Physician heal yourself

This well-known proverb from the Gospel of Luke takes an exciting transformation in this month's *Journal Club* to

Ribosome heal yourself

The possibility of this modified proverb coming to fruition as a potential treatment for certain patients with DBA came one step closer with the publication of a recent manuscript entitled: "Ataluren treatment of patients with nonsense mutation dystrophinopathy." While the disease addressed in this Phase 2b clinical trial was muscular dystrophy, it seems reasonable to envision that the results from this trial may extend to certain patients with DBA.

The patient sub-population in inherited genetic diseases that may benefit from the drug Ataluren is patients with nonsense mutations in protein coding genes. In the trial with Ataluren the gene involved encodes the protein dystrophin, but the hope is that the effects may be generalizable to virtually any protein-coding gene causing a human disease, including the genes affected in DBA.

I've discussed the types of mutations that occur in protein coding genes in this venue before. These mutations affect the readout of the genetic information from mRNA to protein. The protein-coding information contained within an mRNA begins with a start codon that initiates the synthesis of a protein. The ribosome then moves down the mRNA reading the genetic code in groups of three bases, adding one amino acid at a time to the growing protein until the ribosome reaches a termination codon. Once the ribosome encounters a termination codon, protein synthesis is halted and the protein is released from the ribosome where it can then perform whatever function it is destined to do within the cell.

Nonsense mutations arise when there is a mutation in the gene that changes a base in the coding region of an mRNA from a codon that encodes an amino acid to a codon that now specifies termination. These mutations fall somewhere between the initiation codon and the normal termination codon, thus resulting in premature termination of protein synthesis. Premature termination often results in a protein that lacks all of its parts and so can result in the loss of a protein's function and cause a myriad of human diseases, depending on the protein affected.

It has been known for many years that certain classes of antibiotics cause the misreading of termination codons allowing the ribosome to pass through a termination codon without stopping. It was therefore thought that these drugs could potentially be used to treat diseases caused by nonsense mutations. These drugs however have side effects that have diminished enthusiasm for their use in treating genetic diseases. This did not, however, stop research into finding safer drugs with similar modes of action.

One such drug was developed by the small pharmaceutical company called PTC Therapeutics, which was begun by University researchers studying the consequences of premature termination in yeast (here I will shamelessly give a shout out to the importance of basic science research and not knowing where such research will lead). The drug, Ataluren, works by an unknown mechanism that allows ribosomes to read through premature termination codons while still allowing normal termination codons to function. This drug showed promising results in model systems of different human genetic diseases and a proof-of-concept Phase 2a clinical trail. The current study goes one step further to a Phase 2b randomized double-blind clinical trial comparing 2 doses of Ataluren with placebo.

The patients in this study were young boys displaying early signs of Duchenne muscular dystrophy. Drug efficacy was addressed by a test of muscle function called the 6-minute walk test. This test measures how far a boy can walk in 6 minutes. The test was performed when the boys entered the study and then again 48 months later after being placed in one of the three study arms: placebo, lower drug dose and a higher drug dose.

These boys were typically at the stage in their lives when muscle function begins to decline and it was expected that boys would show a decline in distance walked between the zero time point and after 48 months. Many of the subjects entering the study had already exhibited muscle decline and so there was a range of distances walked at the zero time point.

The study included 174 patients randomized in the three study arms. The results from the study arm receiving the low concentration of the drug look very encouraging from a number of different perspectives. First, the drug is well tolerated. There were no serious side effects and the milder side effects that were observed were similar between subjects receiving the drug and those receiving placebo.

Concerning efficacy, patients receiving low concentrations of the drug showed a decline in distance walked over the 48 week period of 12.8 meters compared to 44.1 meters for patients receiving placebo. This difference, 31.3 meters, or approximately 34 yards, corresponds to about the third of the distance of a football field. This difference between study groups did not achieve statistical significance because the number of subjects wasn't high enough, but it did show an encouraging trend toward being efficacious.

There are a number of ways to evaluate data, taking into account different variables in study population. One of these variables was the distance a subject was able to walk at the zero time point. Those subjects that fell below 350 meters in the 6-minute walk test, likely represent the patient population where disease progression was most severe prior to the study being initiated. When these patients were evaluated independently the difference between placebo and low drug groups at 48 months was 68.2 meters, a difference that did reach statistical significance.

A number of additional parameters, referred to as secondary endpoints were also measured in the study groups. All of these secondary endpoints showed positive trends in reducing disease progression with Ataluren. Based on the results from this trial, Ataluren has received conditional approval for use in patients with nonsense mutation Duchenne muscular dystrophy in Europe. Results from a larger trial this summer could lead to approval in the US, should the outcomes in the larger patient cohort be positive.

An obvious question at this point is how well the results obtained in patients with Duchenne muscular dystrophy will translate to other diseases caused by nonsense mutations in protein coding genes. In this regard, encouraging results with Ataluren have also been reported for patients with cystic fibrosis.

A nuance to this story is that there are three different codons that specify termination in the genetic code. Fortunately, Ataluren does not appear to discriminate among these three codons and so could potentially be used for patients with nonsense mutations created with any of the three termination codons.

Another question is whether Ataluren can be expected to work on each and every person with any of the three premature termination codons. The answer here is perhaps not. When a ribosome reads through a premature termination codon it inserts an amino acid that is likely going to be different from the natural amino acid that was coded at that position before the mutation caused the premature termination codon occurred. A change of one amino acid for another in a protein occurs through a different type of mutational event referred to as a missense mutation. I have mentioned before that these mutations are more difficult to predict in terms of whether they will affect protein function or not. Thus, it is possible that for some patients with premature termination codons, these codons may be at positions that are absolutely critical for a protein's structure and function and that the read through that occurs in the presence of Ataluren may insert an amino acid that still leads to a non-functional protein. These patients may therefore not benefit from the drug. In this regard, however, it should be noted that the subjects used in the Duchenne muscular dystrophy study had mutations at various positions throughout the dystrophin protein and so, the overall positive results in this diverse patient population suggest that despite this theoretical concern the majority of patients still benefit from the drug.

In summary, the results from this trial demonstrate that Ataluren enhances the ribosome's ability to read through premature termination codons found in the dystrophin genes of Duchenne muscular dystrophy patients to the point where these patients make enough protein for clinical benefit. If similar results could be obtained in DBA patients where the ribosome was now able to read through premature termination codons in ribosomal protein genes and make more functional ribosomes, we could indeed have a situation where the ribosome is healing itself……..with, of course, a little help from the drug Ataluren.

Bushby et al (2014) Ataluren Treatment of patients with nonsense mutation dystrophinopathy. *Muscle and Nerve* **50**,477-487