Biologics: Targets and Therapy

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REVIEW

Therapeutic Oligonucleotides for Patients with Inflammatory Bowel Diseases

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Irene Marafini
Giovanni Monteleone

Department of Systems Medicine, University of Rome "Tor Vergata", Rome, Italy

Introduction: The better understanding of the molecular mechanisms, which drive the pathological process in the gut of patients with Crohn's disease (CD) and patients with ulcerative colitis (UC), the major forms of inflammatory bowel diseases (IBD) in humans, has facilitated the development of novel therapeutic compounds. Among these, antisense oligonucleotides (ASOs) have been used to inhibit the expression of molecules, which sustain the IBD-associated mucosal inflammation.

Areas Covered: In this short review, we summarize experimental and clinical data on the use of ASOs in IBD.

Expert Opinion: Preclinical work indicates that the modulation of specific inflammatory pathways through the use of ASOs is highly effective and associates with low risk of adverse events. Initial clinical studies have confirmed the benefit of some ASOs even though no compound has yet reached the market. Further experimentation is warranted to establish the optimal route of administration for each ASO, ascertain whether and how long ASOs maintain their activity following administration, and identify which patient can benefit from specific ASO treatment.

Keywords: Crohn's disease, ulcerative colitis, ICAM-1, Smad7

Key Messages

- Experimental and initial clinical data show that antisense oligonucleotide-based strategies are promising in inflammatory bowel diseases.
- Antisense oligonucleotides-based drugs showed a good safety profile.
- Changes in drug formulation and/or route of administration are fundamental to maximise antisense oligonucleotide efficacy.

Introduction

Inflammatory bowel diseases (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), are chronic disorders of the gastro-intestinal tract.[1] The aetiology of both IBD is unknown and therefore no curative therapy is available. However, a better understanding of the basic mechanisms underlying the pathological process in IBD has facilitated the development of several therapeutic compounds, including biologics and small molecules.[2] Unfortunately, not all the patients respond to the available therapies, and some of them lose response over time or develop drug-related complications,[3–5] suggesting the necessity of additional drugs.

Antisense oligonucleotides (ASOs) are short, single-stranded nucleotides that selectively inhibit RNA sequences through multiple mechanisms, including

Correspondence: Giovanni Monteleone
Dipartimento di Medicina dei Sistemi, Università di Roma "Tor Vergata", via Montpellier, 1, Rome 00133, Italy
Tel +390620903702
Fax +390672596391
Email Gi.Monteleone@Med.uniroma2.it

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modulation of RNA splicing, disruption of molecules regulating RNA stability, inhibition of RNA translation into protein and catalytic degradation of target RNA by the recruitment of RNase H.[6,7] Due to the not favourable natural physicochemical properties, the therapeutic use of ASOs is strictly dependent on strategies, which improve the absorption, distribution, metabolism, and elimination of these agents.[8] Without chemical modifications, ASOs are rapidly degraded by nucleases abundantly present in plasma and tissues.[9] The most frequent chemical modification to stabilize nucleotides against degradation is the substitution of sulphur for oxygen to generate phosphorothioate internucleotide linkages. This facilitates the binding of the ASOs to plasma proteins and the consequent up-take by several organs. ASOs are rapidly cleared from the circulation so that only a fraction of them reaches the target tissues.[10] Therefore, intra-ocular, oral or rectal administrations of ASOs have been adopted to enhance tissue concentration and consequently the therapeutic effect of these compounds.[11–13] In this short article, we review the available data about the role of antisense oligonucleotides (ASOs)-based therapies in IBD.

ASOs-Based Therapies in IBD: From Bench to Bedside

Alicaforsen, a phosphorothioate ASO that selectively inhibits intercellular adhesion molecule (ICAM)-1 expression, was the first ASO to be tested in IBD. ICAM-1 is a transmembrane glycoprotein involved in the recruitment of activated leukocytes from the bloodstream to the gut wall. The levels of ICAM are increased in the inflamed tissue of IBD patients and experimental evidence supports the involvement of this molecule in the amplification of detrimental inflammatory responses in the gut.[14,15] Indeed, ICAM-1 inhibition with a specific ASO was sufficient to dampen inflammation in experimental models of colitis and treatment associated with no signs of toxicity.[16] In the first clinical trial conducted in 1998, intravenous alicaforsen was effective in active, steroid-dependent/resistant CD patients.[17] Unfortunately, these encouraging results were not confirmed in Phase 2 and 3 clinical trials, and the development of alicaforsen was eventually discontinued in CD.[18,19] In contrast, knockdown of ICAM1 was associated with benefit in patients with UC.[20–23] In particular, in a Phase II randomized, double-blind, placebo-controlled trial, alicaforsen enema (240 mg/day) was effective in 40 patients with mild to moderate distal UC.[23] In another randomised clinical trial, alicaforsen enema (120 or 240 mg) and mesalamine enema (4 gr) had comparable 6-week response rates, but alicaforsen showed a more durable effect.[24] Similar results were obtained in a subsequent phase 2, double-blind, placebo-controlled study in patients with acute mild to moderate left-sided UC. In this study, patients receiving 240 mg alicaforsen enema had a significantly lower rate of relapse as compared to placebo, although there were no differences in terms of clinical response between the two groups.[20] Moreover, alicaforsen was well tolerated and effective when administered to 12 patients with pouchitis for 6 weeks.[22] In 2016, a retrospective analysis of alicaforsen enema in chronic refractory pouchitis was conducted. Clinical improvement was reported in 11/13 alicaforsen-treated patients, but almost all of them experienced an early clinical relapse.[25] A Phase 3 randomized placebo-controlled clinical trial conducted in patients with pouchitis refractory to treatment with antibiotics has been recently concluded, but results are not yet publicly available. Collectively, the results in UC and pouchitis led the FDA to consider alicaforsen as an orphan drug, which can be prescribed as an unlicensed medicine in accordance with international regulation in patients with pouchitis and patients with left-sided UC.

A specific ASO targeting the p65 subunit of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) was also tested and showed promising results in in vitro studies and in murine models of colitis. However, no data about the use of NF-κB ASO in IBD patients has been published so far.[26–28]

Smad7 is an intracellular inhibitor of TGF-β1, a cytokine whose activity is central in the negative control of immune responses at the mucosal level.[29] To inhibit Smad7, we developed a 21-base single-strand phosphorothioate ASO that hybridizes to the Smad7 mRNA and facilitates RNase H-mediated RNA degradation through a classic antisense mechanism. Studies conducted in IBD lamina propria mononuclear cells showed that knockdown of Smad7 with this ASO restored TGF-β1-dependent suppression of inflammatory cytokine production.[30] Smad7 was also found to be upregulated in the colon of mice with experimental colitis and oral administration of Smad7 ASO restored TGF-β1 activity and reduced mucosal inflammation.[31] Later on, clinical trials were conducted in CD patients. For these studies, a Smad7 ASO-containing pharmaceutical compound, termed Mongersen, was used. Mongersen is a solid oral dosage

form protected by an external coating that determines gastro-resistance and allows the compound to be mainly released in the terminal ileum and right colon. In a Phase 1 study, Mongersen showed a very good safety profile and a good clinical response in patients with active, steroid-dependent /resistant CD.[32] Subsequently, a phase 2, multicentre, placebo-controlled clinical trial in 166 patients with active, steroid-dependent/resistant CD, showed that Mongersen was more effective than placebo in inducing clinical remission.[33] These data were confirmed by another phase 2 study showing clinical and endoscopic response in patients treated with Mongersen.[34] However, a phase 3 clinical trial was then suspended due to an interim analysis documenting the lack of efficacy of Mongersen.[35]

DNAzymes are single-stranded DNA molecules, which specifically cleave RNA after appropriate binding.[36] A specific DNAzyme targeting GATA3 was tested in pre-clinical models of colitis. Expression of GATA3, a transcription factor involved in T cell development, is increased in UC patients and in murine models of colitis. Mice given intra-rectally anti-GATA3 DNAzyme exhibited reduced expression of inflammatory molecules and were less susceptible to oxazolone and trinitrobenzene sulfonic acid-induced colitis.[37] A phase 2a, double-blind placebo-controlled clinical trial investigating the efficacy of GATA3-specific DNAzyme enema formulation (SB012) in UC patients has been recently completed, but the results of this study are not yet available.[38]

More recently, STNM01, a synthetic, double-stranded RNA oligonucleotide directed against carbohydrate sulfotransferase 15 (CHST15), an enzyme that catalyses sulfation of chondroitin sulfate and is supposed to contribute to the production of collagen and development of fibrosis was tested in CD. CHST15 levels are increased in the inflamed colon of CD patients[39] and STNM01 was effective in mice with dextran sulphate sodium (DSS)-colitis.[40] In a phase 1 clinical trial, 18 CD patients with mucosal lesions refractory to conventional therapies received a sub-mucosal injection of STNM01 or placebo during colonoscopy. STNM01 was well tolerated, improved endoscopic scores and reduced the histological extent of intestinal fibrosis.[41]

Conclusions

The recent decades have witnessed the enormous effort of the researchers to identify molecules involved in the perpetuation and amplification of the detrimental inflammatory response in IBD and to develop compounds, which can suppress the function of such molecules. ASOs represent an interesting novelty in this field, as they inhibit the expression of specific targets with a good safety profile. Nonetheless, no ASO developed for IBD patients has yet reached the market.

Expert Opinion

Data from in vitro studies with human tissue samples of IBD patients and experiments performed in animal models of colitis have largely advanced our understanding of the mechanisms that propagate the IBD-associated mucosal inflammation. Consequently, many strategies have been employed to target key molecules involved in the tissue damaging-immune response in such disorders.[2,42] Among these, various ASOs-based compounds have shown promising results in pre-clinical studies and, therefore, were subsequently tested in IBD patients.[43] Unfortunately, however, intravenous administration of Alicaforsen, the first ASO used in IBD patients, was not effective in patients with active CD.[18,19] Effective delivery of ASOs to their intracellular sites of action remains a major issue. Since ASOs are largely taken up by the liver and lymphoid organs following systemic administration,[44] it is plausible that the intravenously administered Alicaforsen did not reach the inflamed gut of CD patients at concentrations sufficiently high to suppress ICAM1. This hypothesis is supported by the demonstration that Alicaforsen, reformulated as an enema to deliver the ASO directly to the inflamed mucosa, was effective in patients with distal and left-sided UC and in patients with chronic unremitting pouchitis.[20–24] Therapeutic failure of Alicaforsen in CD could also rely on the fact that ICAM-1 is just one of the various molecules that control leukocyte trafficking, and therefore, even in its absence, other integrins can promote the recruitment of inflammatory cells in the gut.[45,46] We cannot also exclude the possibility that ICAM-1 plays a critical role in the amplification of the inflammatory cascade in UC but not in CD.

The encouraging results seen in phase 1 and phase 2 studies, in which knockdown of Smad7 with Mongersen was accompanied by clinical and endoscopic benefits, were not confirmed by a recent phase 3 study, which was prematurely discontinued due to lack of efficacy.[32–35] The reasons for such a discrepancy remain unknown. A possibility is that changes in the selection of the patients may have contributed to the different results between the initial phases 1 and phase 2 studies and the larger phase 3 study. Moreover, the therapeutic failure of phase 3 could somehow rely on physical/chemical changes of Mongersen, which are known to occur during the large-scale oligonucleotide synthesis. Nonetheless, it remains relevant to examine whether

knockdown of Smad7 with Mongersen is therapeutic in specific subsets of CD patients (eg prevention of post-operative recurrence) as well as in UC as Smad7 is up-regulated in inflamed mucosa of UC patients.[47,48]

Overall results derived from clinical trials indicate that ASOs are safe and well tolerated by most patients. This suggests that ASOs strategy could be ideal for specific categories of patients (eg elderly) or for the longer maintenance phases.

The ASO therapeutic field has seen remarkable progress over the last years with promising developments in pre-clinical models of colitis and initial stages of clinical trials in IBD patients. Further work is, however, needed to improve the delivery of the ASOs as well as their cellular uptake and intracellular trafficking.

Disclosure

GM has filed a patent related to the treatment of inflammatory bowel diseases with Smad7 antisense oligonucleotides, while the IM has no conflict of interest.

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