

HD Insights™

A Huntington disease research periodical

Issue Nº 3 -- Fall 2012

Research Round-Up

Dr. Lise Munsie
overviews recent
developments in HD
research.

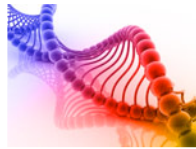
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An Interview with Dr. Nancy Wexler: Discovering the Huntington Disease Gene

By: Kristin Darwin

One morning in 1968, Dr. Nancy Wexler's mother, Leonore Wexler, had jury duty in downtown Los Angeles. As Leonore crossed the street on the way to the courthouse, a policeman yelled to her, "How can you be drunk so early in the morning?" Leonore realized that she had been staggering – an obvious sign that something was wrong.

Soon after, Leonore was diagnosed with Huntington disease (HD). Nancy Wexler, who was pursuing a PhD in clinical psychology at the time of her mother's diagnosis, devoted her life to the study of HD. In 1979, Wexler and her colleagues began a research project in Venezuela to search for the HD gene. They surmised that finding the gene was the most direct route to the development of treatments, even cures! They developed a pedigree of over 18,000 individuals and collected more than 4,000 blood samples from the largest extended family with HD ever to have been discovered. Their data led to the identification of the gene responsible for HD.

Today, Wexler continues her involvement with HD as the Higgins Professor of Neurophysiology at Columbia University, and as President of the Hereditary Disease Foundation. At the November 2011 Huntington Study Group meeting, Wexler told HD Insights about the series of events that led to the discovery of the gene responsible for HD.

(continued on Page 2...)



Nancy Wexler, PhD

Dr. Wexler is the Higgins Professor of Neuropsychology in the Dept.s of Neurology and Psychiatry of the College of Physicians and Surgeons at Columbia University, as well as the President of the Hereditary Disease Foundation.

Nancy Wexler, cont...

Wexler says that her father, Dr. Milton Wexler, was an invaluable contributor to the discovery of the HD gene. She and her father, who was a clinical psychologist and an expert group therapist, began HD workshops the same year her mother was diagnosed. These workshops were the foundation for the Hereditary Disease Foundation that is still active in HD research today. Wexler recalls, "We had a workshop for two days and came up with a research agenda. My dad took what he learned from group therapy, and from creativity, and off-the-wall thinking, and put it into these workshops. And in the 1970s, [the study of] DNA was kind of just being born. So we said, 'Does that have an answer for us?'"

Wexler collaborated with Dr. David Housman to organize a workshop at the National Institutes of Health to discuss the feasibility of finding DNA markers for the HD gene. "There were very prestigious scientists at the workshop," Wexler says. It was the early 1970s. A big question was, "How are you going to go from here to the moon if you don't even know where the moon is, and you have to build your own spacecraft?" Additionally, genetic studies were difficult – all restriction fragment length polymorphisms were hand-crafted. Polymerase chain reaction had not yet been invented. Wexler continues: "At the workshop, there were lots of arguments about how many markers you needed to evenly cover the genome. We had to hand craft each marker and find enough to evenly cover the human genome. And everybody talked about families. The criterion for a family critical for gene mapping was a very large family in which you had both grandparents, both parents, and 10 or 14 kids." At the time of this workshop, such an HD family was believed not to exist. Some in attendance at the workshop even told Wexler it was unethical to publicly announce that a search had begun for the HD gene.

"They're not drunk. They're sick."

They estimated a realistic timeframe for HD gene discovery was 50 to 100 years.

In fact, the large HD family Wexler and her colleagues sought had already been discovered by Venezuelan physician Dr. Americo Negrette, who in 1955 practiced in the small Venezuelan town of San Luis, near Lake Maracaibo. According to Wexler, "As he walked around the streets, he thought, 'These people are drunk all the time! The nursing mothers are drunk, the fathers are drunk, everybody is drunk. What's the matter with them?' Finally, a woman pulled him aside, and said, 'You're such an arrogant doctor! Have you even looked at these patients? Have you smelled their breath? Nobody's drinking. They're not drunk. They're sick.'"

Negrette realized that the woman was correct. He began to write up cases and make pedigrees of individuals from San Luis and its neighboring villages around Lake Maracaibo. He concluded that the people had HD. Negrette and colleagues made a video of the affected people in the villages and presented it at the 1972 World Federation of Neurology Research Group on Huntington's Disease. Nancy and her father Milton Wexler were both in the audience.

Negrette's video presentation and data were central to Nancy Wexler's determination that she should travel to Venezuela to study HD. With the support and guidance of Negrette, in July 1979, Wexler and colleagues began a search in Venezuela for a person who was an HD homozygote. Her search for a homozygote was inspired by revelations in familial hypercholesterolemia. Wexler explains, "The godsend was that Dr. Michael Brown and Dr. Joseph Goldstein had found the gene causing familial hypercholesterolemia by studying homozygotes for the gene. Without the normal protein, it was more obvious

what the abnormal protein was doing." Wexler and her colleagues began compiling pedigrees and collecting blood samples from HD families. She and colleague Dr. Tom Chase eventually found many children with juvenile HD in a small stilt village in Lake Maracaibo. One two-year-old child had a "giant expansion of 109 CAGs – a very dramatic expansion." Wexler began to collect blood samples from "all these layers and layers and layers of families, from great-grandchildren all the way up." Homozygous families and the children with juvenile HD were both essential to finding the HD marker and the gene.

Wexler gave the blood samples she had collected to Dr. James Gusella, Dr. David Housman and Dr. Michael Conneally for benchwork analysis. In 1983, Gusella and Conneally determined that the gene responsible for HD is located at the tip of chromosome four.¹ Wexler was working at the National Institute of Neurological Disorders and Stroke (NINDS) when they made their breakthrough. "It was incredible," she says. "I just started screaming at the top of my lungs: 'We found the gene!' I called my dad. I said, 'Dad, we did it' and he started crying. I called my sister. It was just euphoric."

Wexler also understood the larger implications of the discovery of the HD gene. "It meant that our strategy would work not only for HD, but for everything worldwide. At that point, we realized we had the human genome in our hand. We said, 'Yes, you can do this, you can find new genes.' We found the marker. That just revolutionized everything."

Today, nearly two decades after the discovery of the HD gene, a successful disease-modifying treatment for HD has yet to be developed despite efforts from researchers worldwide. However, Wexler remains hopeful. When *HD Insights* asked what she thought would be the next euphoric moment in HD, Wexler replied, "When we cure it!"

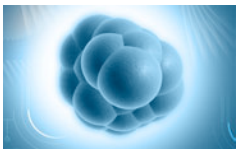
1. Gusella JF, et al. A polymorphic DNA marker genetically linked to Huntington's disease. *Nature*. 1983 306:234-238.

Research Round-Up

By: Lise Munsie, PhD

In the beginning...

In the past year, researchers have published on the use of stem cells to model Huntington disease (HD) and on investigations of stem cell use as a treatment for HD.



Sadan and colleagues¹ induced mesenchymal stem cells into neurotrophic factor-secreting (NTF) cells,

then transplanted the NTF cells into rats that had striatal lesions induced by quinolinic acid (QA). The NTF cells, whether derived from an HD patient or a control, survived transplantation and maintained an NTF-secreting phenotype leading to improved striatal volume and behavioural phenotypes.

Ma and colleagues² showed that human embryonic stem cells can be directed into an enriched population of GABA medium spiny neurons. When GABA neurons were implanted into the striatum of a QA lesion mouse model, they maintained functionality, leading to phenotypic improvements in the mice.

An and colleagues³ corrected the genetic mutation in HD patient-derived induced pluripotent stem cell (iPSC) lines using homologous recombination, and found this also corrected many of the aberrant phenotypes associated with neural stem cells (NSC) derived from these iPSC lines. Genetically corrected NSCs were able to populate the striatum of the R6/2 mouse post-transplantation. Patient-specific cell replacement therapy with corrected mutant huntingtin (mHTT) CAG lengths may be feasible in the future.

The HD iPSC consortium⁴ generated and studied 14 iPSC lines from HD patients and controls. Investigators in different labs found clear and reproducible phenotypes associated with the disease-causing mutation, consistent in multiple lineages. These cell lines may be used in future assay development and screening in HD drug discovery efforts.

¹ Sadan O, Shemesh N, Barzilay R, et al. Mesenchymal stem cells induced to secrete neurotrophic factors attenuate quinolinic acid toxicity: a potential therapy for Huntington's disease. *Experimental neurology*. 2012 Apr;234(2):417-27.

² Ma L, Hu B, Liu Y, et al. Human embryonic stem cell-derived GABA neurons correct locomotion deficits in quinolinic acid-lesioned mice. *Cell stem cell*. 2012 Apr;10(4):455-64.

³ An MC, Zhang N, Scott G, et al. Genetic correction of Huntington's disease phenotypes in induced pluripotent stem cells. *Cell stem cell*. 2012 Aug;11(2):253-63.

⁴ ThHD iPSC induced pluripotent stem cells from patients with Huntington's disease show CAG-repeat-expansion-associated phenotypes. *Cell stem cell* 2012 Aug;11(2):264-78.

In the lab...

There is a vast breadth of recently published basic research in HD.

Dong and colleagues⁵ recently identified additional post-translational modifications in huntingtin. They coupled tandem affinity purification and 2D nano-LC mass spectrometry and found novel phosphorylation sites at serines 431 and 432. Phosphorylation at these sites was specific to polyglutamine-expanded huntingtin when N-terminal fragments were overexpressed in 293 cells.

Milnerwood and colleagues⁶ developed a new protocol for studying excitatory transmission onto GABAergic medium



spiny neurons, with specific focus on the effects of mutant huntingtin on the cortico-striatal pathway. Using striatal and cortical co-cultures, NMDAR-induced cell death

increased in cultures derived from YAC128 mice. The researchers further showed this is associated with the misregulation of phosphorylated cAMP response element binding protein (CREB) leading to decreased transcription of pro-survival factors. The dysfunction was significantly reduced using specific NMDAR subunit inhibitors.

Kordasiewicz and colleagues⁷ used transiently administered antisense oligonucleotides (ASOs), designed to suppress the expression of huntingtin, in HD mouse models and in non-human primates. In mouse models, ASOs were administered into the lateral ventricle and led to specific knockdown of the mHtt transgene and some phenotypic reversal. The knockdown was sustained in both animal models for up to three months post treatment, suggesting that ASOs may provide a clinically relevant strategy for combating HD.

⁵ Dong G, Callegari E, Gloeckner CJ, et al. Mass spectrometric identification of novel posttranslational modification sites in Huntingtin. *Proteomics*. 2012 Jun;12(12):2060-4.

⁶ Milnerwood AJ, Kaufman AM, Sepers MD, et al. Mitigation of augmented extrasynaptic NMDAR signaling and apoptosis in cortico-striatal co-cultures from Huntington's disease mice. *Neurobiol Dis*. 2012 Oct;48(1):40-51.

⁷ Kordasiewicz HB, Stanek LM, Wanciewicz EV, et al. Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. *Neuron*. 2012 Jun;74(6):1031-44.

In the clinic...

Important studies reviewing and analyzing previously obtained clinical data give new insights into disease mechanisms and assess current treatments for HD.

Ji and colleagues⁸ conducted a population-based study assessing the incidence of cancer in Swedish patients with HD and other neurological diseases. The standardized incidence of cancer was significantly lower in patients with polyglutamine disease. The largest difference (0.47) was associated with HD gene carriers. Polyglutamine disease seems to be protective against benign and metastatic cancer.

Armstrong and Miyasaki⁹ looked at the data available for current pharmacological options for treating chorea. Their analysis showed that tetrabenazine, the only drug approved by the FDA specifically for the treatment of chorea, remains the best performing drug at a dose of 100 mg/day. Amantadine and rizuclole give modest benefit. It is important to have multiple effective drugs available for HD patients due to tetrabenazine's potential adverse effects and the necessary close monitoring of patients who receive the drug.



Unschuld and colleagues¹⁰ examined brain metabolite alterations, using magnetic resonance spectroscopy, in prodromal HD gene carriers with no gross brain morphological changes, and compared

the metabolite alterations to controls. Eleven metabolites had decreases in N-acetyl aspartate and glutamate levels associated with prodromal HD. Future studies are needed to determine the utility of these results as longitudinal biomarkers for HD disease progression.

⁸ Ji J, Sundquist K, Sundquist J. Cancer incidence in patients with polyglutamine diseases: a population-based study in Sweden. *Lancet Oncol*. 2012 Jun;13(6):642-8.

⁹ Armstrong MJ, Miyasaki JM. Evidence-based guideline: Pharmacologic treatment of chorea in Huntington disease: Report of the Guideline Development Subcommittee of the American Academy of Neurology. *Neurology* 2012 Aug 7;79(6):597-603.

¹⁰ Unschuld PG, Edden RA, Carass A, et al. Brain metabolite alterations and cognitive dysfunction in early Huntington's disease. *Movement Disorders*. 2012 Jun;27(7):895-902.

Highlights from the CHDI HD Therapeutics Conference



The conference was held this year in Palm Springs, California.

By: Lise Munsie, PhD

At the 7th Annual CHDI HD Therapeutics Conference in Palm Springs, California, scientists from academia and industry convened to discuss the current state of drug development research aimed at slowing the progression of Huntington disease (HD). **Robert Pacifici**, CHDI's chief scientific officer, opened the conference.

The first session of the meeting reviewed the relevance of systems biology to HD research. The opening talks by **Lee Hood** and **Keith Elliston**, CHDI's vice president of Systems Biology, outlined the systems biology field and suggested whole genome sequencing of HD families to gain more genetic information about the processes that affect HD phenotypes. Elliston specifically described ways that CHDI is already using this technology. **Jim Gusella** discussed the discovery of genes that modify the age of onset in HD. The session ended with **Hanchuan Peng**, who described the "3D neuronal atlas" model that his group is developing to quantitatively measure synapses.

The next session focused on post-translational regulation of the huntingtin protein. **Melissa Moore** opened the session by reviewing recent work that examines the toxicity of CAG expansions in RNA; the possibility of toxic RNA in HD; and posed the idea of targeting the RNA to knock down mutant huntingtin (mHtt) levels. **Naoko Tanese** reviewed recent reports of huntingtin functioning at sites of local translation in distal parts of the cell, and indicated that huntingtin traffics RNA to these sites. **Lisa Ellerby** and **Dimitri Krainc** examined the classic post-translational modifications of huntingtin and how these modifications may be drug targets. **Marcy MacDonald** ended the session by describing how her group has screened the full-length protein for novel post-translational modifications and has assigned signatures to mHtt vs. wildtype huntingtin.

Session three highlighted current efforts to silence the expression of mHtt. **Beverly Davidson** showed published results that highlight the safety and efficacy of RNA interference in the primate brain. **Frank Bennett** (*see interview on page 8*) from **Isis Pharmaceuticals** described his work on using antisense oligonucleotides (ASO) to silence

the expression of mHtt, and discussed possible efficacy of ASOs in the brain, administered by infusion into the cerebrospinal fluid. **Steve Zhang** highlighted **Sangamo BioScience's** successful efforts in using zinc finger protein transcription factors in other diseases and talked about the recent efforts of the company in using this technology in allele-specific silencing. **William Kaemmerer** described the biomarkers necessary to test the efficacy of these therapies in humans. **Neil Aronin** described the efforts of his group to refine technical procedures that will enable therapies utilizing adeno-associated viral delivery of small RNAs to become a reality.

The keynote address was given by **Ann Graybiel**, who described her efforts to understand the cortico-striatal pathways and their potential for manipulation. Following her talk, a session on small molecule drug discovery featured efforts by different drug companies, such as **Pfizer's** previous work on phosphodiesterase inhibitors. CHDI's **Varhi Beaumont** described their assays in HD mice testing Pfizer's drugs and discussed the collaboration between their two organizations. **Ladislav Mrzljak** described CHDI's investigations on kynurenic 3-monooxygenase inhibitors. To finish the session, **Graeme Bilbe** from **Novartis** highlighted recent efforts in characterization of an mGlu5 receptor agonist that has previously been effective in multiple models of neurodegeneration.

The final session gave an excellent overview of clinical perspectives of HD. **Cristina Sampaio** from CHDI described the group work currently in place to achieve the company's goal of running "smart trials". **Sarah Tabrizi** outlined the three-year results from the TRACK-HD study, including endpoints that can be used in clinical trials, and introduced the follow-up Track-On-HD study. **Mark Guttman** discussed the clinical onset of HD symptoms and asked whether current diagnostic criteria should be changed.

In the final talk, **Michael Hayden** discussed the actual prevalence of HD. A detailed study conducted in British Columbia revealed a gross underestimation of the prevalence of HD in that population.

Clinical Trials Status Report

SPONSOR	STUDY AGENT	PHASE	PRINCIPAL INVESTIGATOR, CONTACT	DESIGN	TRIAL LENGTH	SITES	STATUS
National Institute of Neurological Disorders and Stroke	Coenzyme Q ₁₀	III	Merit Cudkowicz, MD, MSc 800-487-7671, www.huntington-study-group.org	Randomized double blind study to see if coenzyme Q ₁₀ is effective in slowing the worsening of symptoms of HD	5 years	47 sites - U.S., Canada, Australia	Enrollment complete, study ongoing
National Institutes of Health	Creatine	III	Diana Rosas, MD, MS	Open label, single group assignment study to further assess the long-term safety and tolerability of up to 30 grams of creatine daily in HD participants	12 months	1 site - United States	Enrollment complete, study ongoing
National Center for Complementary and Alternative Medicine	Creatine	III	Steven M Hersch, MD, PhD 800-487-7671, www.huntington-study-group.org	Randomized double blind study to test whether high-dose creatine can slow the progressive functional decline that occurs in adult persons with early clinical features of HD	3 years	60 sites - U.S., Canada, Australia, New Zealand	Currently enrolling
Assistance Publique - Hôpitaux de Paris	Olanzapine, Tetrabenazine and Tiapride	III	Anne-Catherine Bachoud Levi, PhD +33 (0)1 49 81 23 01	Randomized controlled study to compare the beneficial and adverse effects of 3 different neuroleptics in HD	1 year	1 site - Europe	Currently enrolling
National Institute of Neurological Disorders and Stroke	Coenzyme Q ₁₀	II	Christopher Ross, MD, PhD, Elaine M Julian-Baros, BS: 585-273-2879	Randomized double blind study testing the tolerability of treatment with 600, 1200 or 2400 mg per day of coenzyme Q ₁₀ in pre-manifest participants carrying the CAGn expansion for HD	20 weeks	13 sites - United States	Enrollment complete, results expected 4Q 2012
National Institute of Neurological Disorders and Stroke	Citalopram	II	Leigh J. Beglinger, PhD Blair Harrison, MPH: 319-353-4411	Randomized double blind study of the effect of Citalopram on tolerability, functional measures, motor performance, and psychiatric status	16 weeks	3 sites - United States	Enrollment complete, study ongoing
Fundacion para la Investigacion Biomedica del Hospital Universitario Ramon y Cajal	delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD)	II	Justo Garcia de Yébenes, MD +34 91 336 8833	A double-blind, randomized, cross over, placebo-controlled Phase 2 clinical trial to assess neuroprotection by cannabinoids in HD	9 months	1 site - Spain	Enrollment complete, study ongoing
Neurosearch A/S	Pridopidine	II	Karl Kiebertz, MD MPH	Open label, single group assignment study to assess the long-term safety of 45 mg of pridopidine in HD participants	2 years	25 sites - U.S. and Canada	Enrollment complete, study ongoing
Siena Biotech	Selisistat, SEN0014196	II	Ralf Reilmann, MD +41 61 324 1111	Randomized, double blind, placebo-controlled parallel-group design at two dose levels of SEN0014196 in early-stage HD patients	3 months	18 sites - Europe	Currently enrolling, results expected 4Q 2012
Charite University	Epigallocatechin Gallate	II	Josef Priller, MD +49 30 450 617209	Randomized double blind study testing the efficacy and tolerability of (2)-epigallocatechin-3-gallate (EGCG) in changing cognitive function in patients with HD	1 year	4 sites - Germany	Currently enrolling
Prana Biotechnology	PBT2	II	Ray Dorsey, MD 800-487-7671 (U.S.) 800-794-669 (Australia)	Randomized, double-blind safety and tolerability study of PBT2 of individuals with mild to moderate HD	6 months	20 sites - U.S. and Australia	Currently enrolling
Charite - Universitätsmedizin Berlin	Bupropion	II	Josef Priller, MD +49 30 450 617209	Randomized, double blind, placebo-controlled prospective crossover trial investigating the efficacy and safety of the treatment with Bupropion in patients with apathy in HD	22 weeks	4 sites - Germany	On hold
Siena Biotech	Selisistat, SEN0014196	I	Francis Walker, MD, Goran Westerberg, PhD: gwesterberg@sienabiotech.it	Open-label, randomized, parallel group design at one dose level of SEN0014196 in patients with early stage HD	14 days	7 sites - United States	Currently enrolling, results expected 4Q 2012

Sources: www.clinicaltrials.gov and apps.who.int/trialsearch/

To update or add a clinical trial, please e-mail editor@hdinsights.org.

The Use of Antisense Oligonucleotides for Gene Silencing

By: Emily Mitchell Sontag, PhD

Huntington disease (HD) is caused by a single gene mutation and is therefore a good candidate for therapeutic gene silencing. While many potential HD therapeutic agents focus on ameliorating toxic effects following intracellular production of the mutant huntingtin protein (mHtt), gene silencing would disrupt the production of mHtt and could be the ultimate disease-modifying therapy for HD by preventing the toxic effects of mHtt. This article highlights some promising data from gene silencing and potential translational hurdles.

Gene silencing offers many potential benefits. First, gene silencing does not require investigators to determine the exact mechanism by which mHtt causes disease. In addition, with gene silencing, the toxic effects of mHtt would be countered by the disruption of intracellular mHtt production. Finally, gene silencing therapies would remove any toxicity associated with mutant mRNA.

The use of antisense oligonucleotides (ASOs) is a promising approach to gene silencing. ASOs are small single-stranded pieces of DNA that bind via complementary base-pair binding to the intracellular mRNA transcript (Figure). In HD, ASOs prevent the transcription of mHtt. ASOs have been found to reduce a number of different mHtt-associated abnormalities in animal models of HD¹.

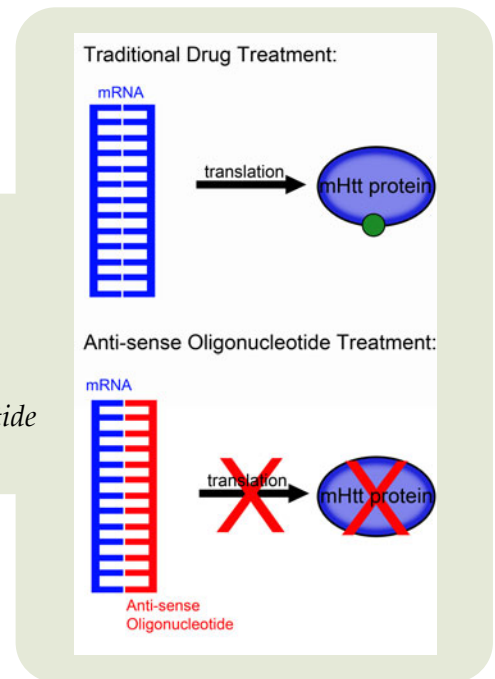
A recent study showed that infusion of ASOs targeting mHtt into the brains of mouse models of HD could alleviate motor symptoms, prevent brain loss and increase survival rates¹. The benefits of the ASO treatment persisted after the production of mHtt had returned to pre-treatment levels.

This effect, termed a “huntingtin holiday” by Carl Johnson of the Hereditary Disease Foundation, suggests that it may be possible for relatively less-frequent ASO treatments to give lasting benefit for patients. The study also found that infusion of ASOs into the cerebrospinal fluid delivered the ASOs to the brain and lowered mHtt mRNA levels in most brain regions in non-human primates. This method of delivery could be safer for human patients than direct intracerebral injection and may affect a wider range of brain tissues. This second point is significant because many research groups have shown that HD neuronal pathology is not limited to the striatum.

Despite the promise of gene silencing, challenges remain. First, the effect of reducing the levels of normal Htt, along with mHtt, is unclear. The majority of people with HD have one copy of the normal Htt gene and one copy of the mHtt gene. Htt is known to be essential in early development^{2, 3} and may be necessary for the survival of particular adult neurons⁴. Even though reducing Htt levels appears to be well tolerated in rodents and non-human primates^{5, 6, 7}, it is possible that the human brain is more sensitive than animal brains to reduced Htt levels.

One solution may be to target only the mutant HD gene for gene silencing, using ASOs or RNAi approaches. Several groups have utilized different approaches, including taking advantage of slight structural differences between mHtt mRNA and

Figure:
Simplified
mechanistic
view of
antisense
oligonucleotide
drugs.



Htt mRNA⁸, or targeting mutations (polymorphisms) other than the expanded CAG repeat in the HD gene⁹. Additionally, other genes in the human genome also contain CAG repeats, but specifically targeting the mutant gene also appears to reduce “off-target” effects.

Delivery is another potential roadblock for gene silencing techniques. It is not known whether spinal infusions will achieve the same widespread distribution of ASO in the much larger human brain, as that achieved in the brain of rodent and non-human primates. Convection enhanced delivery (CED)¹⁰ is a potential delivery approach that uses high pressure to deliver molecules deep into the brain. CED requires the insertion of tubes through the skull and into the brain, after which a pump is attached. This approach is obviously challenging from the patient’s perspective. It may also prove difficult for surgeons to accurately place tubes into the brains of symptomatic HD patients, who typically suffer significant loss of brain volume.

The Use of Antisense Oligonucleotides, cont...

The optimal timing and treatment regimen for HD patients is also not known. Studies suggest that relatively early treatments are more beneficial; however, tracking the benefits of a treatment administered before the appearance of symptoms remains difficult. Groups worldwide are working to establish more quantifiable indicators of early symptoms of HD. Further, even though ASO treatment in animal models reduced symptoms for longer than expected, human patients must deal with the disease for decades, and it is not known how often a “booster” might be needed for continued benefit.

A number of clinical trials utilizing ASOs have been completed, and more are currently underway. Previous trials have used ASOs for the treatment of various cancers, asthma, arthritis, Duchenne muscular dystrophy, Crohn's disease, heart disease and familial amyotrophic lateral sclerosis (ALS). The ALS clinical trial is of particular interest because it uses spinal infusion for the delivery of ASOs. Phase I of this clinical trial was completed in early 2012, and its results may provide information on the safety of ASO spinal infusion.

Many different gene silencing techniques are being used to lower

mHtt expression in animal models of HD. RNA-based strategies, including short-hairpin RNAs (shRNA)¹¹; small interfering RNAs (siRNA)²; and microRNAs (miRNA)³, also show promise in alleviating mHtt-mediated phenotypes. Some of these strategies are moving toward human clinical trials.

Despite the challenges of gene silencing, ASO therapy and other mHtt knockdown approaches for HD remain exciting avenues of treatment. Each new development brings us closer to the discovery of disease-modifying therapy for HD. discovery of disease-modifying therapy for HD.

¹ Kordasiewicz HB, Stanek LM, Wancewicz EV, et al. Sustained Therapeutic Reversal of Huntington's Disease by Transient Repression of Huntingtin Synthesis. *Neuron* 2012 Jun 21;74(6):1031-44.

² Nasir J, Floresco SB, O'Kusky JR, et al. Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. *Cell*. 1995 Jun;81(5):811-23.

³ Zeitlin S, Liu JP, Chapman DL, et al. Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. *Nature Gen*. 1995 Oct; 11(2):155-63.

⁴ Dragatsis I, Levine MS, Zeitlin S. Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. *Nature Gen*. 2000 Nov;26(3):300-6.

⁵ Boudreau RL, McBride JL, Martins I, et al. Nonallele-specific silencing of mutant and wild-type huntingtin demonstrates therapeutic efficacy in Huntington's disease mice. *Molecular therapy*. 2009 Jun;17(6):1053-63.

⁶ Grondin R, Kaytor MD, Ai Y, et al. Six-month partial suppression of Huntingtin is well tolerated in the adult rhesus striatum. *Brain*. 2012 April;135(4):1197-209.

⁷ McBride JL, Pitzer MR, Boudreau RL, et al. Preclinical safety of RNAi-mediated HTT suppression in the rhesus macaque as a potential therapy for Huntington's disease. *Molecular therapy*. 2011 Dec;19(12):2152-62.

⁸ Gagnon KT, Pendergraft HM, Deleavay GF, et al. Allele-selective inhibition of mutant huntingtin expression with antisense oligonucleotides targeting the expanded CAG repeat. *Biochemistry*. 2010 Nov 30;49(47):10166-78.

⁹ Carroll JB, Warby SC, Southwell AL, et al. Potent and selective antisense oligonucleotides targeting single-nucleotide polymorphisms in the Huntington disease gene / allele-specific silencing of mutant huntingtin. *Mol Ther* 2011 Dec;19(12): 2178-85.

¹⁰ Stiles DK, Zhang Z, Ge P, Nelson B, et al. Widespread suppression of huntingtin with convection-enhanced delivery of siRNA. *Exper Neurol* 2012; 233(1):463-71.

¹¹ Harper SQ, Staber PD, He X, et al. RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. *Proc Nat Acad Sc USA*. 2005 Apr;102(16): 5820-5.

¹² DiFiglia M, Sena-Esteves M, Chase K, et al. Therapeutic silencing of mutant huntingtin with siRNA attenuates striatal and cortical neuropathology and behavioral deficits. *Proc Nat Acad Sc USA*. 2007 Oct;104(43):17204-9.

¹³ McBride JL, Boudreau RL, Harper SQ, et al. Artificial miRNAs mitigate shRNA-mediated toxicity in the brain: implications for the therapeutic development of RNAi. *Proc Nat Acad Sc USA*. 2008 Apr;105(15):5868-73.

Upcoming HD Events

DATE	EVENT	ABSTRACT DEADLINE	LOCATION
October 7-10, 2012	American Neurological Association Meeting	Closed	Boston, MA
October 13-17, 2012	Society for Neuroscience Annual Meeting	Closed	New Orleans, LA
November 8-10, 2012	Huntington Study Group Annual Meeting and Huntington Disease Clinical Research Symposium	Closed	Seattle, WA
February 17-19, 2013	Clinical Neurology and Neurophysiology Update Symposium	November 20, 2012	Jerusalem, Israel
April 8-11, 2013	CHDI HD Therapeutics Conference	Not yet posted	Venice, Italy

To have your conference added for future publications, please e-mail HD Insights at editor@hdinsights.org.

Meet the Company

VITAL SIGNS

NAME: Isis Pharmaceuticals

HEADQUARTERS: Carlsbad, California

STOCK PRICE AS OF 8/21/12: \$13.67

52 WEEK RANGE: \$6.25 - \$14.05

MARKET CAPITALIZATION AS OF 8/21/12: \$1.4 billion

EMPLOYEES: Approximately 340



Dr. C. Frank Bennett

Isis Pharmaceuticals is a biotechnology company that develops therapeutic agents that target and modulate RNA. Isis's primary platform is exploiting antisense oligonucleotide technology to selectively reduce the expression of a target RNA. Co-founder and Senior Vice President of Research, Dr. C. Frank Bennett, spoke with HD Insights about Isis's RNA-based targeting in HD. Edited excerpts from the discussion are below.

INSIGHTS: Can you tell us a little bit about Isis?

BENNETT: The focus of the company since its inception has been on novel therapeutic agents that target RNA. We use a technology called antisense oligonucleotides (ASOs) that uses short synthetic nucleic acid analogs designed to bind to a target RNA via Watson-Crick base pairing.

Once our ASOs bind to the target RNA, they can then be a part of a range of mechanisms to modulate the RNA. For the Huntington disease (HD) Project, the primary mechanism that we're exploiting is through an enzyme called RNase H. RNase H selectively degrades the RNA that is bound by the oligonucleotide. Wherever the oligonucleotide binds, it recruits RNase H to that RNA, and the RNase degrades the RNA. The oligonucleotides are then recycled within the cell. This is a novel catalytic mechanism that we are using to very selectively reduce the expression of a target RNA. In this case, we're targeting RNA that is expressed by the huntingtin gene.

INSIGHTS: Since most humans have two copies of the huntingtin gene and in HD-affected individuals only one allele has the CAG expansion, how can you target the production of mutant huntingtin and not wild-type huntingtin?

BENNETT: We're taking several different mechanistic approaches. The most advanced project we are using does not distinguish between wild-type and mutant huntingtin. Our pre-clinical research, performed in collaboration with Dr. Don Cleveland at University of California San Diego, suggests there are no deleterious effects associated with reducing wild-type huntingtin, and published research supports our understanding that wild-type huntingtin protein is essential for normal development before birth, but has a largely undefined role in adults. So we are counting on humans having some tolerance for reduced expression of both mutant and wild-type huntingtin.

However, we also have two different approaches that we're using to get selectivity in case it's needed. One approach is a project with Dr. Michael Hayden and his colleagues at the University of British Columbia, in which we are targeting single nucleic-type polymorphisms that co-associate with the expanded CAG triplet on the mutant allele. We are able to distinguish a single nucleotide- change, which causes the loss of the mutant allele while sparing the wild-type allele. In our preclinical studies we have been able to demonstrate a greater than 50-fold selectivity of the mutant versus wild-type, based upon that same nucleotide- change. The caveat is that there are a number of haplotypes that all have expansions of repeats, so a single drug would at best treat about 50 percent of HD patients. Multiple drugs must be available to treat all the patients who would be amenable to this therapy.

Another approach is to use all the oligonucleotides that bind to the expanded CAG repeat itself. Working with another collaborator, Dr. David Corey, at University of Texas Southwest Medical Center, we have shown that the expansion of that CAG repeat provides more binding sites for the oligonucleotide. Using this technology, we are able to distinguish between wild-type CAG repeat links and the expanded CAG repeat that occurs in a disease. This technology has an advantage that could treat a larger number of patients with a single drug, but there is also concern because other genes have CAG repeats. We are currently working to identify or characterize the risk of the CAG targeting approach versus the polymorphism approach.

Meet Isis Pharmaceuticals, continued...

INSIGHTS: What are the specific opportunities for antisense oligonucleotide therapies for HD?

BENNETT: Our technology can be broadly applicable to a variety of human diseases. We have demonstrated that intrathecal dosing results in a very broad distribution of a drug into CNS tissues and can influence expression of mutant huntingtin in the areas of the brain that are affected in HD.

INSIGHTS: A number of these therapies you mentioned are further along in development. What is holding back development of therapies for HD?

BENNETT: Mainly it is determining the optimal drug to take forward. Clinical trials are quite expensive, and we want to bring forward the drug that has the best chance of being successful in the clinic. We have narrowed down the list to a small number of drug candidates, and we are doing additional studies to characterize the behavior of those candidates in our preclinical studies. Our hope is that we'll have that completed by early next year and then start the process of doing the toxicology studies that are necessary to bring that drug forward into the clinic.

INSIGHTS: Thinking about the clinical development of a drug for HD, are there any specific challenges in HD that are different to other conditions?

BENNETT: I think there are two separate endpoints that we would concentrate on: deciding which drugs make more sense for early clinical programs to show proof of concept, and then which clinical measures would make the most sense for demonstrating that the drug is clinically effective.

INSIGHTS: What do you envision would be the clinical population to which the drug would first be given, in terms of development of clinical HD symptoms?

BENNETT: Since this drug is delivered by intrathecal dosing, we would likely start clinical trials in currently symptomatic patients rather than in volunteers who are in the pre-symptomatic stages of HD. The question is just how advanced would the disease be in patients we would be comfortable in selecting for dosing.

INSIGHTS: You mentioned dosing. How frequently do you think an ASO therapy would need to be dosed in a disease like HD?

BENNETT: Based on our work in other programs, I hypothesize that at a minimum, it would be an intrathecal injection every three months.

INSIGHTS: In those symptomatic patients, what clinical endpoints do you think are best for consideration?

BENNETT: I think we have to build in cognitive changes, maybe imaging measures, and then some motor function measures as well. A big challenge for the project right now is identifying clinical measures that correlate with reduction in intracellular levels of mutant huntingtin.

INSIGHTS: Are there any safety concerns?

BENNETT: At this point in the program it's too early to say. We haven't identified any safety concerns with our preclinical studies, and it's something that we are investigating in our toxicology studies. The purpose of those studies is to help identify if there are any safety concerns that we would need to monitor in the clinic. However, ASO therapies have been very well tolerated in our amyotrophic lateral sclerosis drug and our spinal muscular atrophy drug. In our clinical programs we see a relatively low incidence of spinal headaches in patients dosed intrathecally. These events are more procedure-related than drug-related.

INSIGHTS: What message do you have for HD patients about the promise of ASOs for HD?

BENNETT: Again, it's important to keep in mind is this is one of many therapies being developed by companies right now. I would not look at any of these therapies as a cure for HD. They may either slow down the process of the disease or mitigate some of the symptoms that patients experience. Ultimately, Isis can develop one of several drugs that will make a big impact in the lives of individuals with HD.

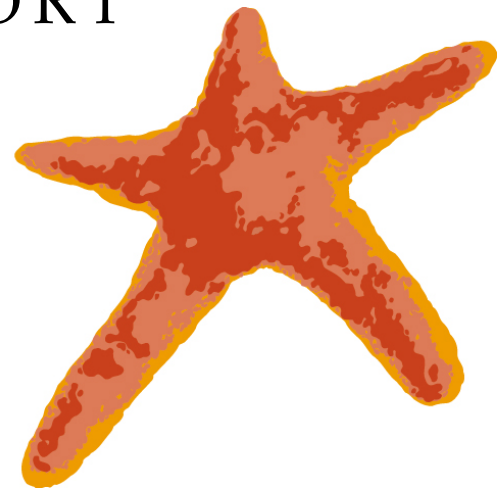
INSIGHTS: How can the research community help you and Isis develop therapies for HD?

BENNETT: One of the big challenges that we and other companies developing therapies for HD face is finding early, measurable clinical biomarkers that correlate with the effects of the drug. I think results from natural history studies will be important for developing drugs and helping us design our clinical trials. We're excited to move these therapies forward. We would like to thank CHDI, the Hereditary Disease Foundation, and the Huntington's Disease Society of America for their support of our efforts.

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HD Research Around the World: Chile



Alleviating Secretory Pathway Stress in Huntington Disease

By: Rene Vidal, PhD and Claudio Hetz, PhD

Many clinical trials that use drugs validated in mouse models of HD have failed to alleviate disease progression in humans. Preclinical studies have been performed in transgenic mice of pure genetic backgrounds that overexpress high levels of truncated forms of mutant huntingtin (mHtt). This mouse model, and other HD mouse models, does not truly replicate HD in humans. Several HD mouse models are available, including many mHtt knock-in mice. However, these mouse models often require the use of homozygous mHtt alleles, because mice carrying only one mutant allele develop very minor phenotypes and fail to express most of the distinctive features of HD. Also, cellular processes known to be important for neuronal function are often altered in HD mouse models. Researchers are developing strategies to identify molecular events that transcend various HD cellular and animal models, and correlate these with alterations observed in human HD-derived samples.

A common molecular feature described in cellular and animal models of HD is the occurrence of protein folding stress responses in the brain, possibly caused by alterations of the protein secretory pathway. Defects in virtually every step of the secretory pathway are observed in HD neurons, such as perturbations in protein folding networks; vesicular transport; the endoplasmic reticulum (ER) and Golgi 3D patterning; protein

quality control mechanisms (i.e. autophagy and the ER-associated degradation pathway); and ER calcium homeostasis. Many alterations of the of the protein secretory pathway generate alterations in the protein folding process and lead to a pathological condition known as ER stress.

Some investigators take a global view of mHtt pathogenesis and hypothesize that strategies aimed at alleviating secretory pathway stress may have beneficial effects in HD. Studies have documented activation of the unfolded protein response (UPR), an adaptive reaction against ER stress, in animal models of HD and human postmortem samples from HD patients. Studies in HD cellular models support the concept that chronic ER stress contributes to HD-related neurodegeneration¹. Lee and colleagues demonstrated that the ER stress sensor IRE1 may govern mHtt aggregation and neurotoxicity through a molecular crosstalk with autophagy, another homeostatic pathway². IRE1 enhanced mHtt degradation by the lysosome-autophagy pathway.

Targeting the stress networks involved in protein homeostasis is an interesting method of disease intervention. We investigated the possible contribution of ER stress to phenotypic HD *in vivo* using a recently generated strain of mice that selectively lack XBP1 in neurons (the downstream target of IRE1).

Despite predictions that XBP1 deficiency would increase the severity of experimental HD, we observed that this genetic manipulation triggered resistance to development of the disease. XBP1 deficiency enhanced neuronal survival and improved motor performance of a full-length mHtt transgenic mouse (the YAC128 model)³. We also validated the effects of XBP1 on mHtt levels in a heterozygous knock-in mouse HD model. The mechanism of protection appears to be related to the upregulation of autophagy and the degradation of full-length mHtt in the lysosomes. In collaboration with Dr. Ana Maria Cuervo (Albert Einstein College of Medicine), we showed that mHtt is delivered to autophagosomes and autophagolysosomes *in vivo* upon targeting XBP1 in the nervous system. This observation suggests that a homeostatic crosstalk between the UPR and autophagy is a response against mHtt pathogenesis that may be manipulated to provide protection against HD. At the molecular level, we found a negative regulation of the transcription factor FoxO1 by XBP1 *in vivo*. FoxO1 is a major protein involved in ageing and operates as a macro-stress integrator of metabolic and stress processes.

Secretory pathway stress in HD could be manipulated by targeting different components of this network, including homeostatic pathways such as the UPR and autophagy.

HD Research in Chile, cont...

General pharmacological strategies may include administration of chemical chaperones such as TUDCA or 4-PBA, modulators of UPR components (i.e. available IRE1 inhibitors), or autophagy enhancers such as trehalose or rapamycin. The use of gene therapy to target protein-folding stress has been applied to other neurodegenerative diseases

with the aim of targeting protein homeostasis, and may be an approach to consider, since HD is a disease that progresses slowly.

The development of network-modifying therapeutic interventions may lead to important protective advances in the HD field. Minor shifts in the protein homeostasis network

may involve the alteration of hundreds of target genes that as a whole may result in beneficial effects in HD. Thus, modulating global homeostatic processes could have broad impact for chronic alterations observed in HD that involve multiple aspects of neuronal physiology and protein homeostasis.

¹ Vidal R, Caballero B, Couve A, Hetz C. Converging pathways in the occurrence of endoplasmic reticulum (ER) stress in Huntington's disease. *Curr Mol Med.* 2011 Feb;11(1):1-12.

² Lee H, Noh JY, Oh Y, et al. IRE1 plays an essential role in ER stress-mediated aggregation of mutant huntingtin via the inhibition of autophagy flux. *Hum Mol Genet* 2012 Jan;21(1):101-14.

³ Vidal RL, Figueroa A, Court FA, et al. Targeting the UPR transcription factor XBP1 protects against Huntington's disease through the regulation of FoxO1 and autophagy. *Hum Mol Genet* 2012 May;21(10):2245-62.

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