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Addressing the Challenges of Treating Patients with Heterozygous Gain of Function Mutations

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Background

Recent progress suggests that many, if not most, genetic rare diseases are genomically far more complex than previously thought. For example, more than 40 potentially causal genes have been identified in the amyotrophic lateral sclerosis population, and within most of those genes, multiple pathogenic mutations have been identified.[1] Arguably less surprising, but nonetheless important, is the genomic diversity associated with more prevalent "multifactorial" diseases. In the Parkinson's disease (PD) population, for example, at least 20 potentially causative genes and >200 genes associated with increased risk of PD have been reported.[2] Our initial experience at n-Lorem underscores the potential scale of the genomic diversity in extremely rare diseases and the impact of these mutations on the lives of patients and families.[3] Approximately 50% of the roughly 130 patients accepted for treatment by n-Lorem express heterozygous toxic gain of function mutations (*TGOFs*) in a very diverse group of genes that result in an equally diverse variety of syndromes (Table 1).

Since cells harboring heterozygous mutations have a wild-type (WT) and a mutant (MT) allele, they also have a WT and MT RNA and protein encoded by the gene with the mutation. Antisense oligonucleotide (ASO) technology has been shown to be a drug discovery platform capable of creating drugs that selectively affect only the products of a mutant allele.[4,5] To achieve allele-selective reduction of mutant RNA and protein, we take advantage of the effects of mismatches on hybridization and the effects of RNA sequences on the cleavage pattern of RNase H1.[6,7] The theoretical maximum selectivity achievable with a single mismatch is about 5-fold,[6] and a selectivity index of >10 is usually desired for targets requiring allele-selectivity. Consequently, to achieve >10-fold selectivity, for MT RNA versus WT RNA, we use the influence of subtle changes in heteroduplex structure induced by RNA sequence. Though we lack a detailed understanding of how heteroduplex sequence affects heteroduplex structure, the impact of such structural changes is reflected in the RNase H1 cleavage pattern. Not only does the number of RNase H1-induced cleavage sites vary as function sequence, but the relative amount of cleavage at specific sites and the total overall rate of cleavage also vary.[7] By positioning a mismatch at appropriate sites in the heteroduplex, we can shift the cleavage pattern and total activity of RNase H1 and gain selectivity.

Specifically, the process used to identify MT allele-selective RNase H1 ASOs requires the identification of nonpathogenic single nucleotide variants (SNVs), and the higher the number of heterozygous nonpathogenic SNVs in a target RNA, the higher the probability of success. To this end, PacBio or Nanopore HiFi long-read, high-resolution whole genome sequencing (WGS) is performed to allow for haplotype phasing. Once nonpathogenic SNVs are identified, multiple phosphorothioate (PS) 2′methoxyethyl gapmer ASOs are designed to each nonpathogenic SNV targeting the MT RNA. These allele-selective ASOs are then screened in patient-derived cells for potency, MT RNA versus WT RNA selectivity, safety, and tolerability.[8] Using this process, notable clinical successes have been achieved.

Typically, for any patient population that expresses one or more pathogenic mutations in a gene, the nonpathogenic SNVs that are used to create allele-selective PS ASOs differ as might be expected as most nonpathogenic SNVs are unlinked to pathogenic mutations.[8] As shown in Table 1, multiple patients with heterozygous pathogenic mutations in the same gene require allele-selective PS ASOs, but many of these patients share no nonpathogenic SNV and, thus, require different PS ASOs. For example, two patients with *SCN2A* require different PS ASOs. Interestingly, two of the three patients with *KIF1A* share the same nonpathogenic SNV and are being treated with the same PS ASO, but the third requires a different PS ASO. Therein lie the opportunity and the challenge.

We have demonstrated that safe and effective allele-selective PS ASOs can be created to treat patients expressing *TGOF* heterozygous mutations. This suggests that we have the tools to treat a patient population that seems likely to grow exponentially as more humans are subjected to WGS, and the opportunity extends well beyond the nano-rare patient population. In fact, in the first four years at n-Lorem, >15 mutations thought to have a worldwide (WW) prevalence, and <30 have proven to afflict many more patients than previously thought.

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Table 1. Gene Targets Requiring Allele-Selective RNase H1 Activating PS ASOs in the n-Lorem Database

Target	*Patients*	*Target tissue*
ADCY5	1	CNS
ASXL3	1	CNS
CACNA1A	2	CNS
CACNA1E	1	CNS
CAMK4	1	CNS
CLCN7	1	Liver; kidney
DHDDS	3	CNS
DNAJC5	1	CNS
DNM1	1	CNS
EIF2AK2	1	CNS
GARS1	1	CNS
GNAO1	1	CNS
GRIA1	1	CNS
IRF2BPL	1	CNS
KCNB1	2	CNS
KCNH1	1	CNS
KCNQ2	3	CNS
KIF1A	4	CNS
MAPK8IP3	2	CNS
NALCN	2	CNS
NARS1	2	CNS
NEFH	2	CNS
PACS2	1	CNS
PFN1	1	CNS
PRPH2	1	Eye
RHOBTB2	2	CNS
SAMD9L	1	CNS
SCN2A	2	CNS
SETX	1	CNS
SLC12A6	1	CNS
SLC37A4	1	Liver
SPTLC1	6	CNS
TARDBP	11	CNS
UBTF	1	CNS

ASO, antisense oligonucleotide; PS, phosphorothioate; Central Nervous System CNS.

For example, patients with *KIF1A* with a TGOF requiring an allele-selective PS ASO are now considered to number as many as 1,000 or more.[9]

The Opportunities and the Challenges

Current experience strongly suggests that as more humans are genomically characterized, a large population of patients with *TGOF* mono-allelic variants will be identified; some will truly be nano-rare, but many will be more prevalent. For patients with pathogenic mutations that cause diseases that are very rare, but significantly larger than 30 patients WW, commercialization may be possible, and a number of approaches to facilitate financing the development and commercialization of novel medicines for such extremely rare diseases have been proposed.[9] However, mono-allelic *TGOF* variants that require allele-selective PS ASOs impose unique challenges because the treatment of all or most of such a population of patients will require more than one PS ASO and perhaps several. A traditional approach in which an initial phase 3 study with a placebo arm vs a single ASO treatmet arm is performed, an new drug application (NDA) A filed and then the next phase 3 initiated[9] may not be feasible. The recruitment of sufficient numbers of patients for whom WGS has proven that each patient has the pathogenic mutation and the necessary nonpathogenic SNV in a reasonable time frame may be difficult to impossible, even for a pathogenic mutation expressed in 1,000 or more patients. More importantly, such an approach would result in most patients needing to wait to be treated for years. This could lead to significant disaffection in this population to be treated, further alienating needy patients from the scientific and therapeutic communities best able to serve them. Of arguably even greater impact is that such a process is likely to mean that commercial approaches provide insufficient returns to justify investment, resulting in a potentially treatable patient population being neglected.

A Potentially Cost- and Time-Effective Solution

Unlike splice switching ASOs for Duchenne's that result in different truncated versions of dystrophin protein being translated, each of which could have quite different levels of benefit,[10] all allele-selective RNase H1 activating gapmer PS ASOs cause reduction of the disease-related RNA and protein and, if subjected to equally rigid preclinical characterization, should have similar efficacy, safety, and tolerability profiles. Consequently, it may be possible to conduct a single phase 3 study stratified by disease severity and targeted nonpathogenic SNV in which each group of patients with a shared nonpathogenic SNV would be treated with the ASO.

At the conclusion of the single phase 3 study in which patients were treated with several allele-selective PS ASOs, the data could be aggregated and compared with placebo. A prespecified statistical plan would, of course, compare the results of each individual ASO with the aggregate result and placebo. Assuming stratification for severity is reasonably successful, each subgroup of patients should display a similar profile, but if, despite efforts to stratify, a subgroup is shown to have been an outlier, that can be analyzed and considered in the registration process. Regulatory authorities would evaluate the aggregate data and the performance of each ASO in each subgroup. They could approve all ASOs or some ASOs or could ask for additional evidence for ASOs approved, but for which questions remain.

The benefits of the approach I suggest could be quite significant. Yes, the costs of performing required preclinical and phase 1 studies would be greater than for a single ASO, but for ASOs, such costs are modest compared with the costs of multiple phase 3 programs. Most importantly, the proposed solution might make the development of several allele-selective ASOs for a patient population sufficiently cost- and time-effective that commercial companies will pursue the treatment of these groups of patients. It should also shorten the time to approval of ASOs that treat most or all of a patient population and avoid alienating patient populations who, quite correctly, would ask why just a few patients are being treated. Furthermore, despite aggregating phase 3 data, companies and regulators can evaluate the performance of each ASO in each subgroup of patients, assuring that only safe and effective ASOs are approved.

One possible extension of this concept might be the use of several ASOs designed to correct mutations in several genes that result in a traditionally defined disease or syndrome. While potentially attractive, at present I do not recommend this because of the variability in phenotype associated with mutations in

different genes. Furthermore, I would think that experience with composite phase 3 trials with allele-selective ASOs for mutations in the same gene will be needed before regulatory agencies would consider extension of this concept to ASOs to multiple genes.

Conclusions

Advances driven by genomic sequencing are changing our understanding of diseases and the way we think of health and disease altogether, and that has been long overdue. In ASO technology, we now have a drug discovery platform that is capable of addressing the needs of patients with heterozygous *TGOF* mutations. The question that remains is if we are clever and nimble enough to be as innovative regulatorily as our patients and technologies demand.

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