbent assay [ELISA] method described in 2.4. All tested samples showed values below the detection limit of quantification [0.5 ng/ml].

2.4. Plasma drug concentrarion

ELISA to detect siRNA in the plasma was performed according to Yu's method.[25] In brief, total RNA was transferred to 5% DMSO-PBS in a polymerase chain reaction [PCR] tube and incubated at 80°C. The 3'-biotinylated template probe [Japan Bio Servies] solution was added to the sample solution and incubated. After hybridization, the sample solution was incubated in a streptavidin-coated 96-well plate. After washing, each well was incubated with the digoxigenin-labelled ligation probe [Japan Bio Services] solution

containing T4 DNA ligase [TaKaRa]. Wells were washed with the washing buffer and with de-ionized water and incubated with the peroxidase-labelled anti-digoxigenin [1:1000 dilution, Roche, Switzerland]. After washing, enzymatic activity was detected using TMB [Sigma-Aldrich] and the absorbance was measured at 450 nm. To construct a standard curve, standards were prepared with known concentrations of STNM01. The concentration of plasma STNM01 was calculated from the standard curve.

2.5. Data analysis and statistical methods

Demographic and other baseline characteristics were summarized by tabulating frequency or providing descriptive statistics for each treatment group. Number of subjects with AEs, incidence [%], and number of events were summarized per treatment group. Segmental SES-CD scores and histological fibrosis scores were calculated for each subject. These scores were tabulated in by-subject listings. Plasma concentrations were tabulated in by-subject listings. The results of the experiments are expressed as mean ± standard deviation [SD]. The result of each concentration of STNM01 was compared with that of placebo by Student's t test using GraphPad PRISM4 [Prism software]. The level of statistical significance was set to $P < 0.05$.

2.6. Ethical statement

The study was performed following informed consent from all subjects and under protocol that was approved by Institutional Review Boards [IRB]. Before initiating the clinical trial, Clinical Trial Notifications were submitted to and accepted [Number: 23–1655] by the Japan Pharmaceuticals and Medical Devices Agency [PMDA].

3. Results

3.1. Patient population and baseline characteristics

A total of 18 CD patients were enrolled into the study [Figure 1]. There was no screrening failure as all subjects showed at least one single mucosal lesion even despite clinical remission status according to the CDAI score. The majority of subjects were male [83.3%]. The major disease location was the rectum. Demographic characteristics were generally balanced among treatment groups, although there was a tendency to lower segmental SES-CD and CDAI scores in the STNM01 250 nM group. The percentage of subjects who received anti-TNF antibodies as a conventional therapy was 66.6% in all treatment groups [Table 1]. Two subjects in the STNM01 and five subjects in the placebo group received corticosteroids and the doses were not changed during the trial. There were no discontinuations.

3.2. Safety

STNM01 2.5 nM, 25 nM, or 250 nM administered submucosally to subjects was generally well tolerated in the majority of subjects. The proportions of subjects who experienced at least one adverse effect [AE] were as follows: STNM01 2.5nM [66.7%], 25 nM [66.7%], 250 nM [66.7%], and placebo [55.6%]. The severity was mild in all AEs in STNM01-treated groups. One moderated AE was observed in the placebo group, which was mild peritonitis caused by faecal impaction in the ileocecal valve. There were no drug-related AEs [Table 2]. Additional PCR analysis and immunohistochemical staining for CHST15 in mucosal biopsies were performed after completion of the Phase 1 trial. Compared with placebo, relative quantity of CHST15 mRNA showed reduction by the treatment with STNM01 at Days 7 and 30 [Supplementary Figure 1, available as Supplementary data at *ECCO-JCC* online]. Mean decrease of CHST15 quantity from baseline in each cohort showed statistically significant silencing effects of 25 and 250 nM STNM01 concentrations [Supplementary Figure 1]. In histological analysis, a significant number of inflammatory cells within the lamina propria and damaged epithelium were positive for CHST15 at baseline [Supplementary Figure 2, available as Supplementary data at *ECCO-JCC* online]. Most cells showed marked CHST15 staining in the entire cytoplasm [Supplementary Figure 2]. In response to STNM01, CHST15-positive cells markedly decreased in numbers and the staining pattern was changed to weak or no expression in the cytoplasm [Supplementary Figure 2]. The CHST15 staining score established by the intensity and extension of staining [Supplementary Table 1, available as Supplementary data at *ECCO-JCC* online] was also reduced by the treatment with STNM01, and reached statistical significance with application of 250 nM STNM01 [Supplementary Figure 2, available as Supplementary data at *ECCO-JCC* online].

Table 1. Demographic and baseline characteristics.

	2.5 nM [$n = 3$]	25 nM [$n = 3$]	250 nM [$n = 3$]	Placebo [$n = 9$]
Male [*n*]	3	3	1	2
Female [*n*]	0	0	2	7
Mean age [years]	29.3	46.0	36.7	34.9
Mean duration of disease [years]	6.3	9	7.6	5.4
Mean segmental SES-CD	4.7	5.0	1.0	3.3
CD location: *n* [%]				
Ileocaecum	0 [0]	0 [0]	0 [0]	2 [22.2]
Transverse colon	0 [0]	1 [33.3]	0 [0]	1 [11.1]
Descending colon	0 [0]	0 [0]	0 [0]	1 [11.1]
Rectum	3 [100]	2 [66.7]	3 [100]	5 [55.6]
Mucosal lesion: *n* [%]				
Ulceration	3 [100]	3 [100]	1 [33.3]	7 [77.8]
Heaviest inflammation without ulcer	0 [0]	0 [0]	2 [66.7]	2 [22.2]
Mean CDAI	143.70	158.7	124.3	134.4
Previous anti-TNF therapy: *n* [%]	2 [66.7]	2 [66.7]	2 [66.7]	6 [66.7]
Systemic corticosteroid: *n* [%]	1 [33.3]	1 [33.3]	0 [0]	5 [55.6]
Immunosuppressant: *n* [%]	1 [33.3]	1 [33.3]	1 [33.3]	3 [33.3]
Previous surgery: *n* [%]	1 [33.3]	2 [66.7]	1 [33.3]	4 [44.4]

SES-CD, Simple Endoscopic Score for Crohn's Disease; CDAI, Crohn's Disease Activity Index; TNF, tumour necrosis factor.

Plasma concentration of STNM01 was below the detection limit of quantification [LLOQ 0.5 ng/ml] at all indicated times in all treatment groups [data not shown]. This was possibly due to the naked nature of STNM01, which was rapidly degraded in the circulation. Altogether, these results support the high safety profile of STNM01 across all tested concentrations.

3.3. Efficacy in endoscopic and clinical responses

Efficacy was evaluated by segmental SES-CD [maximum score was 11 in the present study]. At baseline, non-healed mucosal lesions remained present despite continuous systemic therapy, but nevertheless, the endoscopic response was rapid as six subjects showed partial MH [two subjects in the 2.5 nM group, one subject in 25 nM, three subjects in 250 nM] at Day 7 already after a single injection [Figure 2A and B]. Two of three subjects in the 250 nM group showed complete MH at Day 7. MH efficacy was further obvious at Day 30, as seven subjects who received STNM01 [two subjects in the 2.5 nM group, two subjects in 25 nM, three subjects in 250 nM] showed partial MH [Figure 2B and C]. Four subjects who received STNM01 [one subject in the 25 nM group, three subjects in 250 nM] showed complete MH at Day 30 [Figure 2C]. All subjects who received placebo [n = 9] did not show any endoscopic response [Figure 2B and C]. A representative endoscopic finding is shown in Figure 2A demonstrating a deep, large and non-healed active ulcer at

Table 2. Adverse events registered during the study, *n.*

Incidence [%]	2.5 nM [n = 3] 2 [66.7]	25 nM [n = 3] 2 [66.7]	250 nM [n = 3] 2 [66.7]	Placebo [n = 9] 5 [55.6]	Grade	Causal relationship
Headache	0	1	0	0	Mild	UN
Insomnia	0	0	0	1	Mild	No
Abdominal pain	0	1	0	0	Mild	UN
Heartburn	0	1	0	0	Mild	UN
Soft stool	1	0	0	0	Mild	No
Diarrhoea	1	0	0	0	Mild	UN
Perianal inflammation	1	0	0	0	Mild	No
Fever	0	0	0	1	Mild	No
Peritonitis	0	0	0	1	Moderate	No
Nasopharyngitis	0	1	1	1	Mild	No
Oral aphtha	0	0	1	0	Mild	No
Herpes simplex	0	0	0	1	Mild	No
Influenza	0	0	0	1	Mild	No
Transient leukocytosis	1	0	0	0	Mild	UN
Knee joint pain	0	0	1	0	Mild	UN

*Causal relationship is classified into four categories: certain, probable, unlikely [UN] and none [No].

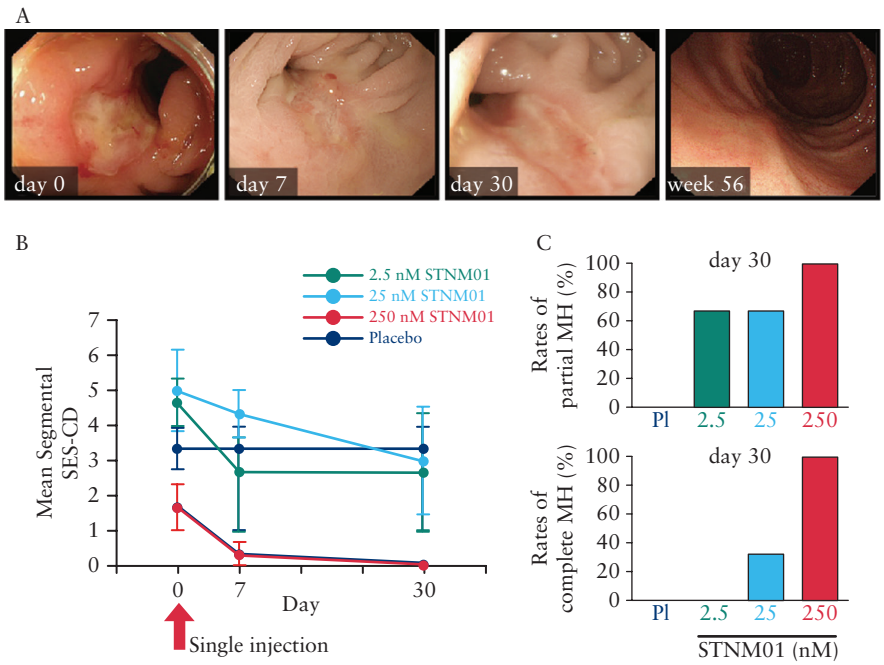

Figure 2. Endoscopic response to STNM01. [A] Representative endoscopic findings depicting the development from an active mucosal ulcer to a scarred tissue area in a patient receiving 25 nM of STNM01. Note that red regenerative mucosa was shown around the diminishing ulcer at Day 7. [B] Changes in mean segmental SES-CD. [C] Rates of partial mucosal healing [MH] and complete MH at Day 30.

baseline in the 25 nM cohort, which was refractory to previous anti-TNF therapies. The non-healed ulcer clearly reduced in size, with appearance of red regenerative mucosa at Day 7, and changed to a scar stage at Day 30 [Figure 2A].

Although we selected patients in clinical remission or with mild disease activity, one subject who received STNM01 2.5 nM showed a change from mild to remission at Day 30. No remarkable change in CDAI was observed in others [data not shown], suggesting that STNM01 did not worsen clinical status and could maintain remission.

3.4. Efficacy in histological response

Efficacy was also evaluated by fibrosis scoring[23] from results of Masson trichrome staining of biopsy specimens. Baseline mucosal histology of non-healer patients was severe, as expected, and all patients showed massive inflammatory infiltrates and fibrosis with damaged epithelium [Figure 3A]. The effect of STNM01 on mucosal fibrosis was rapid, as inflammatory infiltrate and fibrosis were almost normalized at Day 7 [Figure 3A]. It was noted that STNM01 normalized epithelial architecture including fine goblet cell morphology [Figure 3A]. At Day 30, seven subjects who received STNM01 [two subjects in the 2.5 nM group, three subjects in 25 nM, two subjects in 250 nM] showed reduction in fibrosis score from baseline [Figure 3B]. Fibrosis score in placebo-treated patients was almost stable, but rather increased at Day 30 [Figure 3B]. The number of inflammatory cells in the lamina propria significantly reduced in the 25 nM and 250 nM STNM01-treated patients compared with those in the placebo group at Day 30 [Supplementary Table 2, available as Supplementary data at *ECCO-JCC* online].

3.5. Long-term observation

Eleven of 18 subjects [six in the STNM01 cohort, five in placebo] underwent follow-up endoscopy. Among six subjects who received STNM01, five subjects maintained the MH of the previously treated lesion over 1 year [Supplementary Figure 3, available as Supplementary data at *ECCO-JCC* online]. There was no change in treatment in four subjects during the observation period, but one subject discontinued infliximab usage based on the good symptom control. One subject [25 nM of STNM01] showed disappearance of intestinal stricture [Figure 2A]. One subject [25 nM of STNM01] who failed to achieve MH at Day 30 did also not show MH in the long-term observation period despite extensive usage of biologics after the completion of the study. There were no subjects showing clinical exacerbations. All subjects who received placebo failed to achieve complete MH. Taken together, STNM01 reduced CHST15 expression as well as fibrosis while enhancing rapid MH after submucosal injection. Non-healer patients who achieved quick MH response by STNM01 showed long-term persistence of the MH effect.

4. Discussion

MH is an important treatment goal that predicts sustained clinical remission and resection-free survival in CD patients.[1–7] Patients who failed to achieve MH [non-healers] are facing poor prognosis, thus a novel treatment to achieve effective MH is needed. In response to tissue injury, the balance between 'adequate' and 'fibrotic' wound healing may determine the disease outcome, and persistent local inflammation and fibrosis may contribute to skew the host defence system towards more 'fibrotic' instead of 'adequate' healing.[8] We hypothesized that inducible expression of CHST15 and its product CS-E plays a role in creating a local fibrotic field[19] and in regulating the balance between MH and fibrosis. Because selective inhibition of CS-E by chemical approaches represents a major challenge for *in vivo* therapy, we synthesized STNM01, double-strand RNA oligonucleotides specifically designed to inhibit the expression of the CHST15 gene,[18,19] and conducted a clinical Phase 1 study in Japan.

Clinically, it is still important to protect siRNA drugs from degradation at sites of interest and to protect patients from unwanted side effects. We previously reported off-target effects of a CHST15 siRNA possessing the same sequence as STNM01.[18] The 7 mer subsequences of the siRNA cleaved at the 5'-end of the antisense chain having CUACUUA as a base sequence caused off-target effects in genes having a complementary sequence of GAUGAAU. Subsequently, four genes whose expressions might be affected as off-target effects were found, such as oxytocin receptor, chromodomain helicase DNA binding protein 7, G protein-coupled receptor 126, and insulin-like growth factor 2 mRNA binding protein 3.[18] Although the above four genes were not detectable in the intestine or fibroblasts, the repression of these genes might cause side effects. To prevent such potential adverse effects, we established submucosal injection as the application route, which allows naked siRNA to remain exclusively within the injected organ site and be degraded immediately after entering into the circulation. The ability of siRNA diffusion within the submucosal space in a pancolonic and circumferential fashion was more pronounced, as expected, and almost the entire colon could be targeted by this approach in mice.[20] The colonic submucosa has been characterized as

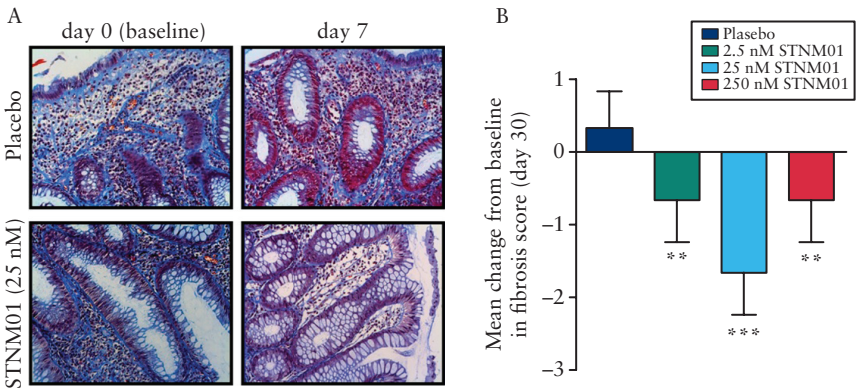

Figure 3. Repression of fibrosis by STNM01. [A] Typical Masson's trichrome [MT] staining of biopsy specimens at Days 0 [baseline] and 7 in patients receiving placebo [upper panels] or 25 nM of STNM01 [lower panels]. Reduction of not only excess fibrosis but also of inflammatory infiltrates was observed by STNM01. Note that epithelial architecture was preserved 7 days after injection with STNM01. Original magnification, x200. [B] Mean changes from baseline in fibrosis score at Day 30. $^{**}p < 0.01$; $^{***}p < 0.001$ versus placebo, Student's t test.

having 'nature's reservoir', enabling naked siRNA to function at sites of local injury with a minimum risk of systemic side effects.

In the present Phase 1 study, safety and pharmacokinetics profiles were evaluated. STNM01 was not detected in the circulation from 5 min to 24 h after a single submucosal injection, tested at various concentrations. There were no drug-related AEs, no abnormal changes in 12-lead ECG or laboratory values [data not shown], supporting the notion that locally injected STNM01 has a minimun risk of systemic side effects. In biopsy specimens there was no evidence of increased inflammation in STNM01-treated groups, suggesting that unfavourable local response was not induced by the tested treatment regimen. We additionally investigated CHST15 mRNA expression in the biospsy specimens. CHST15 mRNA expression was detected at baseline in all patients. We found that STNM01 reduced the expression of target CHST15 mRNA at local mucosal sites at least 30 days after a single injection. Although the measurement of mucosal drug concentrations by ELISA was not performed, due to the limited biopsy specimen size, the results suggest that submucosally injected STNM01 acted at the site of the target lesion but not systemically. The characteristics of nature's reservoir are considered to be applicable also to clinical practice, in view of safety and pharmacokinetics.

An impressive finding was the rapid endoscopic response after a single injection of STNM01. Six out of nine patients who had active mucosal lesions, despite previous extensive usage of systemic drugs including anti-TNF agents for several years, dramatically showed quick endoscopic responses only 7 days after treatment. Red regenerative mucosa was clearly shown similarly in the healing stage of peptic ulcers upon treatment with H_2 receptor antagonists or proton pomp inhibitors,[26] suggesting that in our participants the healed response was induced by exogenously added STNM01. Endoscopic findings improved even more at Day 30 as evidenced by further reduction of segmental SES-CD, where 7 of 9 patients achieved MH effects. Endoscopic MH was also supported by histological findings such as reduction of fibrosis as well as inflammatory infiltrates and appearance of well-preserved epithelium, including goblet cells. Although the possibility that injected drugs can prevail in the colon for 30 days is not completely ruled out, we consider that the MH effect can persist even after disappearance of the drug. Once the ulcerative lesions achieve MH, the region can be protected from further pro-inflammatory stimuli, leading to resolution of inflammation. Since CHST15 is inducible in response to inflammatory stimuli which are provided by luminal pathogens and locally-accumulated inflammatory cells, successful normalization of the mucosal microenvironment would shut down these stimuli, prevent further expressions of CHST15 and CS-E at local sites, and would maintain MH effect for a longer period.

Findings of long-term therapeutic efficacy were unexpected; a single dose of STNM01 could maintain the local MH effect over 1 year in some individuals. Interestingly, patients were clearly divided into two groups. Patients who achieved MH at Day 30 maintained long-term MH, whereas patients who failed to achieve MH at Day 30 did not. Thus, the unexpected phenomenon could be explained in part by the above description that achievement of quick MH can protect hosts from further local stimuli and maintain MH without performing repeated injections. Disease duration may be one of the key factors, as the patient without benefit of STNM01 application had long-standing disease for more than 20 years and experienced several unsuccessful biologic treatments, whereas disease duration of the other eight subjects was less than 10 years. We consider that the 'time to MH' will be a novel and important parameter to predict the outcome of CD.

Although the precise mechanism of CHST15 siRNA is not fully elucidated, we have initially focused on anti-fibrotic action as inhibited activation of fibroblasts was shown to promote epithelial healing in experimental colitis models.[19,27] *In vitro*, CHST15 siRNA inhibited the activation of human colon fibroblasts as evidenced by reduced production of IL-6 and the signal intensity of epithelial-mesenchymal transition [EMT]-related pathways.[20] Since activated fibroblast-derived mediators impact on the activation status of neighbouring inflammatory cells and vice versa, CHST15 siRNA-mediated blockade of overactivation of fibroblasts may lead to suppression of inflammatory cells as well.[12] In addition, CHST15 siRNA reduces CS-E and may thus block the signalling of CS-E-binding molecules including chemokines, adhesion molecules, and RAGE,[17] leading to suppression of inflammation as well as fibrosis at the local site. If the host has regenerative capacity, reduced inflammation and fibrosis may contribute to skew the balance towards epithelial healing. CHST15 siRNA's effect of reverse EMT may also contribute to epithelial healing. The possible impact on the intestinal microflora is also of high interest. As CS-E also binds pathogenic microorganisms,[12] long-term MH achieved by only one single STM01 injection may also be explained in part by modulation of the intestinal microflora of CD patients by inhibiting CS-E. Further studies to investigate the mechanism of action of CHST15 in CD patients will be needed.

There were some limitations to the conducted clinical study. First, the small sample size [n = 3 per each cohort in STNM01] was not sufficient for full statistical analyses. Interpretation of the results needs to be done cautiously, as the observed findings are based on the small sample size. To compensate for the limited number of examined patients and sample size, endoscopic examinations were performed repeatedly in all subjects, and short-term follow-up endoscopy [Day 7] clearly evidenced ongoing regenerative response. A series of biopsy-based tissue examinations supported the endoscopic findings that CHST15 siRNA possessed both MH-inducing and antifibrotic effects in non-healer patients. Second, the target non-healed mucosal lesion at baseline included both ulcerated and active inflammatory lesions without signs of ulceration. In the absence of ulcerations, baseline segmental SES-CD was 1, although histologically severe inflammation was evident in these lesions. Since dynamic allocation was conducted in the 3 + 3 trial design, only the high-dose group among STNM01 applications resulted in the inclusion of patients with a baseline segmental SES-CD of 1. Although all patients achieved complete MH in STNM01 250 nM treatment, it may partly be due to the weakness of baseline scores and may not allow conclusion that the 250 nM dosing had a superior effect compared with 25 nM of STNM01. Third, the therapeutic target in the present study was a single mucosal lesion, and the lesion located closest to the anal verge was selected if the subject showed two or more lesions of comparable inflammatory activity. As most of the patients had rectal lesions at the time of screening, the majority of the STNM01 applications were done in the rectum. Remote effects on other segments after treatment of the rectal lesion were not investigated in the present study. Fourth, this was the first-inpatient study with a novel concept and there was thus a possibility that traditional measures [CDAI and SES-CD] were not adequate to assess the therapeutic efficacy of STNM01. The effects of STNM01 seemed to be rapid appearance of regenerative mucosa and reduction of fibrosis, which are not included in traditional outcome measures. These are nevertheless important factors to improve quality of life [QOL] for non-healer patients, in view of the recently accepted concept that CD is a progressive bowel destructive disease. Together with the fact that CD often shows multiple mucosal lesions, future investigations by increased sample size and variability of application will be needed to further clarify the efficacy points described above.

Local treatment approaches against refractory ulcers resistant to previous systemic therapies are a valuable therapeutic option in CD patients. When applying this mode of application in clinical practice,

it needs to be established how to select the most relevant target lesion[s] in the case of multiple ulcers. This problem is currently also under discussion in regard to cell or organoid transplantation for the treatment of IBD.[28,29] Considering the patient's perspective on the antifibrotic effects of CHST15 in blockage, one possibility is to prolong the time to or reduce the rate of surgery in fibrotic diseases. Deep ulcerations covering more than 10% of the mucosal area were shown to be associated with a high risk of colectomy,[30] and may thus be suitable for specific local treatment. Based on recently proposed treat-to-target algorithms, endoscopic observations at 6-month intervals would be useful for monitoring of active disease[31] and would represent a good opportunity for local STNM01 application. In addition, orally active CHST15 siRNA would be another valuable approach to delay tissue fibrosis and destruction in the future.

In conclusion, the present first-in-humans study shows that targeting of distinct ECM proteins, CHST15 and CS-E, can be achieved in patients with CD by submucosal injection of the siRNA STNM01, which was safe and well tolerated. Our findings suggest that trials to induce 'non-fibrotic' MH emerge as a novel treatment approach for non-healer patients with CD. The results support the continued development of STNM01 for the treatment of CD.

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Conflict of Interest

HY founded Stelic Institute & Co., Inc., in 2004. Stelic filed and issued patents related to the treatment of intestinal fibrosis/inflammatory bowel disease with oligonucleotide-based medicine targeting CHST15. KS is an inventor of a patent related to submucosal injection. Other authors have no conflict of interest.

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Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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