Safety of Antisense RNA Oligonucleotides in Treatment of Cystic Fibrosis

Elizabeth Robinson (ECR_854)

Erin & Katie, Tues 1:30 – 5:30

Cystic fibrosis (CF) is a deadly, heritable lung disorder. Patients with CF lack activity in (or lack altogether) the cystic fibrosis transmembrane regulator (CFTR), a chloride ion channel responsible for keeping lung mucus moist—without proper moisture, the lungs develop breathing difficulties and frequent infections (Boyd et al., 2020). Because it is a genetic disorder, research conventionally focuses on creating gene therapies to supply CFTR DNA. While gene therapies are effective, they often cause adverse side-effects from integrating into unpredictable areas of the genome. Gene vectors may accidentally place the CFTR DNA into a tumor suppressor gene, putting patients at risk for developing cancers (Boyd et al., 2020). Recently, researchers have looked into RNA therapies, such as antisense oligonucleotides (ASO), for combating cystic fibrosis without need for integration. Unlike gene therapies, ASOs cannot integrate into the cell's DNA, and therefore are much less likely to interrupt a proto-oncogene and cause cancer. ASOs are small RNA molecules that block mRNA translation by physically binding to the mRNA (Boyd et al., 2020). Therefore, they can downregulate expression of genes that may cause or exacerbate CF symptoms. For instance, the epithelial sodium channel (ENaC) remains active while the CF patient's missing chloride channel is inactive, contributing to unbalanced moisture in the lungs (Crosby et al., 2017). ASOs targeted at genes encoding ENaC, such as *Scnn1a*, might therefore alleviate or even reverse CF symptoms (Crosby et al., 2017). Conversely, ASOs can be used to block translation regulation elements in the mRNA for CFTR itself, therefore upregulating CFTR translation levels (Boyd et al., 2020). CFTR upregulation through ASOs has advanced into the clinical stage, where researchers are assessing its tolerable dose and adverse side-effects (Drevinek et al., 2020). Due to the low incidence and severity of adverse side-effects in clinical trials, antisense oligonucleotide therapy for treating cystic fibrosis offers a far safer alternative to conventional gene therapy.

The earliest trials assessing CF-treating ASOs' side-effects were conducted in non-human animal models. In 2017, Crosby et al. published their findings on the safety and efficacy of ASO treatment for cystic fibrosis in mouse models. The team's ASO targeted ENaC present in the lungs, but this channel is also active in the colon, sweat glands, and kidneys. Therefore, Crosby et al. 2017 wanted to assess the effects of their ASO treatment in the colon and kidneys to make sure that an aerosolized, inhalable version of their ENaC-targeting ASO would be safe in clinical trials (Crosby et al., 2017). An ENaC ASO targeting mRNA from the *Scnn1a* gene and a control ASO were tested in two separate mouse models: one group of mouse pups engineered to mimic human cystic fibrosis, and one group of adult mice given a separate ASO to cause them to display CF symptoms—such as mucus accumulation in the lungs and airway hyper-reactivity (AHR). The mouse pups inhaled their experimental treatments (the ENaC ASO or the control ASO at 10 microgram/g of saline) daily for eleven days, and the mice with CF symptoms induced by ASOs inhaled theirs three times a week for three weeks. To measure whether or not the ENaC ASO was effective at treating CF symptoms, the group assessed the AHR of the mice over time, the ENaC mRNA levels collected from different tissues, and electrolyte levels in the serum and urine of the mice (to test for altered kidney function) (Crosby et al., 2017). CF symptoms did not worsen in either group of mice receiving the ENaC ASO; in fact, their symptoms improved. Mice in the treatment groups had significantly reduced levels of accumulated mucus in the lungs, as well as reduced airway reactivity. This suggests that the ENaC ASO is an extremely effective treatment for cystic fibrosis, especially compared to non-genetic treatments such as saline inhalation, which tends to only slowdown the disease's progression. Likely, the heavily improved lung functions are due to the lowered levels of ENaC mRNA (~70% reduction), which suggests lower levels of transcription of the *Scnn1a* gene.

There were no reported adverse side-effects, and no mice experienced abnormal kidney functions, as shown by no significant changes in their serum/urine electrolyte levels. This makes sense, as the ENaC mRNA levels in the mice's kidneys did not change by treatment group. This evidence suggests that an ENaC ASO would not lead to kidney disfunction in human patients. However, one major concern revealed by this study could be the ASO treatment's effect on colon function: mice in the ENaC ASO treatment groups had reduced *Scnn1a* mRNA levels by 80 to 90%, a significant difference from the control groups (Crosby et al., 2017). While the authors did not report any resulting defects and did not discuss effects on the colon at length, this could be cause for concern in human clinical trials. This, mixed with the fact that mice models of this human disease are imperfect, suggests that caution is required when moving forward.

To more accurately evaluate ASO effects in humans instead of mouse models, Sermet-Gaudelus et al. 2019 conducted a clinical trial in human patients, assessing whether an ASO drug named eluforsen would be effective at combatting cystic fibrosis caused by a CFTR mutation. They also observed the type, incidence, and severity of side-effects (Sermet-Gaudelus et al., 2019). They recruited patients who were either homozygous or heterozygous for the CFTR mutation and gave each of them 12 doses of eluforsen over the course of three weeks. They measured subsequent chloride channel activity by membrane potential, as cystic fibrosis is caused by unbalanced ion potentials in the first place (Sermet-Gaudelus et al., 2019). No patient's cystic fibrosis symptoms worsened during treatment, which in itself implied that the treatment was at least partially successful. But in patients homozygous for the mutation, the eluforsen even improved symptoms and membrane potential ($p = 0.039$), meaning that the treatment was particularly effective in a subset of the subjects. After halting the treatment, the chloride channel activity weakened from the potential recorded before starting the treatment ($p =$

0.072), suggesting that the treatment was not long-lasting (Sermet-Gaudelus et al., 2019). These results imply that the ASO treatment was effective, but only for the homozygous group and only for as long as it was administered. This brevity was not unexpected, as ASOs are inherently less permanent than gene therapies. The ASO had myriad negative side-effect, mostly in the respiratory or digestive systems. Almost 90% of the patients reported mild negative symptoms, such as nausea, fever, cough, and headache. However, less than one third of those were found to be connected to the eluforsen treatment. No subjects had symptoms severe enough to leave the trial (Sermet-Gaudelus et al., 2019). While the side-effects were not severe, the eluforsen ASO treatment did cause more adverse effects in patients than previously hoped. Overall, the researchers' findings made eluforsen's promise of having lowered side-effects ambiguous, but established that CFTR could effectively improve symptoms in cystic fibrosis patients with a homozygous CFTR mutation (Sermet-Gaudelus et al., 2019).

In a later double-blind, phase 1b clinical trial, Drevinek et al. 2020 assessed the number and severity of adverse side-effects resulting from the eluforsen ASO in various doses and time-frames (Drevinek et al., 2020). They only recruited patients homozygous for the CFTR mutation and split them into three groups: the controls, the single-dose, and the multi-dose groups. The single-dose group was given a single dose at the beginning of the trial. The multi-dose group was given eluforsen 12 times over the course of three weeks. In addition to testing for adverse effects, they also measured whether the patients' symptoms had improved using both collected samples and self-reports—the latter was measured using a pre-established Respiratory Symptom Score (RSS) protocol (Drevinek et al., 2020). In the single-dose group, symptoms did not significantly improve and no negative side-effects were observed. Compared to the control group, three of the four multi-dose experimental groups showed significant RSS score improvement ($p = 0.0592$,

0.0074, and 0.0408, respectively). Conversely, the fourth experimental group did not change significantly from their baseline symptom severity at any timepoint (lowest $p = 0.4614$), implying that the treatment may not be universally effective. Adverse side-effects were minimal: none of their dosage sizes were determined to be intolerable, even up to 50 mg doses, which will allow the drug to move forward in future clinical trials. Therefore, eluoforsen was effective at halting CF symptoms in most cases. 22% of patients in the placebo group experienced at least one adverse side effect (including cough, congestion, and joint pain), and the experimental group with the highest percentage of patients with side-effects stood at 85.7% affected patients (including fever diarrhea, and throat pain). However, the vast majority of these side-effects were mild-to-moderate, suggesting that the treatment may be worth the side-effects. Additionally, only 33% of these side-effects were confirmed to be due to the ASO or its delivery device. One patient in an experimental group did experience side-effects severe enough to stop participating in the study, but so did one patient in the placebo group, and these effects (abdominal pain and hypoglycemia) were not found to be drug-induced: therefore, eluforsen did not cause any severe side-effects in this study. (Drevinek et al., 2020). In summary, there were side-effects, but the ones that were due to the drug (and not other medical issues) were few and mild enough for the eluforsen ASO to continue into further clinical trials. Overall, this trial was successful enough in terms of effectiveness and safety for the eluforsen ASO to proceed into later phases and larger sample sizes (Drevinek et al., 2020). The Dreinek paper revealed the fewest and least severe side-effects of the papers in this review, while also having the most robust methodology and sample size.

Not all recent publications in the side-effects of CF-treating ASOs have dealt with human subjects: Boyd et al. 2020 published a review covering the efficacy and safety of all the various

molecular medicines being studied to treat cystic fibrosis in non-human models. These medicines included CRISPR/Cas9, gene therapies delivered either with viral or non-viral vectors, xenotransplantation, and microRNA therapies like ASO, mRNA supplementation, siRNA, and miRNA. In each case, the review outlines the scientific theory behind the treatment and cites studies that have attempted treatment in cell lines, non-human animal models, and/or clinical trials (Boyd et al., 2020). They did not review any clinical trials for ASOs, but studies in animal models and human cell lineages found that ASOs can cause cytotoxicity. However, their sheer efficacy helps their safety, because ASOs do not need vectors and therefore tend not to cause immune reactions—an issue that arises with virtually every other therapy mentioned, including other RNA therapies (Boyd et al., 2020). The Boyd group's review suggests that, while they are effective, ASO treatments should be handled with caution, as they may damage a patient's cells. It may be subjective whether the cytotoxicity caused by ASO treatments outweighs their potential benefits, especially when considering their most common alternative: gene therapies that have carcinogenic potential.

After assessing an experiment in mouse models, clinical trials in human subjects, and a review of trials conducted in non-living models, I find that, while studies conducted in non-human animal models or human cell lines suggest greater potential side-effects, more recent evidence from trials in actual patients supports antisense oligonucleotide therapy as being a safer and viable alternative to conventional gene therapy. Two clinical trials assessed here both used the eluforsen ASO to target a specific type of cystic fibrosis, but the more recent study uses a larger sample size and more diverse methods of evaluating patients' symptoms: while we cannot conclude that ASOs will have no adverse reactions in patients, more evidence suggests eluforsen is safer than other conventional therapies while still being effective. This contrasts with the

review article and the ENaC ASO study conducted in mice: however, these papers did not deal with actual human bodies, which might explain why the review reported more severe adverse effects than the actual clinical trials. It is important to note that, of the adverse side-effects reported in the clinical trials, one of the most common were gastrointestinal issues: the 2017 Cosby paper found that their ENaC ASO downregulated epithelial sodium channels in the colon, and this may suggest a mechanism behind the appearance of these gastrointestinal side-effects. The Cosby et al. 2017 pre-clinical trial searched for adverse kidney effects in their mouse models, but did not dedicate much time to evaluating gastrointestinal effects. As for efficacy, both clinical trials confirm that the eluforsen ASO is effective in patients homozygous for the CFTR mutation, whereas both the review article and the ENaC ASO study corroborate ASO's high efficiency even without the need for a vector. Overall, these results are highly promising. Future studies should narrow their focus onto human subjects, with particular attention paid to ASOs' effects on the large intestine, but ASOs are already demonstrating their safety advantage over gene therapies.

Works Cited

Boyd, C. A., Guo, S., Huang, L., Kerem, B., Oren, Y. S., Walker, A. J., & Hart, S. L. (2020). New approaches to genetic therapies for cystic fibrosis. *Journal of Cystic Fibrosis*, *19*, S54–S59. https://doi.org/10.1016/j.jcf.2019.12.012

Crosby, J. R., Zhao, C., Jiang, C., Bai, D., Katz, M., Greenlee, S., Kawabe, H., McCaleb, M., Rotin, D., Guo, S., & Monia, B. P. (2017). Inhaled ENaC antisense oligonucleotide ameliorates cystic fibrosis-like lung disease in mice. *Journal of Cystic Fibrosis*, *16*(6), 671–680. https://doi.org/10.1016/j.jcf.2017.05.003

Drevinek, P., Pressler, T., Cipolli, M., De Boeck, K., Schwarz, C., Bouisset, F., Boff, M., Henig, N., Paquette-Lamontagne, N., Montgomery, S., Perquin, J., Tomkinson, N., den Hollander, W., & Elborn, J. S. (2020). Antisense oligonucleotide eluforsen is safe and improves respiratory symptoms in F508DEL cystic fibrosis. *Journal of Cystic Fibrosis*, *19*(1), 99–107. https://doi.org/10.1016/j.jcf.2019.05.014

Sermet-Gaudelus, I., Clancy, J. P., Nichols, D. P., Nick, J. A., De Boeck, K., Solomon, G. M., Mall, M. A., Bolognese, J., Bouisset, F., den Hollander, W., Paquette-Lamontagne, N., Tomkinson, N., Henig, N., Elborn, J. S., & Rowe, S. M. (2019). Antisense oligonucleotide eluforsen improves CFTR function in F508del cystic fibrosis. *Journal of Cystic Fibrosis*, *18*(4), 536–542. https://doi.org/10.1016/j.jcf.2018.10.015