**Script – Antisense (How We Do It at n-Lorem)**

**Introduction**

If it sounds impossible to you that any drug discovery technology could discover and develop a new medicine for a specific patient in 12-15 months, then provide it for free for life, you can imagine my reaction more than 4 years ago when I first realized that it might be possible for the technology that was created under my leadership. But it is possible, and we have already proven that it can be done. Given that I have already provided a basic framework to understand how drugs work and come to be, and discussed the basics of ASO technology, in this podcast, I will focus on a more detailed step by step explanation of how we make the impossible possible at n-Lorem.

**What makes n-Lorem so special?**

If you have listened to any of our podcasts or any of my presentations, you have heard me argue that the only organization that is fully capable of discovering the best personalized ASO for a single patient efficiently and expeditiously is n-Lorem. I would imagine that you have wondered why. Of course, the reason is that we inherit and benefit from more than 3 decades of pioneering efforts to create ASO technology. The first step in creating the optimal ASO for a patient is to identify the best sites to target in the specific RNA that needs to be altered for that patient. Since RNAs are many thousands of nucleotides long, we evaluate ASOs designed to bind to hundreds, to thousands of sites. We call that process ASO site screening, so let’s begin there.

**Screening**

Why must we screen hundreds to thousands of sites in an RNA to find optimal sites? There are several reasons. RNAs are very large, and they have very complex three-dimensional structures. The ideal site for an ASO to bind is an area in the RNA that is unstructured. RNAs are so complex that it is not possible to predict the structures of RNAs in cells. The problem is even greater because RNAs do not exist as RNAs in cells, they have many proteins that are bound and form a structure called ribonucleoprotein or RNP. Therefore, we screen not RNA, but the target RNA in a very complex three-dimensional structure composed of RNA and proteins that we call RNP. In fact, the same RNA can have different structures at different times and be bound by different proteins, so screening is essential.

Over the last 3 decades we have screened many millions of ASOs of a wide range of chemistries and designs. We have screened those ASOs in scores of cell lines, including human derived cell lines. Those efforts have been followed up in thousands of studies in various animal species and of course in tens of thousands of patients. All that data has been integrated into an enormous database coupled to machine learning. That has created an ever more sophisticated algorithm that helps us rapidly design the right sorts of ASOs. Further, ASO screening is highly automated so we can screen hundreds to thousands of sites very rapidly and inexpensively. We have proven repeatedly that the more sites screened the better the performance of the ASO. Therefore, this uniquely capable approach is critical to enhancing the potential for success in treating nano-rare patients.

But if we had only screened ASOs and not invested broadly and consistently in understanding the molecular mechanisms that explain the observations made during screening, in animals and in humans, it is unlikely that RNA targeted drug discovery technology would exist today and it certainly would not be capable of discovering, developing and providing personalized experimental ASOs to patients with nano-rare diseases for free for life. The leverage to advance the technology and steadily improve the performance of ASOs derives from the seamless integration of ASO medicinal chemistry with an ever deeper understanding of the molecular mechanisms by which ASOs produce their effects coupled to massive parallel screening and A.I. analytics with all that has been learned in animals and man. This cycle of innovation results in constant feedback from which we learn constantly, and that process has been in play for more than three decades and is even more productive today.

To understand the final element that makes it possible to meet the needs of some patients with nano-rare diseases, one must understand that within a chemical class, all the ASOs of that class have very similar behaviors because they differ only in their genetic zip code or sequence. Consequently, we can predict the behavior of an ASO that we just discovered based on the behaviors of hundreds of ASOs of the same chemical class that preceded it. That means that before we ever begin development of a personalized ASO for a patient we have a good idea of the right dose to use, the right route of administration and how often to administer the ASO. Finally, because we have constructed databases that summarize all the safety observations from all controlled clinical trials of each of the chemical classes that we use at n-Lorem, we can predict the possible side effects to watch for. Given that the safety databases have been published, treating physicians, patients, and parents can be informed as well, and the FDA has access to these databases as desired. This then means that we can be confident that we are exposing n-Lorem patients only to prudent risk.

The choice we have made to focus solely on RNA targets in the Liver and Kidney after systemic dosing, the eye after direct injections in the eye, the CNS after injection of the ASO into spinal fluid, and the lung after aerosolized administration assures that low doses will be needed and that we really understand what we are doing, further enhancing the potential benefit and reducing risk.

**What happens when an application is submitted?**

What happens when an application for treatment is submitted? Prior to the application being submitted a great deal of work has taken place. The patient has been identified as potentially having an ultra-rare mutation and referred to a tertiary care center to be genetically and phenotypically characterized. This work identifies the mutation and characterizes the type of mutation. For example, is it a null mutation? A gain of toxic function mutation? a splicing mutation? The function of the gene product is defined. The other major effort is to define the phenotype and identify the principal manifestations of the mutation. Numerous tertiary care centers have submitted applications, and, on some occasions, we have had to do additional work including added genomic sequencing, characterization of mutations and sometimes we must do added studies on gene function. As we gain experience, and personalized medicine centers gain experience, I am optimistic that this process will become progressively more efficient.

The first step in processing an application is to assure the patient identity is protected. Then, we must do a search of the scientific literature to learn more about the gene, the prevalence of the mutation, the nature of the mutation, and the ASO strategy to employ. Once we have all the necessary information, we prepare a detailed presentation with a recommendation to be presented to our Access to Treatment Committee. If the committee and the executive committee of n-Lorem agree that the patient is potentially amenable to ASO treatment, we go to work.

First, we work with the investigator to define the treatment goals and clinical measures to be used. We then ask that the investigator and patient or parent to perform a detailed natural history study while we discover and develop the ASO.

Then the screening process that I have described is initiated. Out of that process we typically have multiple candidate ASOs that undergo further testing in cells and in animals. When those studies are completed, we assemble all the data and select the best looking ASO to be manufactured. The special FDA guidance for n-of-1 patients allows us to perform a single 3-month toxicology study in rodents. If the results of that study are satisfactory, the ASO is deposited in sterile vials at our partner, Argonaut’s sterile facility. During this process we file the necessary documents with the FDA to permit the initiation of experimental treatment.

From the patient’s perspective, the first dose of an experimental ASO is the beginning, but getting there requires a great deal of work and could not be done well without all our knowledge and experience. During treatment, our task is to evaluate the performance of the ASO and assure that we and all stakeholders learn as much from the treatment of that patient as possible.

As you can imagine, completing all of this complex experimental scientific work in 12-15 months is challenging. If we encounter problems, such as the ASO we select having issues in the toxicology study, delays can happen. On occasion, we may even be unable to identify a personalized ASO that we feel is satisfactory for a patient. For these reasons, we cannot promise that we will be successful or that we will achieve our goal of treating a patient within 15 months of approval of the application. What we can and do promise is that we will do everything possible to meet the needs of our patients.