THERAPEUTIC ANTISENSE TARGETING OF HUNTINGTIN

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Abstract

Antisense oligonucleotides are a relatively new therapeutic entity which utilizes short, chemically-modified strands of DNA in targeted interactions with RNA to modulate the type or amount of resultant protein. This brief review summarizes the preclinical, translational and early clinical development of an ASO designed to reduce the production of the disease-causing protein in Huntington's disease, an inherited neurodegenerative disease.

Introduction

Huntington's disease (HD) is an autosomal dominant, neurodegenerative disorder. Approximately 30,000 people in the United States have HD, and many more are at risk of developing HD. Disease onset usually occurs in mid-adult life and follows a 15- to 20-year inexorable course to death. A less common form – juvenile HD – begins in childhood and progresses more rapidly, typically resulting in death approximately 10 years from motor symptom onset. Current treatments for HD are limited to symptomatic therapies, as no treatment has been shown to prevent onset or to slow progression. (Bates *et al.*, 2015)

HD is often depicted as a combination of Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) because it manifests in physical changes (abrupt involuntary movements, coordination deficits, speaking and swallowing difficulties), cognitive decline (deterioration in communication, planning, decision-making, memory) and behavioral changes (irritability, aggression, mood swings, apathy). Like PD, AD and ALS, the pathology of HD is characterized by aberrant aggregation of protein in brain cells. Unlike these other diseases, every case of HD is caused by a single genetic mutation – a CAG repeat expansion in *HTT*, the gene that encodes the huntingtin protein. The abnormal gene results in production of mutant huntingtin protein (mHTT) containing an expanded polyglutamine tract, which leads to neuronal dysfunction and death through toxic gain-of-function mechanisms.

Traditional drug discovery is a resource intensive effort with pharmacologists, cell and molecular biologists, medicinal chemists, pharmacokineticists and toxicologists all serving important roles. With the use of high-throughput screening techniques, thousands of small molecules can be evaluated for activity toward specific biological targets to identify leads worthy of further testing (Dandapani *et al.*, 2012). However, these methods are low yield, and targets are generally limited to select classes of proteins with known structures and well-defined binding sites. Fortunately, advances in genomics have accelerated our ability to link genetic targets to human disease and

development of new therapeutic platforms are expanding the number of druggable targets, creating an environment conducive to intelligent design of highly specific, potentially disease-modifying drugs.

RNA-targeted therapeutics, such as antisense oligonucleotides (ASOs), epitomize informed drug design. Over the last 2 decades, ASO technology matured through foundational research on ASO mechanisms, pharmacokinetics and safety; and the technology attained viability through medicinal chemistry inventions imparting desirable drug-like properties (Crooke *et al.*, 2018). Recent new drug approvals – including nusinersen, an ASO for the treatment of spinal muscular atrophy – demonstrate the potential of ASOs to effect disease modification in a neurological disease.

Given the monogenic nature of HD, where disease pathology is primarily due to production of the mutant HD protein (mHTT), inhibition of *HTT* expression represents a promising therapeutic strategy. ASO technology provides a direct route to inhibition of *HTT* expression by targeting *HTT* RNA for destruction, thus preventing translation of mHTT and targeting the primary disease mechanism.

Antisense Oligonucleotides

ASOs are short, single-stranded oligomers comprised of chemically-modified nucleotides that bind to RNA to modulate its function. The order of nucleotides can be designed to enable high specificity to a target RNA, and selection and location of chemical modifications along the ASO dictate post-binding functional modulations. Broadly, ASO mechanisms can be categorized as either promoting the target RNA's degradation through endogenous enzymes, such as RNase H1, or interfering with the target RNA's function without promoting degradation, such as translation arrest or modulation of RNA processing (Bennett *et al.*, 2017). Figure 1 depicts the ASO RNase H1 mechanism, which is utilized in this program to degrade *HTT* RNA.

Foundation for Clinical Testing in HD

Evidence supporting pharmacologic lowering of HTT as a promising therapeutic strategy for HD has accumulated over the last decade, with data emerging from multiple laboratories using various methods to lower HTT in animal models of HD (reviewed by Keiser, Kordasiewicz and McBride) (Keiser *et al.*, 2016). In a series of preclinical experiments, ASOs were shown to effect long-lasting reduction of *HTT* RNA and protein throughout the central nervous system; effects were dose-dependent with a maximal achievable reduction of ~75%, though even modest reductions of ~35% were associated with phenotypic and survival benefits (Kordasiewicz *et al.*, 2012; Stanek *et al.*, 2013; Southwell *et al.*, 2018).

Prior to testing an HTT-lowering ASO in humans, extensive toxicology, pharmacokinetic (PK) and pharmacodynamic (PD) preclinical testing of the human candidate ASO was undertaken. ASO potency and tolerability can vary widely depending on nucleotide sequence and chemical modifications, so a broad screening of ASOs – all homologous to human *HTT* – was conducted to select a suitable human candidate. Additional animal testing was conducted to allow for construction of a preclinical PK/PD model relating ASO dose level to changes in HTT in brain tissue and in cerebrospinal fluid (CSF). This model was used to inform selection of the ASO doses to be used in the clinic and to allow for extrapolation from PD effects that are measurable in the clinic

(such as change in CSF HTT) to PD effects of interest (such as change in HTT RNA and protein in brain tissue).

In parallel with preclinical testing of the human candidate ASO, a clinical development plan was created. While large, long clinical trials will ultimately be necessary to determine if the candidate ASO is efficacious and safe, valuable insights into the ASO's promise – and to the overriding concept of HTT lowering as a therapeutic strategy for HD – can be assessed in a much smaller setting. Accordingly, a short, first-in-human, safety trial was designed to evaluate the tolerability of repeated intrathecal injections of the ASO and to obtain exploratory measures of ASO pharmacology.

The design of the first clinical trial was a randomized, double-blinded, placebo-controlled, multi-ascending-dose, multi-center, phase 1/2a study conducted in 46 HD patients with early-stage disease at 9 international centers (NCT02519036). Study participants were assigned to ASO (HTT_{Rx}, also known as ISIS 443139 or RG6042) or placebo (artificial CSF) at a ratio of 3:1 within each of 5 dosing cohorts (10 mg, 30 mg, 60 mg, 90 mg or 120 mg). Each participant received 4 lumbar intrathecal bolus injections of HTT_{Rx} or placebo at 4-week intervals followed by a 4-month untreated follow-up period. A CSF sample was collected prior to each study drug injection, and an additional CSF sample was collected either 4 or 8 weeks after the last dose of study drug. The primary objective was evaluation of the safety and tolerability of HTT_{Rx}. Other objectives included characterization of CSF pharmacokinetics of HTT_{Rx} and exploration of the effects of HTT_{Rx} on pharmacodynamic biomarkers and clinical endpoints relevant in HD, including mHTT concentration in CSF and functional assessments commonly used in HD.

Key Results of the Clinical Trial

The trial and all results are described in detail in Tabrizi et al (Tabrizi *et al.*, 2019). Overall, HTT_{Rx} was well tolerated. All patients received all scheduled doses of assigned treatment, and all patients completed the trial according to the protocol. Adverse events were generally mild and unrelated to study drug. The only serious adverse event was admission of a patient in the placebo group for a transient postural headache determined to be unrelated to study drug. There were no clinically-relevant adverse laboratory parameter changes and no evidence of renal toxicity, hepatotoxicity or thrombocytopenia during the study.

HTT_{Rx} treatment resulted in significant, dose-dependent reduction in CSF mHTT. At the two highest doses, 90 and 120 mg/month HTT_{Rx}, a mean reduction in CSF mHTT of 40% was observed, with a maximum individual reduction of more than 60% (Figure 2A, B) and a downward trajectory suggesting steady state maximal reduction of CSF mHTT was not reached during this short study (Figure 2A, C).

HTT_{Rx} concentrations were well-aligned with predictions from the preclinical PK/PD model. Using the model to estimate ASO effects in brain tissue, 40% reduction in CSF mHTT is expected to reflect 55-70% lowering of mHTT in cortical tissue and 20-35% lowering of mHTT in striatal tissue; and 60% lowering in CSF mHTT is expected to reflect 70-85% lowering of mHTT in cortical tissue and 35-50% lowering of mHTT in striatal tissue. Sustained mHTT reduction of this magnitude in tissue reaches or exceeds the threshold shown to produce significant improvements in motor function and survival in transgenic mouse models of HD (Kordasiewicz *et al.*, 2012; Keiser *et al.*, 2016). Thus, the CNS mHTT lowering achieved in the high dose groups in this study is believed to be pharmacologically relevant.

Functional, cognitive, psychiatric and neurological clinical outcomes were generally unchanged during the study, and no differences were observed between placebo-treated patients and any HTT_{Rx} dose group. This short study was not designed or sufficiently powered to allow for evaluation of the effect of HTT_{Rx} on disease biomarkers or clinical measures of HD. HD is a slow-progressing disease, with changes on standard outcomes generally occurring over years, not weeks.

Conclusions

Since the discovery of the *HTT* gene in 1993, methods to reduce its toxic product, mHTT, have been sought. The clinical study described here was the first to demonstrate pharmacologically-induced reduction of mHTT in CSF, which likely reflects a reduction of mHTT in CNS tissue. Reaching this milestone is the result of a comprehensive drug discovery and preclinical program to identify an ASO that safely, potently and specifically suppresses HTT production, thus demonstrating that robust early development efforts can facilitate successful translation to the clinic. Larger, longer-term studies are underway to determine whether HTT_{Rx}-mediated CNS mHTT reduction effects meaningful changes to disease course (NCT03342053, NCT03761849; sponsored by Hoffman-La Roche).

The observations in this study may have important ramifications well beyond the HD field. This study was not just the first to demonstrate ASO-mediated protein suppression in the CNS of patients with HD, but the first to demonstrate ASO-mediated protein suppression in individuals with any neurodegenerative disease. Recently, similarly promising results have been reported for an SOD1-targeting ASO in ALS patients (Miller *et al.*, 2019). HD is often depicted as a combination of PD, AD and ALS – a stark reminder of the current lack of disease-modifying treatments for any of these conditions. In the coming years, we look to ASOs as a promising therapeutic modality to meet this need not only for HD but also for PD, AD, ALS and other neurodegenerative diseases associated with aberrant protein production or accumulation.

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