



Smad7 Antisense Oligonucleotide-Based Therapy in Crohn's Disease: Is it Time to Re-Evaluate?

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Abstract

Abundant preclinical work showed that in Crohn's disease (CD), the defective activity of the immunosuppressive cytokine tumor necrosis factor (TNF)- β 1 due to high levels of the intracellular inhibitor Smad7 contributes to amplify the tissue-damaging inflammatory response. Consistently, phase I and II studies documented clinical and endoscopic benefit in active CD patients treated with mongersen, an oral antisense oligonucleotide targeting Smad7. However, a multicenter, randomized, double-blind, placebo-controlled, phase III study was prematurely discontinued as a futility analysis showed that mongersen was not effective in CD patients. The reasons why the phase III study failed despite the fact that previous clinical trials documented the efficacy of the drug remain unknown. The primary objective of this Viewpoint was to provide clues about the factors explaining discrepancies among the clinical trials. We illustrate the recent data indicating that the various batches of mongersen, used during the phase III program, are chemically different, with some of them being unable to downregulate Smad7 expression. Overall, these findings suggest the necessity of new clinical studies to further evaluate the efficacy of chemically homogenous batches of mongersen in patients with inflammatory bowel diseases (IBDs), and, at the same time, they can help understand the failure of other clinical trials with antisense oligonucleotides in IBD (i.e. alicaforsen).

Key Summary Points

1. High Smad7 sustains inflammatory pathways in the gut.
2. Phase II/III studies with a Smad7 antisense oligonucleotide (mongersen) have provided conflicting results in patients with Crohn's disease (CD).
3. Diastereomers of mongersen batches generated during the manufacturing process may account for the different results seen in trials with CD patients.

1 Introduction

Crohn's disease (CD) and ulcerative colitis (UC), the major forms of inflammatory bowel diseases (IBDs), often require lifelong medical therapy for inducing and/or maintaining remission and limiting the risk of complications. For patients unresponsive or intolerant to conventional anti-inflammatory drugs, treatment with monoclonal antibodies targeting inflammatory cytokines (i.e. tumor necrosis factor [TNF] and interleukin [IL]-12/IL-23p40) or the α 4 β 7 integrin, a molecule involved in the recruitment of specific subsets of effector immune cells from the blood to the inflamed gut, or small molecules inhibiting detrimental signalling pathways, has markedly improved the way clinicians manage these pathologies [1–3]. However, there are unwanted therapy-associated adverse effects and more than one-third of patients show primary non-response or lose response over time to initiated treatment [4, 5]. The development of drugs that can selectively target specific inflammatory pathways and avoid unwanted systemic adverse effects, is worth pursuing.

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2 Mongersen

Antisense oligonucleotides (ASOs) could represent a good example of this therapeutic approach as preclinical studies in experimental colitis models documented the good tolerance, safety profile, and efficacy of ASOs [6]. There were attempts to translate such successes into clinics. For instance, initial studies in clinically active CD patients either dependent or resistant to corticosteroids showed efficacy of mongersen, an ASO targeting Smad7, a regulator of gut inflammation and immunity [7–11]. However, a futility analysis on 560 patients (421 receiving the active drug and 139 receiving placebo) of a multicenter, randomized, double-blind, placebo-controlled, phase III study did not confirm the impressive positive results seen in phase II studies, thus leading to discontinuation of the mongersen program [12]. It has been suggested that phase III could have failed because more stringent inclusion criteria (i.e. documented endoscopic activity of lesions) were adopted than those used in the phase II studies [13]. However, this single issue is unlikely to have accounted for such a huge disparity, given that the inclusion criteria of the phase III studies were similar to those of a previous endoscopic phase II study, which documented the clinical and endoscopic efficacy of the drug [14].

Mongersen is a phosphorothioate (PS) 21 nucleotide-long ASO. Compared with the native phosphodiester linkage, PS modification confers a higher degree of metabolic stability from nuclease-mediated degradation. In addition, the PS modification enhances ASO protein-binding properties, thus facilitating the ASO distribution and internalization into tissues without the aid of complex delivery vehicles or formulations. PS substitution converts the achiral phosphodiester linkage into a chiral PS center having two distinct stereochemical configurations, designated Sp and Rp. All PS-substituted ASOs comprise mixtures of up to 2^n individual drug molecules, with n being the number of PS linkages present in the PS-modified oligonucleotides. These diastereomers can have different biological activity [15]. Even small changes in the manufacturing process can result in considerable differences in the chiral composition of PS linkages.

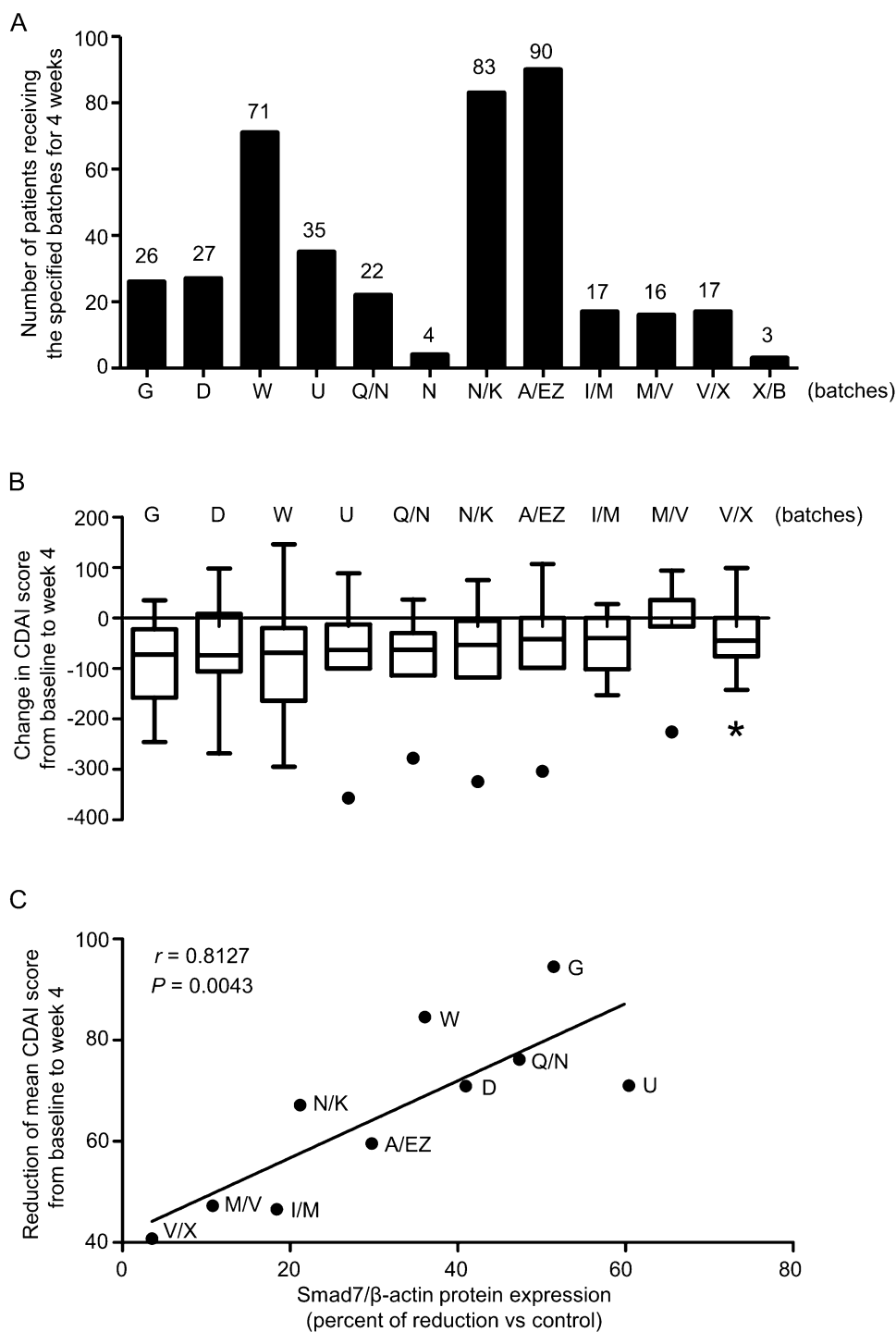
During the advanced clinical program, many batches of mongersen were manufactured and then used in the clinical trial without controlling PS stereochemistry. By assessing Smad7 RNA and protein content in colonic epithelial cells, we have recently documented a marked difference in the ability of those batches to downregulate Smad7, with some of them exhibiting no inhibitory action and most of them exhibiting minimal inhibitory effect compared with the batch used in the previous phase I and II studies [16]. It is plausible that throughout the clinical development

program, there was a lack of reproducibility of the drug substance (DS) manufacture, which could not be detected during quality control testing and by employing several techniques aimed at characterizing the composition and chemical/physical properties of the batches. Solution ^{31}P -NMR spectroscopy, which has the unique ability to provide information about the chemical environments of the phosphorus atoms, showed that each mongersen preparation had a distinct ^{31}P -NMR profile, indicating that PS chirality was not homogeneous across the batches used in the clinical trials. Principal component analysis helped identify clusters of the batches with similar ^{31}P -NMR spectra, and, interestingly, preparations with the same ^{31}P -NMR spectrum profile had a similar *in vitro* inhibitory effect of Smad7 [16]. Post hoc analysis of 411 patients enrolled in phase III and receiving mongersen for at least 4 weeks allowed to identify 12 cohorts, each receiving either a single batch ($n = 5$) or two mixed batches ($n = 7$) of the active compound (Fig. 1a). Clinical efficacy of the drug was assessed at week 4 only, as after such a time point each of the 12 cohorts of patients was treated with mixed batches, making it difficult to ascertain any relationship between the efficacy of the drug and the batch of the DS. There were differences in the clinical response among the cohorts (Fig. 1b), and the greatest reductions in the Crohn's Disease Activity Index (CDAI) score, a clinical symptom score commonly used in CD drug trials [17], were seen in CD cohorts treated with batches showing the more powerful *in vitro* activity (see Fig. 1c and Arrico et al. [16]). At this time point, neither biochemical parameters of inflammation nor endoscopy were available to support the clinical data.

It is thus conceivable that variability in the diastereomeric distribution of the PS linkages among batches may have generated differences in the *in vitro* inhibitory effect of the various ASOs, as well as attenuated the clinical efficacy of most batches. This enables us to rationalize one of the possible causes that may have contributed to the failure of the phase III program. However, it is difficult to corroborate this hypothesis with more stringent experimental data and we leave it to further study and discussion.

If this comes to be true, then the critical question is why mongersen worked very nicely in the previous phase II trials. Although we cannot definitely answer the question, it is noteworthy that the overall manufacturing process of PS-ASO is stereo-reproducible only when it is performed under rigorously identical conditions. Changes in the manufacturing conditions and batch size to accommodate the increasing DS demand for large clinical trials were introduced over time. For the phase I and II studies, batch size was quite similar and the DS was prepared in a very short time frame. All these batches efficiently inhibited Smad7 expression *in vitro* [11, 16].

Fig. 1 **A** Number of Crohn's disease patients receiving the specified batches of mongersen in the phase III study. **B** Box plot graph depicting the median change and interquartile range in CDAI score from baseline to week 4 in distinct cohorts of Crohn's disease patients receiving either a single batch or mixed batches of mongersen. All 411 patients were included in the analysis (intention-to-treat population; last observation carried forward analysis). One-way ANOVA followed by Tukey's post hoc test; batch G vs. batch V/X, $p < 0.05$. **C** Correlation analysis between the mean CDAI score reduction in patients treated with the indicated batches and the ability of such batches to inhibit Smad7 protein expression in vitro (as reported by Arrico et al. [16]) [$p = 0.0043$, Pearson's test coefficient $r = 0.8127$]. For CD cohorts receiving a mix of two different batches, the in vitro activity was calculated as the mean value of the in vitro activity of the single batches (designated as the percentage of reduction of Smad7/ β -actin protein expression vs. control). ANOVA analysis of variance, CDAI Crohn's Disease Activity Index



Further analyses are needed to clarify which variations in the manufacturing protocols and/or in the starting materials led to changes in the diastereomeric composition of the batches used in the clinical development program and how we can prevent such changes. At the same time, these findings support new clinical experimentation to further assess the efficacy of chemically homogenous batches of

mongersen in CD as well as in UC and refractory chronic pouchitis. Future work will also tell us if the issue outlined above is either restricted to the mongersen batches used in the phase III program or ubiquitous in the context of PS-modified oligonucleotides, thereby contributing to explain the failure of previous clinical trials with ASOs in IBD (i.e. alicaforsen) [18].

3 Conclusion

Overall, these findings suggest the necessity of new clinical studies to further evaluate the efficacy of chemically homogenous batches of mongersen in patients with IBDs.

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Declarations

Conflicts of Interest Giovanni Monteleone served as a consultant for First Wave BioPharma and has filed a patent related to the treatment of inflammatory bowel diseases with Smad7 antisense oligonucleotides. Carmine Stolfi and Irene Marafini have no conflict of interests to declare. Raja Atreya received research grants from, or served as a member of advisory boards or speakers' bureaus, for AbbVie, Amgen, Arena Pharmaceuticals, Biogen, Boehringer Ingelheim, Bristol Myers Squibb, Celgene, Celltrion Healthcare, Dr Falk Pharma, Eli Lilly, Ferring Pharmaceuticals, Fresenius Kabi, Galapagos, Gilead, GlaxoSmithKline plc, InDex Pharmaceuticals, Janssen-Cilag, Kiniksa Pharmaceuticals, MSD, Novartis, Pandion Therapeutics, Pfizer, Roche Pharma, Samsung Bioepis, Stelc Institute, Sterna Biologicals, Takeda Pharma, Tillotts Pharma AG, and Viatrix. Markus F. Neurath served as a consultant for Pentax, Abbvie, Boehringer, PPM, Janssen, and Takeda.

Ethics and consent Not applicable.

Author contributions GM: Literature search, data collection and interpretation, and writing of the manuscript. CS, IM, RA and MFN: Critical revision of the manuscript.

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Data availability Data describing the chemical properties of the mongersen batches are available in PubMed and can be accessed via the following doi link: <https://doi.org/10.1089/nat.2021.0089>. The post hoc analysis data shown in Fig. 1 were provided by Nogra Pharma Ltd. Data will be shared on request to the corresponding author with the permission of Nogra Pharma Ltd.

Code availability Not applicable.

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