

## Important advances in next generation genome editing tools for Huntington's Disease



Work with genome editing techniques (zinc fingers and CRISPR) brings these tools closer to use in HD clinical trials

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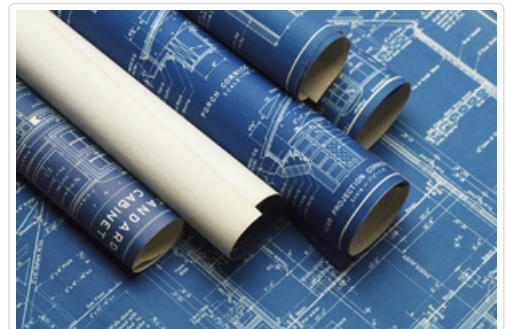
*Recent days have seen a slew of news emerging regarding the use of something called genome editing as a potential therapy for genetic diseases like Huntington's Disease. These approaches, which include exotic sounding tools like zinc finger nucleases and CRISPR/Cas9, differ from more traditional ways reducing the impact of the HD mutation on cells. What's new in this exciting area of research?*

### Huntingtin Lowering Refresher

There has been lots of excitement recently surrounding the advancement of a new **huntingtin lowering**, sometimes called **gene silencing**, treatment for Huntington's disease. The first of these drugs to reach human clinical trials in HD, called **antisense oligonucleotides** (ASO's), reduce the amount of harmful mutant huntingtin protein that is created by a cell and ultimately limit the damage that it can cause.

Those who have been following along know that the first trial assessing the safety of ASO's in human HD patients is currently underway (<http://en.hdbuzz.net/204>), but some readers might also remember that ASO's are not the only player in town when it comes to innovative ways to reduce the harmful huntingtin protein.

In fact, two other new therapeutic techniques known as **zinc finger nucleases** and **CRISPR** have been generating some buzz recently. Although we have covered both of these approaches previously (<http://en.hdbuzz.net/023>), a quick review of how they operate and the differences between them will be useful in understanding some new findings.



Your DNA is like a master blueprint with the instructions for building you, while mRNA is like the individual copies the blueprint that is given to the workers to build efficiently

You probably recall from your earliest biology classes that your DNA provides a detailed set of instructions for how to build, well, you! Each cell in your body is a lot like a construction site, and your DNA is the master blueprint with instructions for taking the most basic building blocks

(called amino acids) and turning them into functioning proteins that contribute to all of your unique features and make sure that things in your body continue to run smoothly.

We call the DNA instructions for building specific proteins *genes*, and we each have two copies of the gene for making an important protein that is called *huntingtin*. The symptoms associated with Huntington's disease occur because one of these sets of genetic instructions has developed a misprint that causes the huntingtin protein to be built improperly. In the vast majority of HD patients, this leads to two types of huntingtin protein – a mutant huntingtin protein that no longer functions correctly, and a healthy huntingtin protein that does.

## Let's Get to Building...

Inside each of the cells in your body, your DNA is stored and protected deep in a location called the nucleus, much like the master blueprint for a building that has been locked away by the construction manager in her office to prevent it from being harmed.

In a real construction site, it would be very slow and inefficient if each worker were required to travel to the construction manager's office to use the same set of instructions when building, and as it turns out the same is true of our cells.

To avoid this this problem, a working copy of the instructions is made that is used as a template for building proteins instead. This working copy is called **messenger RNA**, or **mRNA**, and it is copied from the original DNA and sent out into the cell where it is used to build many copies of the same protein(s) at a larger scale.

If our DNA is like the original master blueprint, then mRNA is a lot like the individual copies of the blueprint that are delivered by the construction manager to their crew so that they can begin to build efficiently. This might seem confusing, but for our purpose all you need to know are the three steps involved in building a protein: DNA -> mRNA -> protein.

## ASO's, Zinc Fingers, & CRISPR: Same Goal, Different Means

It's important to make the distinction between the HD gene in DNA and in mRNA because they are differently targeted by rapidly developing Huntingtin lowering therapies. These include a zoo of different techniques like **ASO's**, **zinc fingers**, and a new approach called **CRISPR/Cas9**. At their core, all of these therapeutic techniques have the same goal in mind – to reduce the amount of harmful mutant huntingtin protein produced in a cell – but they go about achieving this goal in very different ways.

Of the three options, ASO's have been around the longest, which might not surprise you since they have emerged as the first to be assessed in human HD patients. ASO's work by telling cells to shoot the messenger, in this case the mRNA intermediate carrying the instructions from DNA to make a protein. In a treated cell, ASO drugs literally stick to the mRNA that provides the instructions for making the harmful mutant huntingtin protein, and convince the cell to chop it up so that the protein can no longer be produced.

Many scientists and those in the HD community are very excited by the advancement of ASO therapy as a treatment option for HD, but the fact remains that ASO therapy does not target the ultimate cause of HD (the faulty HD gene encoded in a person's DNA) and remains one step removed by targeting the mRNA. Because the mutant gene is still present in the DNA, mutant mRNA and protein would continue to be produced in ASO-treated cells. This means, as far as we understand it today, that treatment with ASOs would have to be continued throughout life.

Unlike huntingtin lowering using ASO's, newer techniques including **zinc finger nucleases** and **CRISPR/Cas9** are both a form of **genome editing** techniques. These amazing new tools allow scientists to target the ultimate source of the problem in HD, the mutant DNA itself. These tools allow scientists to precisely target a specific location in the DNA (like the instructions for making the huntingtin protein), and then carry out a number of useful tricks.

One of these tricks they can do is to serve as a sort of stop sign for the cell. When the machinery that normally reads DNA arrives at the mutant HD gene, appropriately designed genome editing tools can call them off - telling them not to do their work at that precise gene. This results in no mutant huntingtin mRNA or protein ever being made. Note that this is different than how ASOs work, which is by degrading mRNA that's already been made.

An important new genome editing tool called **CRISPR/Cas9** has recently made a lot of people very excited. These tools, borrowed from certain bacterial species who use them as a kind of immune system, allow cells to insert foreign DNA sequences into their own DNA. Really clever humans took those tools from bacteria and re-engineered them to allow scientists to make precise cuts in DNA at specific sequences.

In theory, and in the lab, CRISPR techniques can be used to cut specific DNA sequences so the cell can no longer read a gene. They can also be used to direct cells to make specific changes to DNA sequences - even, in theory, repairing mutations like the ones that cause HD. Targeting the cause of HD at its root (the HD gene) would ensure that that both the mutant huntingtin mRNA and mutant huntingtin protein are no longer made, and can no longer cause harm.

## **Safety First!**

If you find yourself wondering why we are not already testing these new tools as drugs, it's because many things need to happen in the drug development pipeline to make sure that the final product is both safe and effective before it can be tested in HD patients.

First, scientists need to figure out ways to get these drugs to the brain where the mutant huntingtin protein does most of its damage. This is difficult – our brains are especially good at keeping things out that might be harmful, and unfortunately it's not about to give these drugs a free pass. If we tried to put them in a pill or inject them into our blood, our bodies would break them down and make them useless long before they reach the brain.

Since ASO's have been around a while longer, scientists have had some extra time to address this problem, although their solution is still far from perfect. The ASOs being used in the human HD trial have to be injected into the fluid that bathes the brain and spinal cord, the cerebrospinal

fluid. We have every reason to believe this will work, but is obviously more complicated than just swallowing a pill.

Genome editing tools like CRISPR and Zinc Fingers are even more complicated to deliver than their ASO counterparts. This is because they are actually proteins themselves, and those are hard to deliver intact into cells.

To get around this problem, researchers use harmless viruses to hitch-hike the DNA instructions for making these proteins into brain cells. From there, cells are tricked into using the same machinery they use to build their own proteins to build genome editing tools, essentially turning cells into factories for their own drugs!

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## What's new with Zinc Fingers for HD?

Not being ones to shy away from a good challenge, many research teams have been hard at work addressing the previously mentioned issues to make progress toward applying these new techniques to HD. Previously, HDBuzz reported that a group of researchers from Spain had tested a new zinc finger drug that showed some beneficial effects in a mouse model of HD in a short-term study (<http://en.hdbuzz.net/103>).

Recently, the same research team – led by Mark Isalan, who now resides at Imperial College in London, U.K. - designed and tested an updated version of their drug to see if they could enhance its effects for a longer period of time and reduce the harmful immune response in the brain associated with delivering the drug using AAV's.

After some intense biochemical tinkering, Isalan and colleagues were able to demonstrate that their new and improved zinc finger candidate was more effective than their previous version at reducing the harmful mutant huntingtin protein, was longer lasting, was more selective in targeting only the HD gene, and displayed a much better safety profile than their previous version.

These are very exciting findings, indeed, and all positive steps forward in making zinc finger therapy a viable option for treating human HD! This slow, patient, work with Zinc fingers is similar to what happened with ASO drugs, who've had a many year head start on genome editing tools.

## What about CRISPR?

Despite being considered the most precise way to conduct genome editing, CRISPR technology is still the new kid on the block and scientists have had much less time to iron out the kinks compared to the other techniques.

In an exciting advancement in utilizing CRISPR as a therapeutic option in HD, a team of researchers led by Jong-Min Lee at Massachusetts General Hospital recently developed a CRISPR construct that can selectively edit out the mutant, but not wild-type, copy of the HD gene in cells in a dish. Taking advantage of the highly-specific targeting ability of CRISPR, they were able to instruct treated cells to cut out the mutant HD gene, while leaving the healthy copy intact.

Of course, it is one thing to show that a drug is effective on cells in a dish, and another thing entirely to show that it can be effective when tested in a living organism. This is especially true with techniques like CRISPR - as far as we know today we need a virus to carry the instructions to each of our **100 billion** brain cells to rescue each of them from the effects of the mutant HD gene.

Another risk is that CRISPR and other genome editing tools modify DNA permanently. This is different than drugs like ASOs which are eventually cleared from the brain, meaning that their effects wear off over time if they're not delivered again.

At first, this idea seems fantastic! If we could cure HD with only a single treatment, we would certainly love to be able to do it. However, we are still unsure about the long-term effects associated with permanently deleting the HD gene and reducing the amount of huntingtin protein that is created, regardless of whether its mutant or not. The possibility exists that permanently deleting the HD gene could lead to some serious health issues of a different variety to pop up later, and we will need to spend a considerable time studying its effects before we know whether it will be safe.

## What Comes Next?

There is still a lot of work to be done before genome editing techniques like zinc fingers and CRISPR treatments will become viable options for Huntington's disease, but the research presented here shows that we've taken some important steps toward accomplishing this feat.

While the recent work shows that zinc finger therapy is effective in a mouse model of HD – whose brains are smaller than a dime – it will be much harder to show that it can be effective in humans, whose brains are much larger, more complex, and present many other challenges to overcome. CRISPR therapy will likely take even more time, since we are only just now getting to the point where we can make plans to test its effectiveness in mouse models of HD.

However, this is no reason to become discouraged, and in fact we think that quite the opposite is true! The most exciting part about the current research is that it shows that we have multiple arrows in our quiver as we try to develop huntingtin lowering treatment for HD. Even if it turns out that one option doesn't work out as we hoped it would, we're making steady progress developing new therapies that may also provide effective treatments for HD.

This idea is one that many are starting to catch on to quickly – recently two pharmaceutical companies, Sangamo Biosciences and Shire Pharmaceuticals, joined forces to accelerate their efforts in developing zinc finger treatment as a therapeutic option for Huntington's disease.

While it will take a while to iron out the kinks, we imagine that it will only be a matter of time before the same becomes true of CRISPR. Personally, we think that the progress that has been made so far gives us a lot to be excited about!

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*The authors have no conflicts of interest to declare. For more information about our disclosure policy see our FAQ...*

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## Article history

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Minor fixes

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## Glossary

**ASOs** A type of gene silencing treatment in which specially designed DNA molecules are used to switch off a gene

**CSF** A clear fluid produced by the brain, which surrounds and supports the brain and spinal cord.

**huntingtin protein** The protein produced by the HD gene.

**gene silencing** An approach to treating HD that uses targeted molecules to tell cells not to produce the harmful huntingtin protein

**Genome Editing** The use of zinc-finger nucleases to make changes in DNA. 'Genome' is a word for all the DNA we each have.

**messenger RNA** A message molecule, based on DNA, used by cells as the final set of instructions for making a protein.

**amino acid** the building blocks that proteins are made from

**CRISPR** A system for editing DNA in precise ways

**wild-type** the opposite of 'mutant'. Wild-type huntingtin, for example, is the 'normal', 'healthy' protein.

**nucleus** A part of the cell containing genes (DNA)

**AAV** a virus that can be used to deliver gene therapy drugs to cells. AAV stands for adeno-associated virus.

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