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ORIGINAL ARTICLE

Lipidated IL-22 Alone or Combined with Immunomodulatory Agents Improves Disease Endpoints and Promotes Mucosal Healing in a Mouse Model of Chronic Dextran Sodium Sulfate-Induced Colitis

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Abstract

Background IL-22 facilitates mucosal healing by directly inducing epithelial regeneration and barrier integrity, which is essential for achieving remission and thereby treating inflammatory bowel disease.

Aims Here, we evaluated efficacy of a novel lipidated IL-22 alone and in combination with immunomodulatory agents in addressing chronic dextran sodium sulfate (DSS)-induced colitis in mice and demonstrated action of IL-22 on mucosal healing.

Methods Mice were treated with DSS, followed by various doses of lipidated IL-22, anti-TNF antibody, fingolimod, or anti-mouse α4β7 integrin antibody. Additionally, gene expression was determined in colonic biopsies from ulcerative colitis patients to assess effects of IL-22 stimulation.

Results Lipidated IL-22 significantly improved all aspects of chronic DSS-induced colitis in mice, with dose-dependent efficacy. Combinations of a range of immunomodulatory agents with lipidated IL-22 showed further additive reductions in disease activity, significantly greater than those of monotherapies. Immunohistochemistry revealed that lipidated IL-22 increased epithelial cell proliferation and reduced $CD3^+$ T-cell infiltration, indicating enhanced mucosal healing. This was further supported gene expression data from colonic biopsies from ulcerative colitis patients after IL-22 stimulation.

Conclusions Given the challenges in achieving long-term remission in IBD due to inflammation and mucosal damage, lipidated IL-22 presents a promising treatment option that directly promotes mucosal healing, unlike traditional immunomodulatory therapies.

Keywords Interleukin-22 · Inflammatory bowel diseases · Pharmacotherapy · Biologics · Intestinal barrier function

Introduction

Inflammatory bowel disease (IBD) is characterized by a recurring cycle of interconnected disease drivers, including inflammation, mucosal wounding, and microbial dysbiosis [1]. Although advances in medical therapies have improved outcomes, remission rates for patients with IBD remain modest at 20% to 30% [2]. Despite the varied landscape of approved therapies for IBD, a substantial proportion of patients do not respond or become refractory to treatment, with approximately 40% of patients who initially achieve deep remission relapsing again within 2 years [3]. Therefore, IBD continues to impose a substantial clinical, economic, and humanistic burden on patients and healthcare systems [4].

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Mucosal damage is a primary driver of harmful inflammation and relapse in patients with IBD [5] and mucosal healing is achieved by less than half of patients with IBD on current medical treatments [5, 6]. Achievement of mucosal healing has been associated with decreased rates of surgical interventions and reduced hospitalizations in patients with IBD [7, 8]. Mucosal healing is a key IBD treatment goal and pivotal in achieving long-term remission, but agents directly targeting mucosal damage remain a significant unmet need [9].

IL-22 has been identified as a master regulator of mucosal homeostasis involved in multiple aspects of epithelial barrier function, including regulation of epithelial cell proliferation and permeability and production of mucus and antimicrobial proteins [10]. IL-22 is secreted in response to damage, infection, or inflammation of epithelia, such as that which occurs in IBD [11, 12]. In contrast to traditional IBD treatment paradigms that involve immunomodulation, IL-22 is an atypical cytokine that selectively targets epithelia and is not directly immunomodulatory [13].

Lipidated IL-22 offers a promising new treatment option for managing IBD as it may directly promote mucosal healing. This is supported by previous data from experimental models of acute colitis using different IL-22 agonistic approaches [14–16]. Native IL-22 has a half-life of a few hours in humans, which through lipidation—a minimal structural modification that protects a protein from clearance and degradation by enabling non-covalent binding to circulating serum albumin—can be substantially prolonged while maintaining native protein size [17]. Optimization of the pharmacokinetic properties of IL-22 via lipidation enables harnessing of IL-22 biology to treat IBD.

In this study, we aimed to test the efficacy of a novel lipidated IL-22 alone and in combination with currently used immunomodulatory treatments in a mouse model of chronic colitis. Furthermore, we aimed to demonstrate the action of IL-22 on mucosal healing and explore how these findings may translate to human ulcerative colitis.

Materials and Methods

Chronic DSS-Induced Model of Colitis

Ethics approval for the study was obtained from the Italian Ministry of Health. 8 week-old C57Bl/6N mice (all females, weight between 17 and 20 g) were obtained from Charles River Laboratories. Mice were treated with three oral cycles of 2% (weight/volume) dextran sodium sulfate (DSS) (molecular mass, 40 kDa; MP Biomedicals), each characterized by 7 days of DSS exposure in drinking water, followed by 7 days of regular drinking water for a total of 42 days.

Experimental groups were selected to investigate the effects of lipidated IL-22 alone and in combination with immunomodulatory agents—an anti-murine tumor necrosis factor (TNF) antibody, fingolimod (sphingosine-1-phosphate [S1P] modulator) or an α4β7 integrin antagonist—to explore potential additive effects. In the first experiment, the experimental groups (8 animals per group / 56 animals in total) received vehicle, lipidated IL-22 (0.05 mg/kg, 0.15 mg/kg, 0.3 mg/kg), anti-TNF (10 mg/kg, 15 mg/kg) or combination lipidated IL-22 (0.15 mg/kg) plus anti-TNF (10 mg/kg). In the second experiment, the experimental groups (9 animals per group / 81 animals in total) received vehicle, lipidated IL-22 (0.15 mg/kg, 0.3 mg/kg), fingolimod (0.1 mg/kg, 0.3 mg/kg), anti-mouse α4β7 integrin antibody (15 mg/kg, 30 mg/kg), combinations lipidated IL-22 (0.15 mg/kg) plus fingolimod (0.1 mg/kg) or lipidated IL-22 (0.15 mg/kg) plus anti-mouse α4β7 integrin (15 mg/kg). In both experiments, the highest doses reflected therapeutic dosing and were included to serve as positive controls. The combination treatments were performed with subtherapeutic doses. From day 18 to day 41, lipidated IL-22 was administered subcutaneously once a day, anti-murine anti-TNF antibody and anti-mouse α4β7 integrin antibody were administered intraperitoneally 3 times per week, and fingolimod was administered once daily by oral gavage. The study endpoints included disease activity index (DAI), colon length and weight, Rachmilewitz histologic score, and an endoscopic score. Pathological evaluation and histological analysis were performed by a blinded pathologist. Colitis severity was monitored throughout the study using a DAI score based on daily evaluation of body weight, stool consistency, and presence of blood in the stools, as determined using Hemoccult SENSA Cards (Beckman Coulter). A modified murine endoscopic index score of colitis severity was assigned in anesthetized animals prior to sacrifice on the basis of colon translucency (0–3 points), granular features of the mucosa (0–3 points), morphology of the vascular pattern (0–4 points), and the presence of fibrin (0–4 points) [18], leading to a cumulative score between 0 (no signs of inflammation) and 16 (endoscopic signs of very severe inflammation).

At sacrifice (day 42), mice colons were excised, weighted, and measured. Colons were fixed, dehydrated, and processed, and sections were stained with hematoxylin and eosin for histologic evaluation. Histologic score was assigned by a blinded pathologist based on an assessment of the distal colons. Sections were scored on the ulceration; extent of ulceration, inflammation, extent of inflammation, and fibrosis using a scoring system as described [19]. Fibrosis scores were not quantified since it has previously been demonstrated that the DSS-induced model of chronic colitis does not induce fibrosis. The resulting total histological score ranges from 0 (no signs of disease) to 20 (histologic signs of severe inflammation). Additional histological

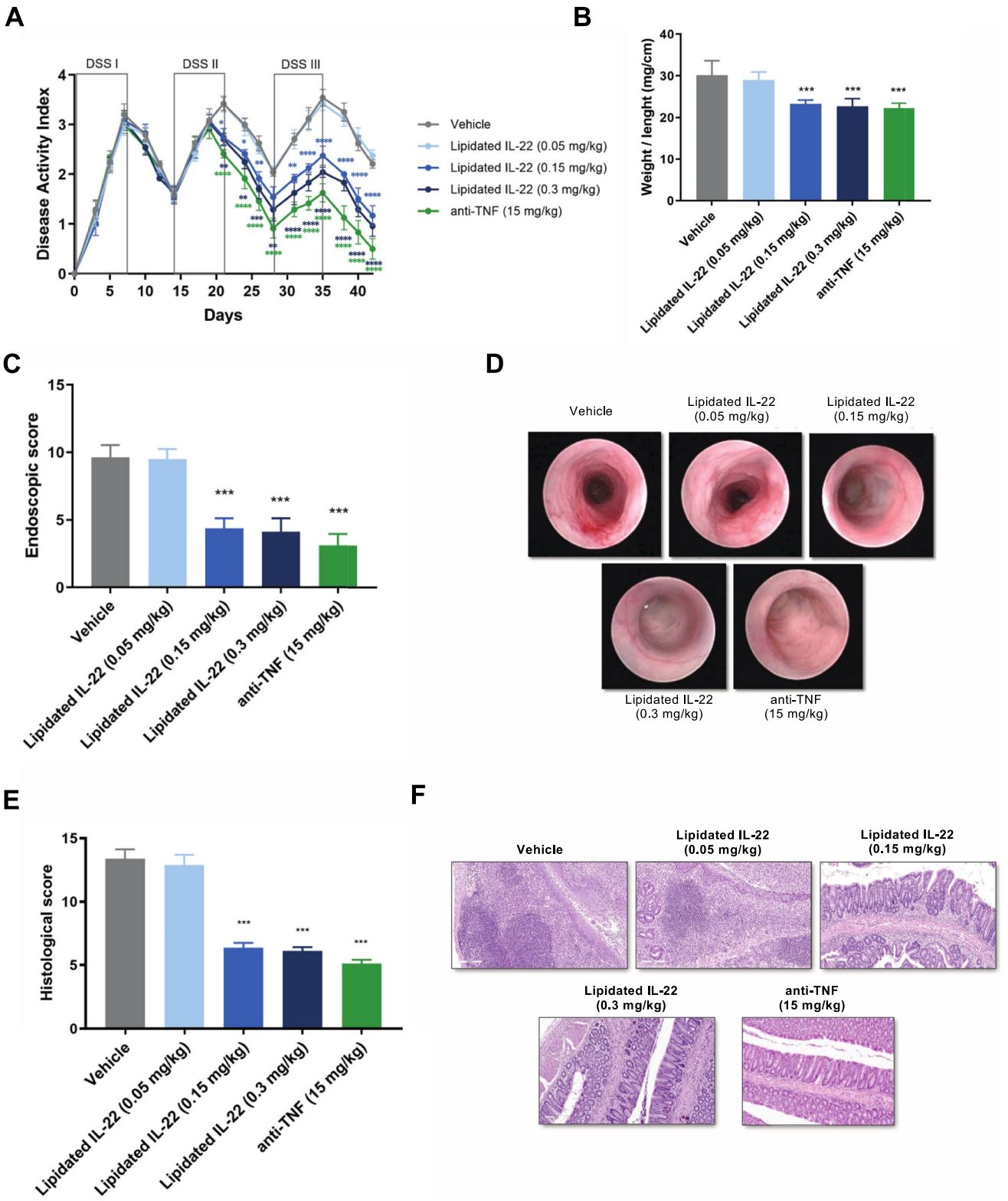
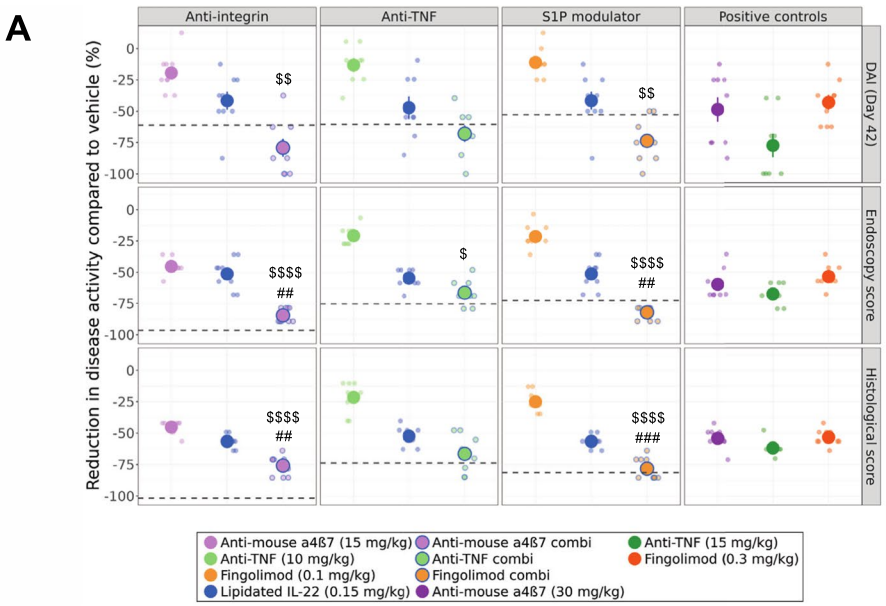
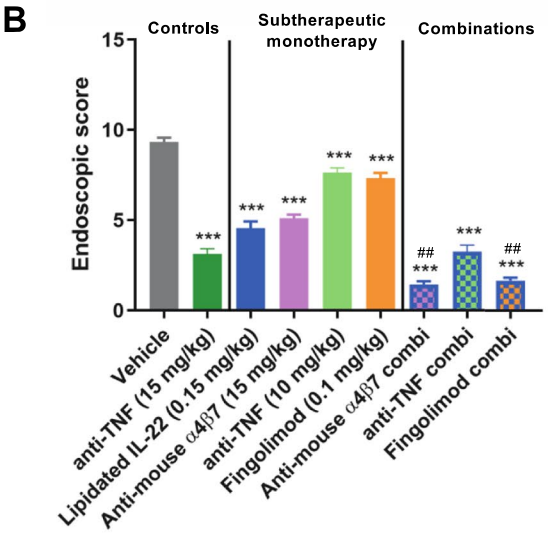
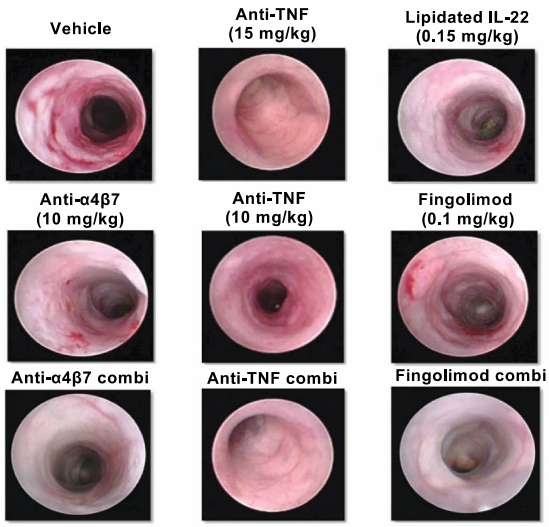
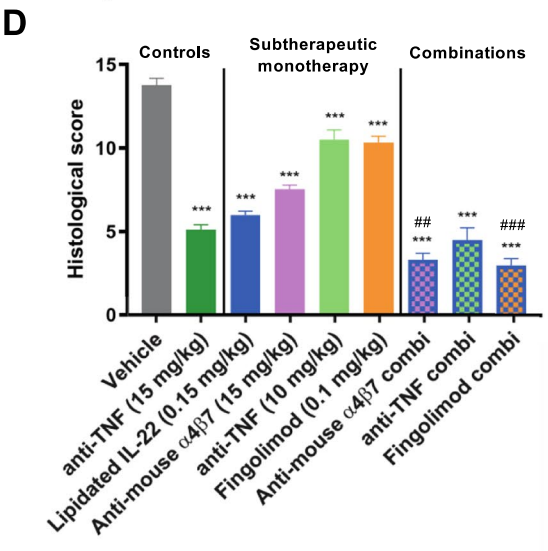


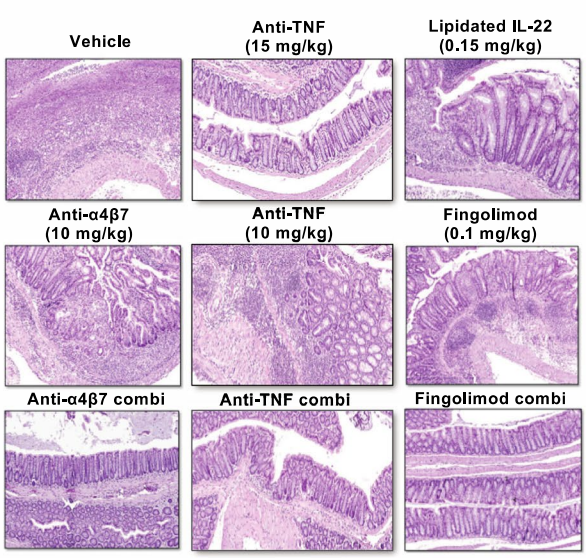
Fig. 1 Effect of lipidated IL-22 on disease endpoints in a murine chronic DSS colitis model. Disease activity index **A**, colonic weight/length ratio **B**, endoscopic scores on Day 42 together with representative images **C** and **D**, and histological scores from distal colon sections at termination together with representative images **E** and **F** following lipidated IL-22 treatment in a chronic DSS colitis model. Data are presented as mean ± SEM in the graphs. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ (Dunnett's one-way ANOVA)


A
Anti-integrin
Anti-TNF
S1P modulator
Positive controls
DAI (Day 42)
Endoscopy score
Histological score
Reduction in disease activity compared to vehicle (%)
Anti-mouse a4ß7 (15 mg/kg)
Anti-mouse a4ß7 combi
Anti-TNF (15 mg/kg)
Anti-TNF (10 mg/kg)
Anti-TNF combi
Fingolimod (0.3 mg/kg)
Fingolimod (0.1 mg/kg)
Fingolimod combi
Lipidated IL-22 (0.15 mg/kg)
Anti-mouse a4ß7 (30 mg/kg)


B
Controls
Subtherapeutic monotherapy
Combinations
Endoscopic score
Vehicle
anti-TNF (15 mg/kg)
Lipidated IL-22 (0.15 mg/kg)
Anti-mouse α4β7 (15 mg/kg)
anti-TNF (10 mg/kg)
Fingolimod (0.1 mg/kg)
Anti-mouse α4β7 combi
anti-TNF combi
Fingolimod combi


C
Vehicle
Anti-TNF (15 mg/kg)
Lipidated IL-22 (0.15 mg/kg)
Anti-α4β7 (10 mg/kg)
Anti-TNF (10 mg/kg)
Fingolimod (0.1 mg/kg)
Anti-α4β7 combi
Anti-TNF combi
Fingolimod combi


D
Controls
Subtherapeutic monotherapy
Combinations
Histological score
Vehicle
anti-TNF (15 mg/kg)
Lipidated IL-22 (0.15 mg/kg)
Anti-mouse α4β7 (15 mg/kg)
anti-TNF (10 mg/kg)
Fingolimod (0.1 mg/kg)
Anti-mouse α4β7 combi
anti-TNF combi
Fingolimod combi


E
Vehicle
Anti-TNF (15 mg/kg)
Lipidated IL-22 (0.15 mg/kg)
Anti-α4β7 (10 mg/kg)
Anti-TNF (10 mg/kg)
Fingolimod (0.1 mg/kg)
Anti-α4β7 combi
Anti-TNF combi
Fingolimod combi

◂**Fig. 2** Effect of lipidated IL-22 in combination with anti-inflammatory agents in a murine chronic DSS colitis model. **A** Analysis of improvements in disease activity index, endoscopic and histologic scores at day 42 relative to vehicle treated animals for the IL-22 combination treatments. Dashed lines indicate the additive combination effect based on the monotherapy arms for the given combination. The endoscopic scores on Day 42 together with representative images **B** and **C** and the histological scores from distal colon sections at termination together with representative images **D** and) is shown following combination treatment with lipidated IL-22 and anti-inflammatory agents in a chronic DSS colitis model. Data are presented as mean ± SEM in the graphs. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ compared to vehicle. $^{\$}p < 0.05$; $^{\$\$}p < 0.01$; $^{\$\$\$\$}p < 0.0001$ compared to each monotherapy arm. $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ compared to each positive control treatment (all Dunnett's one-way ANOVA)

analysis included quantification of $Ki67^+$, $CD3^+$ T cells, and goblet cells.

The full analysis set for all endpoints included all animals included in both studies, except for the additional histological analysis which was only performed in the animals from the first study. No animals or data points were excluded. Data were summarized as means ± standard deviation or ± standard error of the mean. For experiments including more groups, a one- or two-way analysis of variance (ANOVA) with multiple comparisons was performed in GraphPad Prism (GraphPad Software, Boston, MA); $P < 0.05$ was considered statistically significant.

Gene Expression Study in Human Colonic Tissue

We performed a cross-sectional, observational study to investigate the effect of ex vivo IL-22 on gene expression in colonic mucosal biopsies from patients with ulcerative colitis. The study was approved by the local scientific ethics committee and undertaken at the Department of Hepatology and Gastroenterology, Aarhus University Hospital from June 2022 to January 2023. Patients aged 18 to 80 years were eligible if they had an established diagnosis of ulcerative colitis and were scheduled for sigmoidoscopy. Patients with a diagnosis of active malignancy (except non-melanoma skin cancer), diagnosis of psoriasis, or who were deemed unfit to participate by the study investigators were excluded. All patients provided informed written consent before participation in the study.

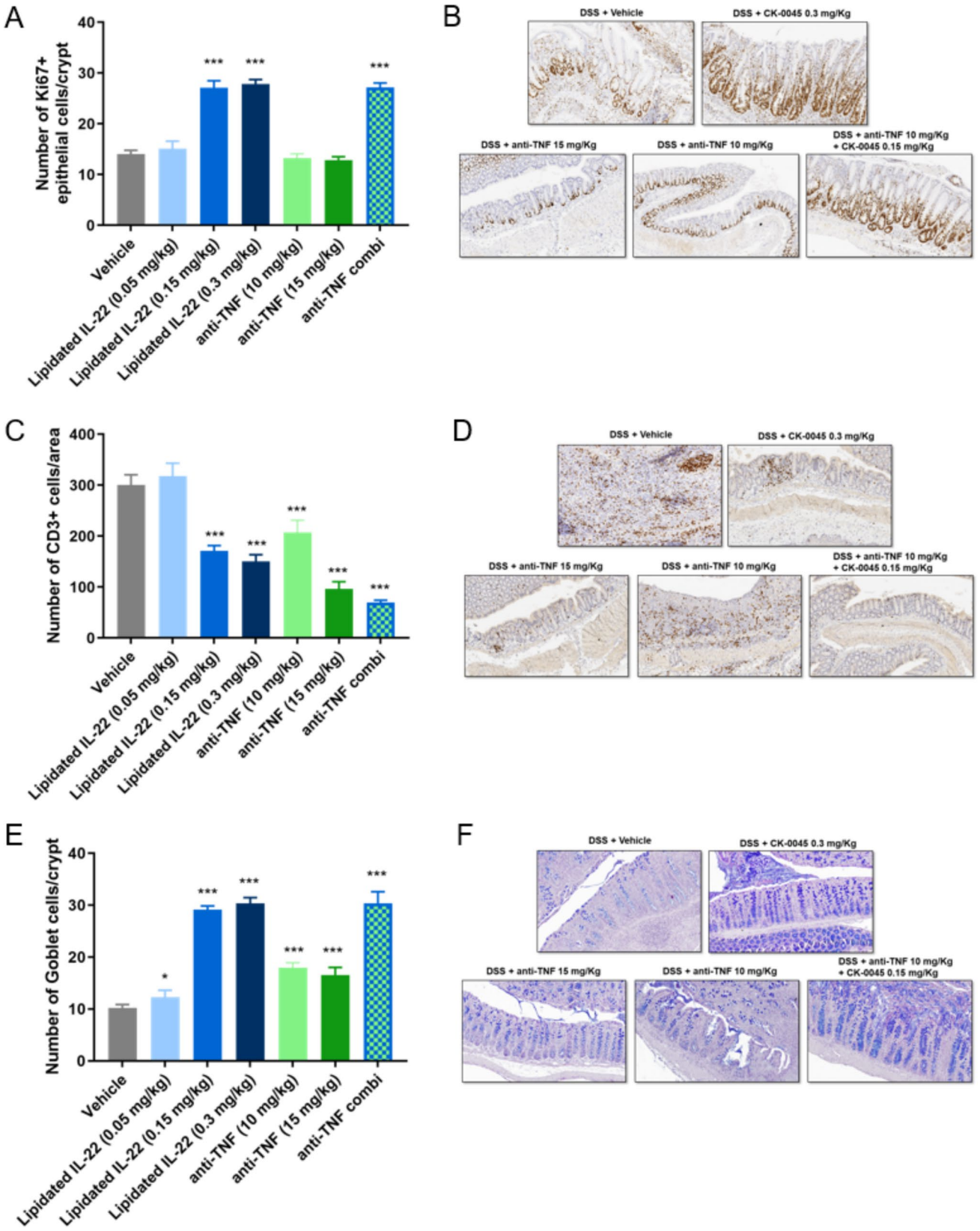
Nine patients who underwent sigmoidoscopy due to a clinical suspicion of active disease were included. The clinical and biochemical characteristics of the patients are described in Supplementary Table 1. Mucosal biopsies were collected during sigmoidoscopy from areas of active inflammation (n = 9), and when possible (n = 3) from areas without active inflammation. Demographic information, medical history, and biochemistry data were recorded in a REDCap database. Biopsies for ex vivo IL-22 stimulation were incubated with or without IL-22 for 4 h. RNA was extracted from the mucosal biopsies, and gene expression was analyzed using qPCR. Previously reported IL-22 expression biomarkers were tested (Supplementary Table 1), encompassing regenerating family member 1 alpha (*REG1A),* serum amyloid A *(SAA),* deleted in malignant brain tumours 1 *(DMBT1),* mucin 1 *(MUC1),* suppressor of cytokine signaling 3 *(SOCS3),* along with reference genes beta 2 microglobulin (*B2M),* actin beta *(ACTB1).* Also, the endogenous expression interleukin-22 (*IL22*), IL-22 receptor (*IL22RA1*), and IL-22 binding protein (*IL22RA2)* were measured in inflamed and uninflamed biopsies. Gene expression levels were reported relative to the mean of the reference genes. Gene expression in IL-22 stimulated and unstimulated biopsies were compared by the Wilcoxon Signed-rank test using GraphPad Prism (GraphPad Software, Boston, MA). Patient characteristics were summarized using Stata 18 (StataCorp LLC, College Station, TX).

Results

Lipidated IL-22 Improves Chronic DSS-Induced Colitis

Lipidated IL-22 has a substantially prolonged half-life of approximately 6 h in mice compared to the ~ 24 min of wild type IL-22 (Supplementary Fig. 1A), while maintaining wild-type-like potency (Supplementary Fig. 1B). To establish the efficacy of lipidated IL-22 in reducing clinical, endoscopic, and histological signs of colitis, three dose levels were tested in a chronic DSS-induced colitis model with treatment starting during the second cycle of DSS. Lipidated IL-22 at 0.15 mg/kg and 0.3 mg/kg dose-dependently and significantly improved clinical signs of DSS-induced chronic colitis as measured by DAI, whereas we observed no change with the minimum dose of 0.05 mg/kg (DAI; Fig. 1A). The dose-dependent improvements in DAI were accompanied by a significant reversal of colon length reduction (data not shown), and improvement in the colon weight/length ratio equal to the effects of the positive control treatment with the optimal preclinical therapeutic dose of murine anti-TNF (Fig. 1B).

In line with the improvements in gross pathology, lipidated IL-22 dose-dependently and at 0.15 mg/kg and 0.3 mg/kg significantly improved both the endoscopic and histologic scores in DSS-induced chronic colitis mice (Fig. 1C–F). Together, these data on the efficacy of lipidated IL-22 in a murine model of chronic DSS-induced colitis support its potential as a stand-alone treatment for managing IBD.


A
Number of Ki67+ epithelial cells/crypt
40
30
20
10
0
***
***
***
Vehicle
Lipidated IL-22 (0.05 mg/kg)
Lipidated IL-22 (0.15 mg/kg)
Lipidated IL-22 (0.3 mg/kg)
anti-TNF (10 mg/kg)
anti-TNF (15 mg/kg)
anti-TNF combi
B
DSS + Vehicle
DSS + CK-0045 0.3 mg/Kg
DSS + anti-TNF 15 mg/Kg
DSS + anti-TNF 10 mg/Kg
DSS + anti-TNF 10 mg/Kg + CK-0045 0.15 mg/Kg
C
Number of CD3+ cells/area
400
300
200
100
0
***
***
***
***
***
Vehicle
Lipidated IL-22 (0.05 mg/kg)
Lipidated IL-22 (0.15 mg/kg)
Lipidated IL-22 (0.3 mg/kg)
anti-TNF (10 mg/kg)
anti-TNF (15 mg/kg)
anti-TNF combi
D
DSS + Vehicle
DSS + CK-0045 0.3 mg/Kg
DSS + anti-TNF 15 mg/Kg
DSS + anti-TNF 10 mg/Kg
DSS + anti-TNF 10 mg/Kg + CK-0045 0.15 mg/Kg
E
Number of Goblet cells/crypt
40
30
20
10
0
*
***
***
***
***
Vehicle
Lipidated IL-22 (0.05 mg/kg)
Lipidated IL-22 (0.15 mg/kg)
Lipidated IL-22 (0.3 mg/kg)
anti-TNF (10 mg/kg)
anti-TNF (15 mg/kg)
anti-TNF combi
F
DSS + Vehicle
DSS + CK-0045 0.3 mg/Kg
DSS + anti-TNF 15 mg/Kg
DSS + anti-TNF 10 mg/Kg
DSS + anti-TNF 10 mg/Kg + CK-0045 0.15 mg/Kg

◂**Fig. 3** Lipidated IL-22 histologically induces mucosal healing in an additive fashion to anti-TNF treatment Histological quantification and representative histological images of the number of $Ki67^+$ epithelial cells per crypt **A** and **B**, number of $CD3^+$ cells per area **C** and **D**, and number of goblet cells per crypt **E** and **F** in the distal colon following treatment with lipidated IL-22, murine anti-TNF or combination in a chronic DSS colitis model. Data are presented as mean ± SEM in the graphs. ***$p < 0.001$ compared to vehicle (Dunnett's one-way ANOVA).

Additive Effects of Lipidated IL-22 in Combination with Immunomodulatory Drugs

Given the primary therapeutic role of IL-22 is mucosal healing, whereas existing drug classes for IBD are all immunomodulatory, there is a strong biological rationale for complementary and deeper benefits of combination treatment. We therefore investigated combination treatment of IL-22 with many of the currently used immunomodulatory drugs—anti-TNF, fingolimod, and α4β7 integrin antagonist—in the chronic DSS-induced colitis model.

The combination of lipidated IL-22 with anti-integrin, anti-TNF or fingolimod reduced all endpoints (DAI, endoscopic score and histologic score) more than the agents individually (Fig. 2A). For lipidated IL-22 combined with anti-integrin and fingolimod, the effect of the combination demonstrated a significant improvement in DAI compared to both monotherapy arms that was greater than the calculated proportional decrease in DAI, indicating a fully additive effect.

Endoscopy confirmed that lipidated IL-22 in combination with anti-TNF, fingolimod, and α4β7 integrin antagonist inhibited chronic experimental colitis. The combined administration of lipidated IL-22 at 0.15 mg/kg with all three immunomodulatory agents significantly improved the composite endoscopic score compared to both vehicle and their respective monotherapy groups, confirming strong additive effects (Fig. 2A–C). The efficacy of particularly the combinations of lipidated IL-22 with the S1P modulator fingolimod or anti-mouse α4β7 integrin antibody led to endoscopic improvements that significantly exceeded those seen with all positive control treated animals.

Histologically, similar findings were observed after treatment with lipidated IL-22 combinations. Combined administration of lipidated IL-22 at 0.15 mg/kg with either fingolimod at 0.1 mg/kg or anti-mouse α4β7 integrin antibody at 15 mg/kg significantly improved total histological scores and all subscores compared to vehicle and their monotherapy groups (Fig. 2A, D-E). As with the endoscopic improvements, the efficacies of particularly the combinations of lipidated IL-22 with the S1P modulator fingolimod or anti-mouse α4β7 integrin antibody were significantly greater than all positive control groups. Together, these findings demonstrate the potential of combination treatment regimens of lipidated IL-22 and existing immunomodulatory treatments to break the current therapeutic ceiling for IBD.

Lipidated IL-22 Stimulates Epithelial Healing in Chronic DSS-Induced Colitis

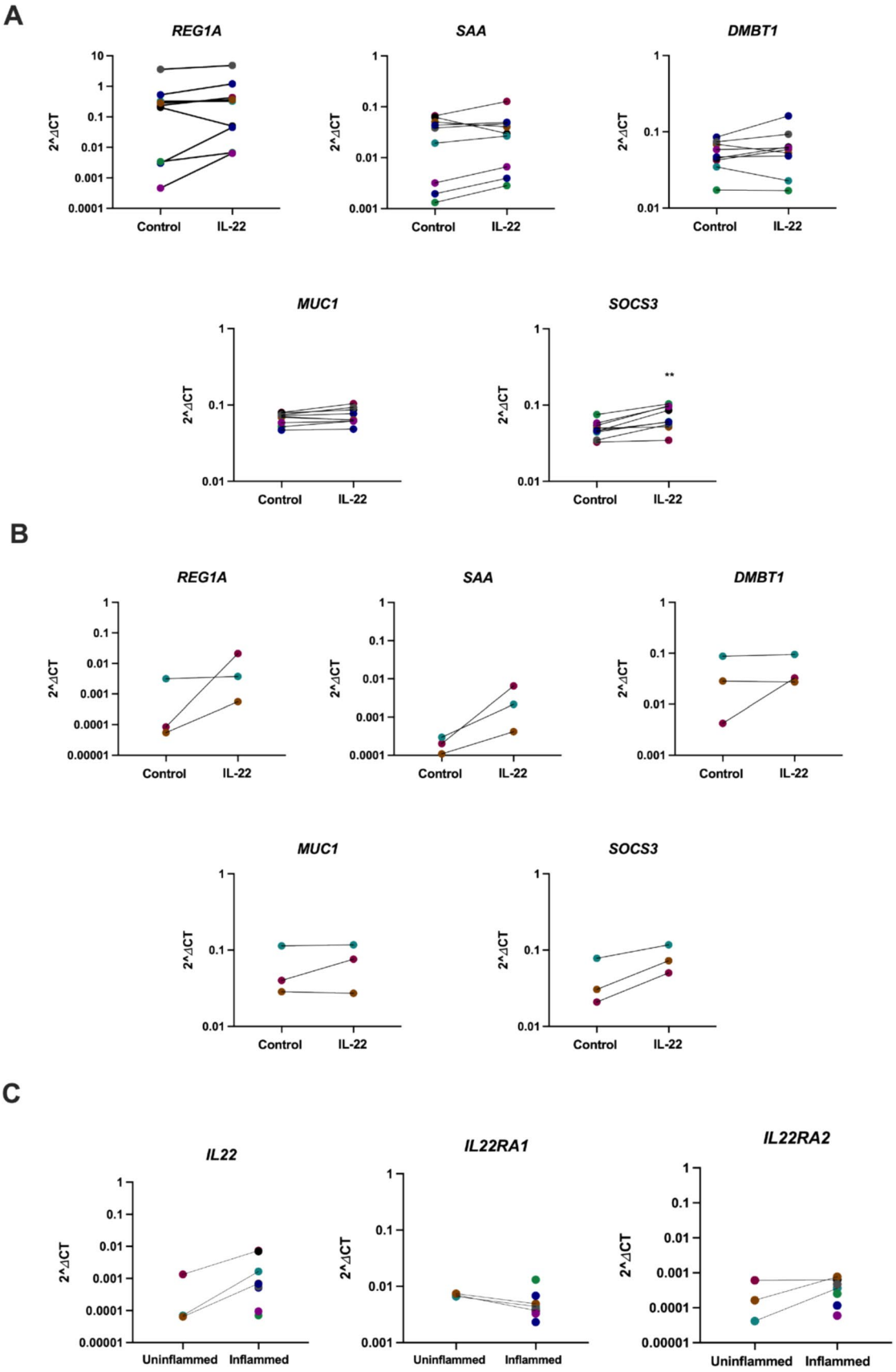
Immunohistochemical stainings were performed to understand the mode of action of lipidated IL-22 in chronic DSS-induced colitis. Indicative of its mucosal healing action, lipidated IL-22 at both 0.15 mg/kg and 0.3 mg/kg significantly upregulated, with comparable efficacy, the number of proliferating epithelial cells per crypt compared with vehicle-dosed DSS-treated mice (Fig. 3A-B). Treatment with lipidated IL-22 was also accompanied by significant dose-dependent reductions in the number of infiltrating $CD3^+$ T-cells in the distal colon compared with vehicle-treated mice (Fig. 3C-D).

In comparison, treatment with anti-TNF at 15 mg/kg more strongly reduced the number of infiltrating $CD3^+$ T-cells as would be expected given its immunomodulatory mode-of-action, whereas no increase in proliferating epithelial cells was observed (Fig. 3). Lipidated IL-22 at 0.15 mg/kg combined with anti-TNF 10 mg/kg significantly reduced the number of $CD3^+$ T-cells more than what was achieved by the compounds individually and increased the number of proliferating epithelial cells, further substantiating the additive effect of the two compounds.

Lastly, all doses of lipidated IL-22 also significantly increased the numbers of goblet cells per crypt compared to both vehicle-treated and anti-TNF-treated animals (Fig. 3E-F). Together these findings demonstrate that the improvements seen with lipidated IL-22 in the chronic DSS colitis model are likely mediated by increased mucosal healing. The induction of mucosal healing was clearly maintained in the combination treatment regimen of lipidated IL-22 and anti-TNF.

IL-22 Stimulates Pathway Gene Expression in Inflamed and Uninflamed Colonic Tissue from Patients with Ulcerative Colitis

Given the therapeutic potential of lipidated IL-22 established in the murine chronic DSS-induced colitis model, we finally sought to investigate whether IL-22 activation would translate to the context of human patients with ulcerative colitis. We therefore recruited 9 patients with ulcerative colitis for colonic biopsy of inflamed and non-inflamed tissue, with clinical and biochemical characteristics of the patients presented in Supplementary Table 2. Of the patients included, 4 were on a combination of two immunomodulatory drugs and one patient was on a combination of three drugs at the time of sigmoidoscopy.


A
REG1A
SAA
DMBT1
MUC1
SOCS3
2^ΔCT
Control
IL-22
**
B
REG1A
SAA
DMBT1
MUC1
SOCS3
2^ΔCT
Control
IL-22
C
IL22
IL22RA1
IL22RA2
2^ΔCT
Uninflammed
Inflammed

◂**Fig. 4** IL-22 pathway activation after stimulation with IL-22 of inflamed and uninflamed colonic tissue from patients with IBD. Relative gene expression in macroscopically inflamed (**A**) and uninflamed (**B**) colonic mucosal biopsies from patients with ulcerative colitis stimulated ex vivo with human interleukin-22 or phosphate buffered saline (Control) for 4 h. **C** Expression of *IL22* and its receptors were measured in unstimulated biopsies from areas with and without macroscopically active inflammation. **p < 0.01 compared to control (Wilcoxon Signed-rank test)

The expression level of the five IL-22 inducible genes tested was higher in the mucosal biopsies from the sites of macroscopically active inflammation compared with mucosal biopsies from areas without macroscopically active inflammation. Ex vivo stimulation with IL-22 of the biopsies from the sites of active inflammation significantly increased expression of *SOCS3* ($P = 0.004$) with further numerically increased expression of *REG1A* ($P = 0.054$), *SAA* ($P = 0.07$), *DMBT1* ($P = 0.20$), and *MUC1* ($P = 0.13$), when compared to unstimulated biopsies (Fig. 4A). Biopsies without active inflammation showed similar directionality of the effects (Fig. 4B). The endogenous expressions of *IL22, IL22RA1,* and *IL22RA2* in unstimulated biopsies were measured in areas with and without macroscopically active inflammation (Fig. 4C), demonstrating no significant changes between the two areas. These findings demonstrate that IL-22 agonism in both healthy and inflamed colonic tissue can induce IL-22 pathway activation.

Discussion

Current treatment algorithms in IBD generally focus on suppressing inflammation rather than targeting the defects in epithelial barrier integrity that promote disease onset and propagation [9]. Despite the emergence of biologics and small molecule drugs addressing IBD, a subset of patients still fail to respond adequately to anti-TNF agents and immunomodulators or experience secondary loss of response, with approximately half of patients treated with anti-TNF agents failing to achieve mucosal healing [20, 21]. This therapeutic ceiling is attributed to the complex pathophysiology of IBD, whereby multiple inflammatory pathways operate simultaneously, which suggests that targeting a single inflammatory pathway may be insufficient for complete disease control [22]. To address this limitation, a dual targeting approach combining IL-22 inhibition with immunomodulatory agents may help to overcome the current therapeutic ceiling and improve overall treatment outcomes in patients with IBD.

IL-22 presents a highly differentiated mechanism of action that promotes mucosal healing by directly inducing epithelial regeneration and improving barrier integrity, including supporting a balanced microbiota [9, 23, 24]. In this study, lipidated IL-22 demonstrated therapeutic efficacy on clinical, endoscopic, and histological endpoints, including significant reductions in the presence and extent of colonic ulceration and inflammation in a model of chronic DSS-induced colitis. Other IL-22 agonistic approaches have been developed, including an IL-22-Fc fusion agonist and probiotics producing bioactive IL-22 [14, 25]. Our lipidated IL-22 approach combine the long half-life necessary for pharmacological treatment, with, unlike for example an Fc-fusion based approach, a wild-type-like molecular size and potency. Furthermore, and for the first time, we demonstrate the complementarity of lipidated IL-22 when combined with most classes of currently used immunomodulatory treatments in IBD and provide evidence of additive effects between the approaches.

The beneficial effects of IL-22 on epithelial repair and host defense have shown promise in preclinical models across a wide range of indications [26, 27]. In this study, we observe dose-dependent efficacy on all measured parameters after 24 days of repeated subcutaneous lipidated IL-22 dosing in a preclinical mouse model of colitis. For all clinical parameters, including the endoscopic and histological scores, the highest dose (0.3 mg/kg) lipidated IL-22 group was indistinguishable from the anti-TNF 15 mg/kg positive control group representing standard therapeutic dosing. These data support that IL-22 holds therapeutic potential in the treatment of IBD. The translatability of these findings is supported by our human data demonstrating responsiveness to IL-22 in colonic tissue from patients with ulcerative colitis.

We explored the combination of IL-22 with currently used immune modulators, as IL-22 theoretically presents a complementary mode of action to currently used therapies, and there is an increasing clinical demand for evidence-based combination treatment particularly for refractory or severe disease presentation [28]. Lipidated IL-22 at 0.15 mg/kg combined with anti-TNF 10 mg/kg was also able to reduce all clinical signs of DSS-induced colitis, as well as the endoscopic and histological scores, indicating an additive effect of the two compounds. The highest therapeutic efficacy was demonstrated with combined treatment of lipidated IL-22 0.15 mg/kg with either fingolimod 0.1 mg/kg or anti-mouse α4β7 integrin antibody at 15 mg/kg. Although efficacy was not significantly different from the combination with anti-TNF, the data for these combinations suggest additive effects and better reductions in clinical signs of DSS-induced colitis than achieved with therapeutic doses of anti-integrin and fingolimod alone.

In agreement with its direct epithelial targeting, we found that lipidated IL-22 dose-dependently increased epithelial $Ki67^+$ and goblet cell numbers accompanied by a dose-dependent reduction in $CD3^+$ T-cells in this model of chronic DSS-colitis, and increased the production of antimicrobial peptide genes in the human colonic biopsies. This

provides further support for the unique ability of IL-22 to induce mucosal healing and improve gut barrier integrity more broadly.

In summary, lipidated IL-22 improved endpoints in a chronic DSS colitis model and promoted mucosal healing and reduced inflammation, with additive effects in combination with immunomodulatory agents on amelioration of colitis. This supports further exploration of the potential benefits of lipidated IL-22 in IBD management, particularly as combination therapy.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10620-025-09007-w.

Author Contributions SS, SD'A, TDS, AKD, SD, MvdB designed the studies. SD'A, SS, AKD acquired the data. SS, MvdB analysed the data. SS, SD'A, TDS, MvdB interpreted the data. SS, MvdB drafted the manuscript. All authors carried out critical revision of the manuscript.

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Data Availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Conflict of interest S.S. and T.D.S. are consultants for Cytoki Pharma. S.D'A has received service fees from Cytoki Pharma, Dompè, Enthera, Ferring, Violicom, Novapep, Surrozen. A.D. has received honoraria as a speaker and/or advisory board member from AbbVie, Janssen, Pfizer, Takeda, and Tillots Pharma. A.L.K., R.J. and M.v.d.B. are employees of Cytoki Pharma. S.D. received honoraria as a speaker, consultant, and/or advisory board member from AbbVie, Alimentiv, Allergan, Amgen, Applied Molecular Transport, AstraZeneca, Athos Therapeutics, Biogen, Boehringer Ingelheim, Bristol Myers Squibb, Celgene, Celltrion, Dr Falk Pharma, Eli Lilly, Enthera, Ferring Pharmaceuticals Inc., Gilead, Hospira, Inotrem, Janssen, Johnson & Johnson, Morphic, MSD, Mundipharma, Mylan, Pfizer, Roche, Sandoz, Sublimity Therapeutics, Takeda, Teladoc Health, TiGenix, UCB Inc., Vial, and Vifor.

Ethical approval All studies in animals have been carried out in accordance with European Union legislation and have received ethical approval from the Italian Ministry of Health. The study involving human subjects has been carried out in accordance with the Declaration of Helsinki and was approved by the Central Denmark Region Ethics Committee (j. no. 1-10-72-362-21).

Informed consent Informed consent was obtained from all participants in the study involving human subjects after the nature and possible consequences of the study were explained.

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