

Milton Wexler Interdisciplinary Workshop
“Curing Huntington’s Disease – Approaches and Plans”

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Prepared by Marina Chicurel, Ph.D.

One of the key messages emerging from the Milton Wexler Interdisciplinary Workshop: Curing Huntington's disease—Approaches and Plans" is the field's increasing need for coordinated teamwork. Participants underscored the importance of focusing on and pursuing the most promising lines of HD research in a concerted manner.

Participants first discussed clinical trials; their current status and the challenges that lie ahead. They then turned their attention to four promising approaches for developing treatments: silencing the huntingtin gene, boosting the clearance of the abnormal protein, manipulating huntingtin aggregation and cellular localization, and targeting some of abnormal huntingtin's downstream effects. Participants also emphasized the importance of continuing studies on the role of wildtype huntingtin in cellular function. In addition to identifying specific strengths and limitations for each of the approaches discussed, participants identified general challenges in the path towards finding treatments for HD. In particular, they noted the increasing need to bridge the gap between cell-based studies and whole animal phenotypes, as well as the need to strengthen the links between animal studies and human clinical trials.

As the meeting drew to an end, participants agreed that a primary goal for the future is to coordinate ongoing efforts so that translation from the lab to the clinic is as streamlined as possible. This is particularly urgent for gene silencing efforts which are closer to the clinic than other candidate treatments. The establishment of committees composed of academic and industrial scientists, as well as FDA officials, was considered key, especially for helping standardize the use of biomarkers, the design of clinical trials, and the prioritization of candidate interventions.

A family stands together in their battle with HD

While the importance of coordinated teamwork to find treatments for HD was emphasized by participating scientists, the value of coming together as a family to battle HD was poignantly revealed by the Behl family. With remarkable intelligence and grace, the Behls described how, by supporting each other and confronting the disease as a team, they have found hope and strength to cope with the devastating disorder.

The father and two of the grown children who attended the meeting described how the mother of the family began showing mood disturbances over 15 years ago, then developed an abnormal gait, and was finally diagnosed with HD approximately 7 years ago. They explained how the family has been affected by this devastating sequence of events in many ways. First, the family faced confusion, anxiety, and sadness as they witnessed the mother's early changes in character and behaviors. Before the diagnosis, the family was bewildered by the mother's sudden outbursts of rage, her withdrawal from her duties as manager of her husband's clinical office, and her, at first dwindling, and then complete lack of, correspondence with her children when they were in boarding school.

After the diagnosis, the family gained understanding of the situation, but they were then confronted with another set of terribly difficult questions: whether, when, and how the children would get tested for their own

HD statut. As a group, they discussed many factors impinging on these questions, but the one thing they never doubted was that they would face the problems together, as a team. They agreed they would get tested because, as the daughter explained, they are “planners”, and they knew they wanted to go through the testing together.

Making this happen wasn't easy, however. Coordinating a date for testing in which everyone could come together—the parents, the three grown children, and two fiancés, all with busy schedules and living in different parts of the country—was the first challenge. Setting up the logistics to perform the group testing was another difficult task. As explained by Yvette Bordelon, the mother's neurologist, getting the required health care practitioners to come together, and finding a room that would accommodate the large group required some effort. The daughter who was present at the meeting said the family was ready to search for a different clinic if their request to be tested as a group was not honored.

Finding a date in which the entire family could be together to learn the outcome of the testing was the next challenge. Indeed, the family had to wait 8 months after the results were ready to ensure all members were available. The wait was excruciating, especially for the son who said he couldn't stop thinking about the results. However, there was one thing he knew he did not have to worry about: the steadfast support from his family. The three children decided before the testing that, as long as one of them had a normal sized HD repeat, they would be fine. They made the commitment to each other that the healthy children would take care of the affected ones. The tests revealed that two of the three children had expanded HD repeats. All of the children are only in their 20's. They have already begun to make arrangements to live closer together.

This extraordinary unity and willingness to face the consequences of HD head-on inspired participants at the meeting. Moreover, the Behls drew even more admiration when they described how their approach to HD contrasts with that of their own relatives in India. The Behls noted that the members of their extended family never mention HD by name, and refer to it obliquely as “the disease.” HD is a matter of deep shame, and practically no attempt is made medically to ameliorate patients' symptoms. Individuals with HD are considered at fault and isolated from others when they begin to show symptoms. In addition, parents try to marry off their children early if they suspect they carry the HD abnormality, without informing the spouses-to-be of the situation. This approach to HD does not stem from ignorance—as explained by the Behls, their relatives are well educated and aware of the genetic basis of the disease. The lack of interest in pursuing palliative treatment appears to be due to the notion that, because there is no cure for the disease, it isn't worthwhile doing anything about it. However, if there was a cure for HD, the Behls predicted their relatives would seek it out immediately.

Clinical Trials

These remarkable accounts—inspiring, on the one hand, and heartbreaking on the other—motivated participants to brainstorm ways in which their joint efforts might accelerate the development of a cure for HD.

Initiating the discussion, Anne Young gave a brief summary of some of the approximately 20 ongoing clinical trials for HD. Young explained that there are two categories of clinical studies: observational and experimental.

Observational clinical studies

Observational clinical trials seek to identify HD-associated alterations and map their progression. The PREDICT-HD study, for example, is designed to define the earliest biological and clinical features of HD before the onset of diagnosable symptoms. It is the largest study of pre-symptomatic individuals and, as noted by Bordelon, in the last year, several of its tests have been refined, providing additional and higher quality data. The PHAROS study is similar to PREDICT-HD, but involves individuals who are at risk for HD. Their genetic status is determined to inform the research and codified. Participants choose not to learn their gene status. This study has compiled prospective data over the past ten years.

Both studies are generating valuable data that promise to contribute to the identification of much-needed biomarkers to assess the efficacy of experimental treatments. They are also revealing the shortcomings of current diagnostic tools. For example, Young noted that observational studies, in particular PHAROS, have shown that standard diagnoses result in as many as 20% false positives. Many of the behavioral abnormalities associated with the early stages of HD (such as HD-like mood alterations, depression, psychosis, apathy, delusions, and mild chorea) have also been reported in individuals not carrying the HD abnormality. Even measurements of involuntary movements, such as saccades, can be misleading, noted Young.

As noted by Jang-Ho Cha, however, the number of incorrect HD diagnoses is much lower than the false-positives associated with other neurological disorders, such as Parkinson's disease. In addition, HD false-positives are rarely random—they can often be attributed to the influence of HD patients on family members. Cha has observed, for example, healthy individuals who unconsciously mimic some of the choreic movements of their spouses afflicted by HD. An extreme example of a false positive was pointed out by Nancy Wexler. She described a woman in Venezuela who had severe neurological, cognitive and psychiatric symptoms, and was diagnosed as having HD by more than a dozen neurologists. Blood samples from her parents and siblings were also redrawn and analyzed anew to test for non-paternity. Her blood sample was also taken several times. Her Huntington's disease allele was measured independently several times. It was entirely normal. Another factor that contributes to misdiagnoses is the malleability of HD symptoms, as noted by David Housman. For example, the mother in the family who attended the workshop showed a marked improvement in her cognitive skills after practicing arithmetic games with her daughter, despite her ongoing cognitive decline.

Remarkably, the Behl family noted that their relatives in India are able to predict with great accuracy when children are as young as 9 or 10, who will develop HD in the future. They explained that 7 out of 9 of their mother's siblings developed HD, and this was anticipated years earlier by the relatives who knew them. The relatives seem to notice that the children who are going to develop HD are less athletic, less academically

accomplished and/or less outgoing than those that don't carry the mutation. However, they did not predict that the mother who attended the workshop (and developed her symptoms later in life) would be afflicted.

Clinical trials to test experimental treatments

Participants also briefly discussed several of the current trials testing experimental treatments for HD. As explained by Young, these can be classified into two categories: those designed to change the course of the disease by stopping or reversing the damage caused by abnormal huntingtin and those designed to treat specific symptoms. Currently, there are only two compounds being tested that fall into the first category: Co-enzyme Q10 (CoQ10) and creatine. Co-Q10 is being tested in a trial with symptomatic individuals, as well as in a trial with pre-symptomatic individuals. The latter is a placebo-controlled study using large doses of CoQ10 (2400 mg/ day) which is scheduled to last 3-5 years. Performing studies to test over-the-counter supplements is challenging because many individuals with HD, or at risk for HD, are already taking these compounds. In addition, potential subjects are often hesitant to take the chance of being assigned to a placebo control group when they can be taking the harmless supplement on their own. Indeed, the Behl son and daughter, who carry the HD expanded repeat and are currently taking CoQ10, said they were interested in participating in clinical trials, but not under those circumstances.

Providing an update on creatine, Young noted that a trial testing this compound's effects in pre-symptomatic individuals is currently enrolling subjects (pre-CREST study). A study with symptomatic individuals (CREST) using 30 g/day of creatine has already shown some slowing of the disease. The Behl family noted that the mother is currently taking 10 g/day of creatine and wondered if they should increase her dose. Unfortunately, noted Young, commercial sources of creatine often have impurities that can cause side-effects, such as diarrhea, when the supplement is taken in large doses. The daughter also noted her own difficulties taking creatine as a presymptomatic individual because of its unpleasant taste. Participants agreed it would be helpful to explore ways to make creatine more palatable. Medical grade creatine is available online through the Avicena Group - <http://www.avicengroup.com/products/pharmaceuticals/index.php>.

Participants also discussed several trials for treatments that target HD symptoms. For example, as noted by Young, there are two ongoing trials to test the effects of memantine, a glutamate NMDA channel blocker used to treat cognitive problems in Alzheimer's disease. The data are still being analyzed, but it appears that, in some people, memantine can worsen motor symptoms. At this point, Young emphasized, physicians must test what works best for each of their patients, rather than following a set formula for all HD patients. Indeed, the Behl family and Bordelon described how they have worked together to find the combination and dosages of memantine, olanzapine, and tetrabenazine that seem to be best suited for the mother of the family.

Other treatments being currently tested in humans were also briefly discussed. For example, Novartis is testing the effects of an inhibitor of the metabotropic glutamate type 5 receptor which has been used to treat side-effects of medications for Parkinson's disease. A D2 dopamine modulator, known as Huntexil or ACR-16,

is another candidate in trials. So far, the observed effects—amelioration of HD-associated dystonia and bradykinesia—are only minor. An HD trial to test Dimebon, a drug approved as an antihistamine in Russia, is also underway. As noted by Carl Johnson, the drug was reported to provide a very minor amelioration of Alzheimer's disease symptoms and then just failed to prove beneficial for HD patients in clinical trials. With very little testing in HD mice to support it, the drug was moved into clinical trials for HD and is now in phase III. [According to the April 17, 2011 press release from Pfizer Inc and Medivation, Inc., who were supporting the Phase 3 HORIZON trial in patients with Huntington disease, "Dimebon did not achieve statistical significance for either of the co-primary endpoints, the Mini-Mental State Examination (MMSE), which measures cognition ($p=0.39$), or the Clinician's Interview-Based Impression of Change, plus caregiver input (CIBIC-plus), which measures global function ($p=0.84$)."] They are stopping the study.]

As noted by Young, trials that test treatments for HD symptoms are less costly and more rapid to perform than trials that seek to change the course of the disease. Thus, they are more attractive to industry and more subject to the influences of commercial interests. Bordelon added that, if a promising symptomatic treatment is identified, it is important to test not only its short-term effects, but its longer-term consequences, since it may confer unanticipated long-lived benefits or toxicity.

Candidate treatments: Gene Silencing

Participants then turned their attention to promising candidates in the preclinical pipeline. Members of four teams working on gene silencing were present at the meeting and provided brief summaries of their current work and future goals. Their summaries highlighted the likely efficacy of this treatment, while also revealing the challenges that remain to be solved. Participants discussed these challenges which included: delivery, distribution, and safety issues.

As noted by Ryan Boudreau, in 2005 Beverly Davidson and colleagues published a proof-of-concept study showing that silencing the huntingtin gene could improve the symptoms of HD in mice. Huntingtin expression was knocked down by approximately 50% using shRNA, resulting in improved motor and neuropathological deficits. Since then, several studies, using various silencing agents and models of HD, have extended and confirmed these observations. Moreover, a couple of recent studies have suggested that it is even possible to reverse HD-associated alterations in symptomatic animals and produce benefits that persist for months after treatment.

For example, Holly Kordasiewicz described work showing that antisense oligonucleotides (ASOs) designed to direct the degradation of huntingtin mRNAs by endogenous RNase H, can reverse motor deficits, extend survival and prevent the loss of brain mass for up to 12 months after treatment of HD mice. Kordasiewicz noted that she and her collaborators have used ASOs developed by Isis Pharmaceuticals (2' O-(2-methoxy)ethyl (MOE)-modified gapmers) that are 20 bases long and specifically hybridize to exon 1 of the

human huntingtin mRNA. Frank Bennett explained that the modification at the 2' position, stabilizes the molecules by acting as a water stake, which helps protect the phosphate groups from protein interactions.

So far, noted Kordasiewicz, the team has performed infusions into the right lateral ventricles of HD mice resulting in a reversal of motor deficits (as assessed by rotarod, open field activity and elevated maze performance) in both R6/2 and BAC-HD symptomatic mice, as well as survival extension and prevention of the loss of brain mass in R6/2 mice. ASOs have been infused as early as 8 weeks in R6/2 mice (it has proved technically difficult to infuse them in younger mice) and as late as 6 months in BAC-HD mice. Kordasiewicz further explained that the beneficial effects take approximately two months to develop and persist for up to 12 months, long after the two-week perfusion and 3-month lifespan of the ASOs *in vivo* has elapsed.

Safety issues

Although these and other gene silencing studies are encouraging, several challenges remain. For example, under some circumstances, gene silencing agents can be toxic. For example, Boudreau noted that high levels of exogenous RNAi substrates can cause toxicity by saturating the endogenous RNAi machinery. To circumvent this problem, Davidson and colleagues have switched to using artificial miRNAs which are expressed at much lower levels than shRNAs. These miRNAs have been shown to mediate potent gene silencing and therapeutic efficacy in a mouse model of HD.

ASOs can also be toxic at high doses causing inflammation. This inflammatory response is commonly mediated by the activation of pattern-recognition receptors (PRRs) expressed by immune cells in the CNS, noted Bennett. Normally, PRRs recognize evolutionarily conserved patterns from pathogens, including nucleic acids, to initiate an immune response to infection. To minimize the activation of these receptors, Bennett and colleagues are designing ASOs that do not include sequences known to be present in PRR ligands.

Chesselet added that some oligonucleotides that appear to be safe can cause regional inflammation which is easily overlooked. Her team has found that certain ASOs which do not cause immediate, overt problems when infused into mouse brains and seem to be safe based on bioinformatic analyses of their sequences, result in local inflammation several months after infusion. This effect is easily missed by commonly-used techniques to monitor inflammation, such as microarray analyses of whole tissues or blood tests. Chesselet is currently examining whether this local inflammation is a consequence of delivering toxic ASOs, or a non-specific response to the delivery process. One advantage of using mouse models of HD, noted Bennett, is that inflammation is usually more severe in rodents than in primates, so current mouse tests are providing worst-case scenarios.

Off-target silencing is another safety concern. As explained by Boudreau, in the case of RNAi-based therapies, off-target silencing primarily occurs when the seed region of a small RNA (nucleotides 2-8) hybridizes with 3' UTR sequences of non-target mRNAs and destabilizes them or represses their translation. In his experience, off-sequence effects are not always immediate and can take about 4 months to become

detectable. However, it is possible to rationally design highly specific silencing RNAs by selecting seed regions that are very rare in the 3' UTRome. The large size of huntingtin is advantageous because there are many sequences to choose from. According to Boudreau's calculations, there are 10-15 huntingtin target sequences that should be safe and potent. Boudreau has already successfully designed a few miRNAs based on this strategy.

As noted by Bennett, avoiding off-target silencing is generally easier to accomplish with ASOs because even a single mismatch greatly reduces RNase H's ability to recognize a substrate. Summarizing his team's strategy to select ASOs, he noted that over 3000 sites in a transcript are examined, first focusing on potency, then testing tolerability in mice and rats, and then performing binding tests. As part of their tolerability tests, Kordasiewicz noted that she has tested the effects of an ASO that specifically targets human huntingtin in a mouse that lacks the human sequence. Analyzing microarray profiles of the striatum and cortex two months after treatment, Kordasiewicz observed slight alterations in the expression of only 7 genes associated with inflammation. These data indicate that off-target effects associated with this ASO are minimal, if present at all, and lend support to the team's sequence selection process

Participants also briefly touched upon the issue of whether it is important to specifically target the abnormal huntingtin allele or not. Consistent with reports from other groups indicating no ill-effects from treating rodents with non-allele specific silencing agents, Kordasiewicz noted that her team obtained indistinguishable results when targeting only the abnormal allele versus targeting both normal and abnormal alleles in BAC-HD mice.

Nevertheless, several groups, such as Neil Aronin's, are developing allele-specific treatments to minimize potential safety risks. Bennett added that Isis is collaborating with Michael Hayden's group to examine the effects of ASOs that target allele-specific SNPs, in addition to their work with non-allele specific ASOs. One limitation of this approach, however, is that it restricts the number of sequences to select from for maximizing potency. Bennett noted that he is also working with David Corey who has devised a novel system to target abnormal huntingtin in which duplex RNAs with 1-3 mismatched bases in their central region are used to block the expression of abnormal huntingtin mRNA preferentially. The technique relies on blocking translation, rather than inducing mRNA cleavage, and can be used to selectively target abnormal huntingtin because the structure of the expanded CAG repeat appears to be a superior substrate for the RNAi machinery. Housman cautioned, however, that the number of CAG repeats in abnormal huntingtin can be similar to that found in other, normal proteins, such as the wildtype TATA box-binding protein (TBP). But Kordasiewicz considered this was unlikely to pose a problem because of the difference in intramolecular context of the two CAG stretches.

Delivery and distribution issues

The challenges associated with delivery and distribution of silencing agents were also discussed. RNAi-based strategies have mostly converged on the use of adeno-associated viruses (AAVs) for delivery. One of the advantages of this delivery system is that the viral particles persist as episomes within their host cells, limiting the invasiveness of delivery to one or a few injections over the lifetime of an individual. Moreover, as described by Aronin, viral technology has advanced substantially in the past few years and continues to be optimized by researchers working on several genetic disorders. Approximately 100 AAV serotypes with different properties, including different cell type affinities, have been characterized.

One serotype which appears to be particularly effective for delivery to neurons in the brain is AAV-9. As noted by Aronin and Paul Patterson, this serotype spreads relatively well through retrograde transport. Johnson pointed out that a key stumbling block in the use of viral delivery systems in the brain has been getting sufficient coverage, but Aronin noted that it is possible to give multiple injections in different brain regions and, given AAV-9's relatively good degree of spreading, it is likely to provide sufficient coverage.

Participants also discussed the safety of AAV. Housman wondered if AAV could elicit immune responses, especially if used repeatedly in multiple doses. Patterson noted that we are all exposed to these viruses without ill effects and Aronin added that different serotypes could be used to minimize secondary immune responses, if multiple dosing is necessary. In addition, several studies have shown safety in patients, although Patterson noted that he was not aware of any post-mortem studies. Aronin said his group is planning to monitor neuropathology, glial activation, viral content and expression in sheep injected with AAV. In addition, Boudreau mentioned that there are ongoing efforts to develop ways to regulate the expression of the delivered genes so they can be turned off if safety issues arise.

Researchers working with ASOs face a different set of delivery challenges. The half-life of ASOs is only a few months in vivo, so they must be delivered continuously (or at least repeatedly) to have long-lasting effects. As previously mentioned, Kordasiewicz studies indicate that the benefits conferred by ASOs outlive the ASOs 3-month in vivo lifespan, but it will still be necessary to deliver new ASOs at regular intervals. As noted by Bennett, two of the basic questions his team is currently addressing are: how often to treat and how to deliver the treatment. As explained by Kordasiewicz it is necessary to deliver the ASOs directly to the CNS because the oligonucleotides are directed to the liver when injected systemically. To decrease the invasiveness of an intrathecal pump, however, Bennett and colleagues are planning to test subcutaneous access ports connected to intrathecal catheters.

Optimizing the distribution of ASOs is another challenge. Kordasiewicz and Bennett noted that they have obtained good distribution throughout the mouse CNS—including cortex and striatum—using cerebrospinal infusions. However, in animals with larger brains, uniform distribution is harder to attain. As explained by Kordasiewicz, the knock down mediated by ASOs occurs as a gradient across the brain, with target reduction being less efficient in deeper parts of the brain. The larger the brain, the more of a gradient. In an experiment with rhesus monkeys, for example, the researchers observed 75% target reduction in the cortex,

but only 25% in the striatum. Distribution seems to improve, however, when ASOs are delivered as a bolus, rather than a gradual infusion.

Based on these considerations, participants stressed the importance of continuing and extending studies in animals with large brains. As mentioned above, Bennett and co-workers are working with monkeys, as is Boudreau's team. The latter have obtained 50% knock down of huntingtin in rhesus monkeys and observed no negative effects using a large number of behavioral tests. Also, as previously noted, Aronin is working with sheep, who have even larger brains than rhesus monkeys. It is possible that sheep will provide a sufficiently good model for preclinical testing, that Aronin will be able to forgo tests in nonhuman primates. As noted by Aronin, an official involved in the review of orphan disease grants, informed him that testing in nonhuman primates is not a requirement for moving a treatment into clinical trials. Another factor that was considered was cost. Johnson wondered if the cost of ASOs would be prohibitive in large brains. Bennett noted that, in fact, the cost is very reasonable—cheaper than protein-based drugs, and comparable to small molecules.

Another important question discussed at the meeting was the location(s) to target silencing agents. On the one hand, as previously mentioned, it is desirable to maximize distribution throughout the brain because HD pathology is so widespread. Imaging studies by Diana Rosas, for example, indicate early pathology across many different brain regions. However, it might not be necessary to treat the whole brain to obtain significant therapeutic benefits. Indeed, as noted by Marc Diamond, it might even be preferable to limit interventions to circumscribed brain areas for safety reasons, especially during initial clinical testing.

Given that the caudate appears to be a primary site of HD pathology, Aronin and co-workers are focusing on this brain region for their initial AAV studies. William Yang added that both the cortex and striatum might be particularly desirable targets because they are key sites of pathology, each apparently responsible for distinct, but interconnected, deficits, as revealed by studies in BAC-HD mice. The benefits of reducing abnormal huntingtin in these two areas appear to be synergistic. In particular, reduction in the expression of abnormal huntingtin in the cortex and striatum ameliorate striatal pathology in an independent and additive manner.

Participants also discussed the possibility of targeting brain areas that are involved in determining specific phenotypic characteristics of HD. For example, Boudreau asked if it would be possible to target a brain region(s) whose pathology correlates with survival. Diamond noted that the causes of death in HD are multifactorial, and thus it is impossible to pinpoint an area as directly linked to death. Kurt Fischbeck then asked whether there were brain areas whose pathology is linked to the symptoms that are most bothersome to patients. As noted by Young, cognitive alterations are probably the most burdensome symptoms, but are difficult to map to a specific brain area and are very variable.

Participants also questioned whether neurons are the only desirable targets, or whether glial cells should also be targeted. Kordasiewicz and Boudreau noted that both ASOs and RNAi seem to reach glia, at least to some extent, but the contribution of these cells to the benefits observed after treatment is unknown.

Setting up clinical trials

Bennett provided participants with a general idea of what a trial to test anti-huntingtin ASOs would entail, but emphasized that his team has yet to make detailed plans. The initial study would probably be a single-dose trial with 4 cohorts, each comprised of 6-8 patients, including placebo groups. The trial would be set up to provide participants with the opportunity to roll into a subsequent multi-dose cohort and, eventually, it might be converted to an open-label study to allow patients who had received the placebo to switch to the ASO treatment.

The multiple-dose trial would last approximately 3 months, in which subjects would receive an ASO dose once a week, for three weeks, to load up the tissue, followed by monthly doses for maintenance. Depending on the results, the subjects might be offered an extension to receive additional doses. Bennett noted, however, that the focus of these initial trials will be on safety, not efficacy. He also stressed that a key part of the studies will be the use of robust biomarkers to track disease status and the effects of treatment. So far, the team has favored motor endpoints, such as rotarod performance in mice, but is very interested in using other, quantifiable biomarkers for both preclinical and clinical studies.

Participants agreed on the importance of sensitive, robust and reliable biomarkers for clinical trials. In particular, they stressed the desirability of measuring huntingtin expression levels in the brain to confirm that the treatments are acting upon their intended target. As noted by Young, researchers at Novartis are currently working on this and, at the HD2010 meeting, Miriam Moscovitch-Lopatin described how her team is now able to quantify huntingtin protein levels, as well as the ratio of abnormal to total huntingtin protein, in buffy coat samples from peripheral blood. Rosas noted that measuring huntingtin levels in CSF reliably, however, has proven difficult. Efforts to overcome these difficulties are currently underway. Young wondered if nanoparticles could be used as labels to monitor the location of treatment agents, such as the viral vectors used for shRNA delivery.

The value of learning from research in other diseases was also discussed. For example, Kordasiewicz and Bennett noted that they plan to apply the lessons they learn from their ongoing phase I trials using anti-SOD1 ASOs for amyotrophic lateral sclerosis (ALS) to subsequent HD trials. The researchers are focusing on safety at this stage, and hope that many lessons will transfer to HD. As noted by Housman, the rapid disease progression of ALS will probably result in a rapid collection of results which can be helpful for the development of ALS treatments, as well as others, including HD. Bennett agreed, but clarified that the HD study will proceed even if the assessment of the efficacies of the other treatments is not yet complete. One of the lessons already learned, noted Bennett, is that the FDA review process is time-consuming and causes interruptions in the studies. If possible, future studies could be set up to deal with these assessments more efficiently. As noted by Jang-Ho Cha, it will be important to include FDA officials in clinical trial planning sessions.

Despite the pre-clinical studies and the above-mentioned strategies to maximize the chances of success, the initial clinical trials may fail, as commonly occurs in drug development. A key question then, posed by Cha, is: What will be done in this situation? What analyses will be conducted to dissect the reasons why the treatment didn't work? To answer this question, Housman urged participants to think about past failures and the lessons derived from them. For example, he mentioned the anti-apoptotic drug CEP-1347 which was tested for Parkinson's disease (PD) and failed in clinical trials. Failure was attributed, in part, to the fact that the animal models used in pre-clinical studies did not recapitulate the human disease closely enough. As noted by Michael Levine, however, there are several animal models of HD that mirror the human disease very well, so the risk is not as great as it is in PD. In addition, it was unclear whether CEP-1347's target, apoptosis, was appropriate for PD, whereas there is no question about abnormal huntingtin expression representing a valid target for HD.

Aronin added that the risk of clinical trial failure is intrinsic to all drugs tested, and the best way to reduce this risk, and analyze failures if they occur, is to carefully monitor anatomy, behavior, huntingtin levels, etc. over time in animal models to get the best possible image of the drug's effects before moving into the clinic. On the other hand, noted Diamond, in the case of gene silencing, it might be more fruitful to use mice to simply optimize the knock down procedure without assessing other outcomes because there is no question that the abnormal huntingtin gene is a valid target, but the responses of mice to the treatment may or may not be predictive of human responses.

In the end, participants agreed that perhaps the most important effort to maximize the chances of learning from and overcoming clinical trial failures is the development of sensitive, reliable biomarkers. As noted above, an assay to measure huntingtin brain levels is highly desirable. Additional biomarkers, that accurately track disease progression and help link animal studies with human clinical trials, are also expected to help, not only for monitoring the effects of gene silencing agents, but for monitoring other candidate treatments (see *Bridging the Gaps Between Cell Studies, Animal Studies, and Human Studies*).

Candidate Treatments: Reducing Abnormal Huntingtin Protein

Although not as close to clinical testing as gene silencing, enhancing the degradation of abnormal huntingtin protein in cells is another promising avenue of research. Cells have two endogenous mechanisms to degrade huntingtin: the proteasome pathway and macroautophagy. As noted by Ana María Cuervo, it is impossible to exactly determine the relative contributions of the two processes, because their relative activities change under different circumstances and vary across cell types. In addition, experimentally separating the two processes can be difficult because they are interrelated. As noted by Joan Steffan, recent reports indicate that several proteins play roles in both proteasomal and autophagic degradation. In addition, inhibiting one pathway can affect the activity of the other. Despite this complexity, studies from Steffan, Brigit Riley, and others

suggest that the proteasome and autophagic pathways are both recruited and contribute in similar degrees to the degradation of abnormal huntingtin.

Enhancing Huntingtin Degradation through the Proteasome Pathway

As explained by Eric Reits, mammalian proteasomes are a primary site for the degradation of misfolded proteins, including abnormal huntingtin. However, they are incapable of digesting polyglutamine sequences. Fred Goldberg and colleagues have shown that the undigested polyglutamine chains are released into the cytoplasm where they can accumulate and form toxic aggregates, particularly when they are greater than approximately 40 glutamines in length. The aggregates formed have a polyglutamine seed, inactive proteasomes within the core, and ubiquitinated proteins, huntingtin fragments, and other polyglutamine proteins in the periphery. (Al LaSpada wondered why this phenomenon is not observed with other expanded polyglutamine proteins which are toxic, but readily degraded by the proteasomal pathway. Reits pointed out that the molecular context of the glutamines might be important and Rick Morimoto added that distinct cellular environments may affect aggregation differentially.)

One type of proteasomes, however, the immunoproteasome— assembled with distinct subunits in response to pro-inflammatory signals such as interferon γ —is not sequestered in huntingtin aggregates and remains active, noted Reits. Labeled immunoproteasomes can be seen coming on and off of aggregates and appear to cluster around the edges of larger aggregates. Of particular interest, overexpressing one of the subunits that is a specific component of immunoproteasomes, the multicatalytic endopeptidase complex-like-1 (MECL-1), results in improved degradation of polyglutamine and huntingtin exon 1. Reits noted that MECL-1 can degrade polyglutamines as part of the proteasome or as an independent dimer residing in the cytoplasm. Johnson added that immunoproteasomes are overexpressed in mouse and human HD brains. However, whether this overexpression results in more efficient degradation of polyglutamines, noted Reits, is unknown.

Reits has also examined the role of non-proteasomal cytoplasmic peptidases in the breakdown of polyglutamine peptides. He noted that puromycin-sensitive peptidase (PSA) and the tripeptidyl peptidase TPPII are capable of cleaving polyglutamine chains released by proteasomes. As described in the HD2010 meeting by Raphael Hourez, PSA appears to, not only degrade polyglutamine chains released by proteasomes, but to promote autophagy. These surprising findings indicate biochemical crosstalk between the proteasomal and autophagic pathways (see *Enhancing Huntingtin Degradation through Macroautophagy*). Aronin noted, however, that he has failed to see any differences in the behavior of HD cells that lack a PSA allele compared to HD cells expressing a normal dose of PSA. It is possible that PSA's autophagic role is only triggered when PSA is highly overexpressed.

Focusing on TPPII, Reits has observed that overexpressing this peptidase results in a decrease of huntingtin aggregate formation. Reits plans to search for ways to enhance the oligomerization, and thus the activity, of TPPII. In addition, as described in the HD2010 meeting, Reits is testing whether TPPII activity can

be increased by chaperones that prevent polyglutamine oligomerization because monomeric polyglutamines are better substrates for TPPII. Indeed, expression of chaperones DnaJB6 and DnaJB8 prevented aggregation and, in the case of DnaJB6, reduced the levels of soluble polyglutamine peptide. Blocking endogenous peptidases prevented the effect, further supporting the proposal that chaperones and peptidases can work together to eliminate toxic polyglutamine fragments.

Extending this work, Reits is setting up screens to search for additional compounds that decrease polyglutamine peptide aggregation. He is using N2A cells that express constructs encoding polyglutamine peptides with a degron to promote degradation. The peptides are expressed in an inducible manner and a fluorescein arsenical hairpin binder (FLASH) system is being used to label the aggregates and quantify their number and size by Förster Resonance Energy Transfer (FRET) or Fluorescence Lifetime Imaging Microscopy (FLIM).

Enhancing Huntingtin Degradation through Macroautophagy

Macroautophagy—a pathway for the bulk degradation of protein aggregates and organelles TKTK—is another target for enhancing the clearance of abnormal huntingtin. Ai Yamamoto explained that autophagy has been shown to target both soluble huntingtin oligomers and aggregates up to 1 μ m in diameter, as revealed by immunoelectron microscopy. Cuervo added that large aggregates are usually surrounded by many autophagosomes. As noted by Yamamoto, macroautophagy is usually a very efficient process, but the system can be overwhelmed when aggregates, which can take days to degrade, start to accumulate.

In an attempt to boost autophagic activity in cell and animal models of HD, David Rubinzstein and colleagues administered rapamycin, an inhibitor of the serine/threonine kinase mTOR which regulates autophagy. Although the results were initially encouraging, the lack of specificity of this approach complicated its interpretation, and raised safety concerns. In addition to regulating autophagy, mTOR regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis and transcription.

Thus, the goal now is to find ways to *selectively* enhance the autophagy of protein aggregates. Participants discussed several signals that have been suggested to promote this type of autophagy, including ubiquitination, oligomerization, acetylation, and SUMOylation.

Oligomerization as a signal for selective autophagy and the involvement of the Nrf2 stress response in aggregate pathology

A central topic of discussion was a recent article by Riley and co-workers in which autophagy and aggregate pathology were examined. As summarized by Riley, the presence of ubiquitin in inclusions and the finding of ubiquitin-positive inclusions in the brains and livers of autophagy-deficient mice, had led to the proposal that ubiquitin chains, in specific topological arrangements, might serve as selective signals for

autophagy. The leading candidate for this recognition is p62, a protein that is also found in inclusions, and contains a ubiquitin association domain (UBA) and a recognition sequence for LC3, an autophagosomal protein.

However, recent data have called into question how much, or even whether, this ubiquitin-based process contributes to huntingtin degradation. As noted by Marian DiFiglia, ubiquitin co-localizes with huntingtin in the aggregates of both HD mice and patients. However, in transgenic animals, both DiFiglia and Riley have seen a delay between the arrival of huntingtin and the arrival of ubiquitin to the aggregates, indicating that the two are not traveling together. Also, as noted by Reits, ubiquitin is usually found only around the periphery of aggregates, suggesting that most of the huntingtin protein in aggregates is not ubiquitinated. In addition, Riley's mass spectrometry studies show that less than 1% of huntingtin is bound to ubiquitin in both R6/2 and human brain tissue (although Mary Kennedy cautioned that the ubiquitinated peptide may be difficult to detect because of ineffective volatilization).

Providing additional evidence for a lack of correlation between ubiquitin and selective autophagic clearance, in their recent paper, Riley and colleagues showed that shutting down autophagy using an inducible genetic system, leads to the accumulation of all types of ubiquitinated topologies. This suggests that there is no specific ubiquitin topology that acts as an autophagy signal. Furthermore, p62 levels did not increase in parallel with ubiquitin chains in autophagy-deficient mice, nor did the researchers find significant amounts of p62 bound to ubiquitin (although as noted by Yamamoto, p62's interaction with ubiquitin is non-covalent and dynamic). Consistent with these findings, Steffan noted that ATG-19, the protein in yeast that mediates selective autophagy and is homologous to p62, does not even contain a UBA domain.

In addition to weakening the ubiquitin-based model of selective autophagy, Riley's recent study suggests an alternative signal for driving autophagic substrate selection: oligomerization. Using a flow-cytometry-based assay to monitor selective autophagy in cell culture, Riley reported that inactivation of autophagy results in the selective accumulation of aggregation-prone proteins and does not correlate with an increase in substrate ubiquitination. The assay relies on cells expressing inducible inhibitors of autophagy, as well as bicistronic constructs comprised of two labeled proteins: a test protein (e.g., abnormal huntingtin) and a reference protein (e.g., wildtype huntingtin or cherry fluorescent protein). Because the synthesis of both labeled proteins is controlled by the same promoter, any differences in amounts can be attributed to differences in rates of degradation. The researchers used two-color flow cytometry to monitor the ratio of relative increments in the two fluorescent labels in response to the inhibition of autophagy. Thus, they were able to assess the autophagic selectivity of different substrates. Their results indicate that, when autophagy is inhibited, an aggregation-prone fragment with an expanded polyglutamine stretch accumulates relative to monomeric proteins.

Steffan noted that oligomerization may also play a role in normal protein clearance. She explained that her examination of wildtype huntingtin by Western blots shows that an N-terminal fragment of huntingtin, possibly the caspase-6 fragment, is present as an SDS-resistant oligomer which is likely cleared by selective autophagy.

Riley's study also provides a possible explanation for p62's involvement in aggregate pathology. In addition to its proposed role in autophagy, p62 plays a key role in stabilizing the transcription factor Nrf2 which acts as a master regulator of a protective pathway against oxidative and electrophilic stress. Thus, Riley and colleagues reasoned that the presence of p62 in inclusions could be due to this latter role, rather than, or in addition to, its role in autophagy. In this case, disrupting autophagy should result in an accumulation of p62, which is normally cleared by autophagy, and a consequent stabilization of Nrf2. Indeed, the researchers found that when they genetically combined the autophagy inducible knockout with a p62 or Nrf2 knockout, the cells fared better. They also observed that the accumulation of poly-ubiquitin chains was abrogated, suggesting that this accumulation is an indirect consequence of p62-mediated activation of the Nrf2 stress response.

Participants discussed technical questions, as well as the implications and potential future directions of Riley's findings. For example, Judith Frydman asked how the genetic inhibition of autophagy in Riley's flow cytometry experiments might affect proteasome function. Riley noted that there did not seem to be much of an effect—inhibiting each clearance pathway resulted in additive effects. Participants also wondered if the aggregate and soluble forms of huntingtin could be tracked independently. Riley clarified that her assay does not distinguish between these protein forms because it is based on flow cytometry and her study did not include microscopy or filter-trap experiments. Participants also noted that care should be taken when using fluorescent protein labels to study autophagy. As noted by Reits, these labels can affect the rate of autophagy in a size-dependent manner. In addition, Steffan noted they can affect nuclear-cytoplasmic distribution. Furthermore, she pointed out that GFP, in particular, can affect the activity of the I-kappa-B kinase (IKK), which is involved in regulating autophagy.

Looking ahead, Riley noted that her team is interested in further examining the role of Nrf2 and how it might influence cell toxicity caused by autophagic shut down. In particular, they would like to investigate the effects of knocking down both p62 and Nrf2 simultaneously. Moreover, the team plans to extend some of their work to study other neurodegenerative disorders, such as Alzheimer's disease.

Yamamoto noted that it might also be interesting to analyze Keap1's interaction with p62. Keap1 is the adaptor protein which normally mediates the ubiquitination of Nrf2 and its subsequent degradation. The interaction of excess p62 with Keap1 is thought to interfere with Nrf2 degradation. Another interaction worth examining, noted Cuervo, is how p62 might affect proteasome activity. Although Riley did not observe significant binding of p62 to ubiquitin, Cuervo noted that other studies have shown that p62 can compete with the proteasome for ubiquitin binding.

Several other observations that relate to Riley's findings were discussed. For example, Cuervo noted that crossing a macroautophagy-deficient mouse with a p62 knockout resulted in improvement in liver, but not brain, pathology. Riley noted that there is a great degree of variability between the responses of different brain regions, so that improvements in certain areas could be masked by global assessments of pathology. Yang added that the effects of Nrf2 activity in the brain are complicated, involving cell-cell interactions. Astrocytes

express Nrf2 at much higher levels than neurons and, thus, neurons can be affected indirectly by astrocytic changes in Nrf2 activity. Yet another observation, contributed by Yamamoto, is that knocking down p62 by itself appears to result in the loss of large aggregates in HD cells. Riley noted that she observed a drop in ubiquitin levels under these circumstances.

Challenges in the development of autophagy-based therapies

Participants discussed the challenges that lie ahead in the development of therapeutic candidates based on the above mentioned findings and considerations. For example, Cha asked how specificity will be achieved, given that there is no known signal to induce the specific autophagy of abnormal huntingtin. Yamamoto explained that it should not be necessary to target abnormal huntingtin as an individual protein. By driving autophagic activity towards the disposal of aggregated proteins, abnormal huntingtin will be more efficiently eliminated, along with other proteins that share the same autophagic signals and which are also destined for degradation. Because these other proteins, including fragments of wildtype huntingtin as previously mentioned, are already tagged for degradation, their enhanced disposal should not pose a problem.

The challenge, however, will be to activate selective autophagy moderately so that it does not recruit an excessive amount of the basic components of the autophagic machinery, which could affect other forms of autophagy and significantly perturb protein synthesis levels. Cuervo noted that improving the efficiency, rather than the magnitude, of selective autophagy could help circumvent these problems. Also, shunting the degradation of abnormal huntingtin aggregates to other autophagy pathways might prove helpful. Cuervo mentioned a recent study by Nukina and colleagues in which constructs containing HSC70 motifs and polyglutamine stretches were directed to lysosomes through the chaperone-mediated autophagy (CMA) pathway. Although as explained by Yamamoto, the goal of this study was to demonstrate that polyglutamines can be digested within lysosomes, Cuervo noted it also suggests the possibility of manipulating autophagic pathway selection. Huntingtin contains many HSC70 motifs, but they do not normally direct huntingtin to the CMA pathway. Whether the motifs can somehow be made to facilitate CMA-mediated degradation, remains to be determined.

Another complication is that autophagy appears to be disrupted in HD. Cuervo's team, for example, has identified a deficiency in autophagosomal cargo recognition in HD which may be due to interference caused by abnormal huntingtin residing in autophagosomal membranes. As noted by LaSpada, trying to activate or enhance the efficiency of a damaged system may prove difficult. Treatments that seek to enhance autophagy must be delivered well before the system is too damaged or too blocked to respond to stimulation.

In addition, work from several labs, including Steffan's and Scott Zeitlin's, indicate that wildtype huntingtin may itself play a role in the mechanism of selective autophagy. Indeed, as noted by Steffan, huntingtin shares sequence homology with the Atg-11 autophagic cargo recognition protein. Thus, enhancing huntingtin clearance could itself cause a deficit in autophagic function. However, because the therapeutic goal is

to only target selective autophagy of oligomerized proteins, the levels of intact, wildtype huntingtin should not be affected. In addition, Boudreau noted that, so far, the knock down of wildtype huntingtin in gene silencing experiments has failed to reveal any obvious autophagic problems and Cuervo observed only a moderate decrease in autophagy when she reduced wildtype huntingtin levels.

Participants also discussed the complexity of manipulating the Nrf2 pathway for therapeutic purposes. As noted by Yang, the activation of Nrf2 has been proposed as a potential therapeutic candidate for neurodegenerative disorders, including HD, because of its protective role against oxidative stress. Yet Riley's data suggest that long-term activation of Nrf2 is likely to be toxic. Johnson noted that, in one set of experiments in mice, constitutive expression of Nrf2 in astrocytes improved the phenotype of ALS, but not of HD.

Promising directions in the development of autophagy-based therapies

Despite these challenges, participants described several encouraging advances in the development of candidate therapies. For example, Yamamoto noted that her team has succeeded in selectively increasing aggregate clearance by driving the expression of Alfy, a protein that appears to be rate-limiting and specific for aggregate clearance. Alfy is a phosphatidylinositol 3-phosphate (PI3P) binding protein which interacts with p62 and the autophagosomal protein Atg5 to coordinate target recognition with site-specific activation of autophagy.

Another promising handle on boosting the clearance of abnormal huntingtin was described by Steffan who pointed out a recent publication by Dmitri Krainc and colleagues showing that the acetylation of abnormal huntingtin tags it for clearance by autophagy. Steffan added that her group has found that nicotinamide can increase the acetylation of substrate proteins and their subsequent degradation, including abnormal huntingtin. Furthermore, Steffan noted that poly-SUMOylation is another regulator of huntingtin clearance which might also provide a target for manipulation. In addition, other candidates for enhancing huntingtin clearance may soon emerge from Riley's flow cytometry assay which provides a robust system for screening molecules that enhance autophagic selectivity. The researchers are currently setting up to screen drug libraries from Elan.

Moreover, to enhance the efficiency of autophagy and correct the apparent disruption caused by abnormal huntingtin, Cuervo's team is searching for peptides to block abnormal huntingtin's interference with autophagosomal cargo recognition.

A means of monitoring autophagy levels, which will be critical for testing autophagy-based therapeutic candidates, was also discussed. Seeking to identify biomarkers of autophagy, Riley described a promising strategy using stable isotope labeling by/with amino acids in cell culture (SILAC). SILAC is a mass spectrometry-based technique that detects differences in protein abundance between cell samples using non-radioactive isotopic labeling. Although, as noted by Cuervo, other researchers have previously used SILAC to compare the protein profiles of normal cells versus those with autophagy gene knockouts, Riley plans to use cells from mice in which autophagy can be turned off genetically in an inducible manner. Participants

encouraged Riley to use neuronal cells, in particular primary neurons, given that neurons are long-lived, post-mitotic cells with unique re-modeling and protein turnover requirements.

Finally, Paul Patterson described an alternative means to achieve the degradation of abnormal huntingtin: through the use of anti- abnormal huntingtin antibodies that are expressed intracellularly (intrabodies), Intrabodies mediate protein degradation through calpain and, in contrast to autophagy-based approaches, they can be readily tailored to target specific proteins. Studies from Patterson's lab and others indicate that anti- abnormal huntingtin intrabodies can completely eliminate the soluble protein and greatly decrease aggregates. Furthermore, when delivered to the striatum using AAV vectors, intrabodies improve pathology, behavior and lifespan in several models of HD.

Candidate Treatments: Manipulating Abnormal Huntingtin to Reduce its Toxicity

Opportunities for manipulating aggregation

Modulating abnormal huntingtin's propensity to form aggregates is another strategy with potential therapeutic value. As noted by Frydman, molecular chaperones are important modulators of the aggregation and toxicity of expanded poly-glutamine proteins, and may serve as therapeutic targets. Her team has focused on the hetero-trimeric chaperonin TRiC (also known as CCT) which was identified as a potent suppressor of huntingtin aggregation and toxicity in an RNAi screen for modifiers of huntingtin toxicity. Frydman first observed that the ability of TRiC to suppress aggregation seemed to be mediated by its direct binding to huntingtin because purified TRiC prevented the aggregation of purified huntingtin exon 1 in vitro. Also, Frydman observed a direct interaction of the proteins in yeast cells.

Frydman's team has now dissected the molecular interactions between TRiC and huntingtin, characterizing the different players and how they interact. TRiC is a double-ring complex, where each ring is composed of eight subunits. Of these subunits, only 2 appear to interact directly with huntingtin and mediate in vivo effects when overexpressed. A single subunit, CCT1, however, appears to be the key recognition site for huntingtin, as it can single-handedly inhibit aggregation in vitro and suppress toxicity in vivo. Using cross-linking experiments, Frydman identified the N-terminal portion of huntingtin, N17, as the major site of interaction with CCT1 (huntingtin's polyglutamine region appears to be only a minor interaction site). N17 exists as a coil-coil alpha-helical structure and seems to normally interact with itself and with polyglutamines. It is also known that N17 enhances huntingtin aggregation and its absence slows aggregation kinetics.

Consistent with these findings, Yang noted that his team has identified TRiC as a huntingtin binding protein in interactome studies and mapped the binding to N17. Using an antibody against polyglutamine, Yang co-precipitated huntingtin and CCT1, but not in abnormal mice tissues expressing huntingtin without the N17 portion. Together, these observations are particularly significant as they confirm Frydman's yeast-based results in mammalian tissues.

Frydman's current model is that TRiC interacts with N17 and interferes with its ability to facilitate aggregation in both wildtype and abnormal huntingtin, thus disrupting the oligomerization process at an early stage. Interestingly, if the hydrophobic region of N17 that binds to TRiC is mutated to alanines, no aggregates form at all, even if the constructs include an expanded polyglutamine stretch. This effect is greater than the decrease in aggregation caused by eliminating N17 altogether. Also, Frydman noted that adding N17 in trans enhances aggregation.

Participants discussed future challenges and opportunities to extend this work. For example, Rick Morimoto encouraged the characterization of additional sites of TRiC-huntingtin interactions. He considered it unlikely for a chaperone to interact with only one or two sites in the entire huntingtin protein. However, Frydman noted that few chaperones appear to interact with huntingtin. In addition, by electron microscopy, it appears that TRiC interacts only at the N17 site and one other point along the huntingtin protein. Frydman added that she is interested in examining the CCT1 binding domain and how it reduces toxicity. She hopes to probe the structural interaction of CCT1 and N17 using techniques such as mutagenesis and nuclear magnetic resonance (NMR) spectroscopy.

Participants also discussed the importance and difficulties of extending Frydman's findings to in vivo mammalian systems. Paul Muchowski noted that N17 is probably associated with membranes in vivo which may affect TRiC interactions. Furthermore, Diamond noted that experiments from his lab indicate that CCT1 overexpression suppresses aggregation of both exon 1 *and* exon 1 lacking the N17 sequence. This is in contrast to Frydman's in vitro studies showing no effect of CCT1 when N17 is missing. As pointed out by Diamond and Kennedy, the effect observed by Diamond could be indirect. For example, CCT1 overexpression might shift the entire balance of the in vivo chaperone network.

Participants agreed that correlating in vitro observations with in vivo studies will be challenging, but constitutes an important next step. Of particular interest, Yang noted that he has mice that overexpress CCT1 which could be crossed with BAC-HD mice. Yang also has mice expressing abnormal or wildtype huntingtin that lack the N17 sequence. Frydman, who has been searching for models to study toxicity in vivo, was encouraged by the availability of these tools. Also, Yang noted his team can create mouse models to perform in vivo mutagenesis analyses to help dissect the molecular interactions between N17, polyglutamines, and CCT1. The establishment of these in vivo mammalian models should also help lay the groundwork for Frydman's future plans to test peptidomimetics of CCT1 as potential therapeutic candidates. As noted by Morimoto, however, it will be important to ensure that such an approach doesn't interfere with TRiC's normal role folding polypeptides. So far, said Frydman, overexpressing CCT1 does not seem to result in dominant-negative effects. It is possible, however, that at higher doses, such effects could surface. Frydman suggested organizing a workshop to discuss chaperones and their interactions with N17, or more generally, as suggested by Diamond, to discuss huntingtin's interactions with chaperones and other related proteins.

The Role of Cellular Location

Participants also discussed the role of cellular location in the toxicity, aggregation, and degradation of abnormal huntingtin. Frydman described her work in yeast characterizing two quality control compartments, JUNQ and IPOD, that appear to play distinct roles in handling damaged or aggregated proteins. The ‘juxtannuclear quality control’ (JUNQ) appears to handle misfolded, but soluble, proteins that can exchange with the cytoplasmic pool, and includes proteasomes. On the other hand, the ‘insoluble protein deposit’ (IPOD) contains non-diffusing, aggregated substrates and is located near vacuoles and autophagic vesicles. Both compartments are close to the nucleus in most cells and both rely on chaperones and, in mammals, microtubules, for their formation. However, the set of chaperones involved in the formation of each is different, although some chaperones participate in both. In addition, each compartment evokes differential transcriptional responses and has different kinetics of protein localization.

As explained by Frydman, there are several factors, besides solubility, that seem to determine where a particular substrate will be directed. Several amyloidogenic proteins, such as Rnq1 and abnormal huntingtin, can be found in the IPOD. However, toxic forms of these proteins appear to be preferentially shuttled to the JUNQ. For example, Frydman has observed a toxic huntingtin construct that lacks the proline stretch in the JUNQ. Similarly, Rnq1 is directed to the JUNQ when it becomes toxic by ubiquitination.

A key question, posed by Diamond, is whether proteins are sent to the JUNQ because they are toxic, or become toxic because they are sent to the JUNQ. Indeed, as noted by Johnson, some proteins can be toxic in one cellular location, while being harmless in another. Diamond added that a protein’s conformation might be a key determinant of its destination. The question is complicated because each of a protein’s features—conformation, post-translational modifications, toxicity, and location—can be affected by the other features. Participants agreed that unraveling the causal relationships will be challenging. As noted by Housman, the use of prion proteins, which adopt toxic and non-toxic configurations, might be helpful to probe these questions.

Another important next step is to examine the compartments in mammalian neurons. DiFiglia noted that she has published work describing how abnormal huntingtin in striatal cells accumulates in “huntingtin bodies” which then converge to reside in the perinuclear region. Huntingtin constructs lacking the proline domain, however, were found in huntingtin bodies that remained dispersed in the cytoplasm.

The effects of the formation of the JUNQ and IPOD compartments on the rest of the cell are also of interest. As noted by Frydman, large amounts of chaperones converge at these locations, which might disrupt the function of other parts of the cell. It is possible, for example, that this chaperone sequestration contributes to the mitochondrial deficits associated with HD. Also, as noted by Morimoto, the activity of the cell’s chaperone network is finely tuned—compromising or altering the function of a single chaperone can disrupt the function of the whole network.

In addition to these compartments, Yang noted the importance of the nuclear-cytoplasmic partitioning of huntingtin. Different types of huntingtin aggregates reside in the nucleus and cytoplasm of HD cells and some

studies have suggested that nuclear aggregates are particularly toxic. Summarizing his recent experiments with BAC-HD mice expressing huntingtin constructs lacking the first 17 amino acids, Yang noted that these mice show earlier nuclear aggregation and have more severe motor deficits. Thus, nuclear aggregates are worth examining in more detail and, as noted by Yang, are likely to include various different types. Based on these open questions, Johnson suggested organizing a workshop to discuss huntingtin's spatial localization.

Candidate Treatments: Downstream Targets

Participants also discussed a few therapeutic targets downstream of abnormal huntingtin. Although target validation becomes more challenging as one moves farther from the primary cause of HD, participants identified several downstream targets with promising therapeutic potential.

Kynurenine Pathway

As explained by Muchowski, one way in which abnormal huntingtin affects neuronal cells indirectly is by activating the kynurenine pathway in immune cells. The kynurenine pathway mediates the degradation of tryptophan and production of nicotinamide adenine dinucleotide (NAD). There are several lines of evidence that link this pathway to HD pathology. For example, one of the kynurenine metabolites, quinolinic acid, causes HD-like lesions when injected into the brain. Furthermore, both quinolinate and 3-hydroxykynurenine (3-HK), another metabolite of the pathway, are elevated in mouse and human brains at early stages of disease. Thus, the enzyme that leads to the generation of these metabolites, kynurenine-3 monooxygenase (KMO), has emerged as a promising therapeutic target for HD.

Although several compounds have been developed to inhibit KMO (KMO is also a target for stroke), Muchowski noted that none of these inhibitors are very effective. Consequently, Muchowski began collaborating with his father, a chemist, to design a better inhibitor. One of the compounds they have developed, JM6, has shown promising results in models of neurodegenerative disease, including HD. In R6/2 mice, Muchowski's team observed neuroprotective effects and a 30-40% increase in longevity. They also observed moderate effects on motor symptoms and a dramatic amelioration of synaptic loss. Similarly, in a mouse model of Alzheimer's disease, the researchers observed neuroprotection, a rescue of spatial memory, ameliorated disinhibition behaviors, and the complete rescue of synaptic loss in several brain areas.

Looking into the mechanism by which JM6 achieves these beneficial effects, Muchowski discovered that, surprisingly, JM6 does not reach the brain. Instead, it appears to mediate its effects by inhibiting KMO in peripheral immune cells. This inhibition causes an increase in kynurenic acid, a metabolite derived from kynurenine, the substrate of KMO. Kynurenic acid is neuroprotective and readily crosses the blood-brain barrier (BBB). In the brain, it inhibits the $\alpha 7$ acetylcholine receptor and, at high doses, acts as a broad-range inhibitor of glutamate receptors. These effects can be helpful for HD given that, as reported by George Rebec, HD

pathology is ameliorated by decreasing glutamate stimulation. Also, increasing kynurenic acid helps normalize the levels of this compound which are low in HD brains. Muchowski added that JM6 can affect the brain quickly—within minutes of its administration, kynurenic acid increases in the brain as assessed by in vivo microdialysis.

The findings made participants re-consider the potential role of immune cells in HD. Muchowski noted that immune cells are affected in HD and abnormal huntingtin appears to upregulate the pathway through transcriptional changes.

Participants also discussed future steps to clinically evaluate JM6, as well as new versions of JM6 currently under development which are BBB-permeable. Johnson considered that the research is at a point in which extensive pre-clinical testing should be performed to prepare for clinical trials. He emphasized that reaching the clinic with a large amount of solid, animal data should maximize the efficiency of clinical testing. Muchowski noted that his team is currently evaluating mice derived from a cross between a KMO knockout and an R6/2 mouse. The researchers have, so far, observed moderate improvements in motor deficits, but have yet to examine brain pathology and cognition. The team is also preparing to study a conditional KMO knockout to avoid the complexity of interpreting results that might be affected by developmental compensation. A surprising indication that such compensation may indeed be occurring is the observation that KMO knockout mice appear to learn better than wildtype mice.

Additional experiments to characterize the effects of blocking KMO were suggested by other participants. For example, DiFiglia recommended doing more studies to link physiology and kynurenine metabolite levels in the brain, and Ali Khoshnan suggested assessing the effects of knocking out KMO on the immune system.

Muchowski also noted that his team recently received an NIH grant for performing safety and toxicological studies over the next 2-3 years. Establishing dosing parameters will be particularly important because there are several immune functions that can be disrupted when kynurenine metabolites are altered. For example, administering kynurenine systemically, noted Muchowski, can cause immunosuppression.

Regarding outcome measures, Muchowski noted his team is particularly interested in monitoring the effects of KMO inhibition on cognitive symptoms. So far, their tests in mice are encouraging. However, in humans, noted Cha, it will be challenging to detect an arrest in cognitive decline, particularly in HD which is so variable. Muchowski agreed, but noted that, according to Jane Paulsen, the cognitive decline that occurs over a single year can be measured reliably, at least in young patients. Furthermore, in this case, researchers will have the ability to monitor the metabolites associated with the inhibitors' activity, which should facilitate the establishment of correlations between drug activity and symptom severity.

Another approach to protect neurons from the harmful effects of abnormal huntingtin is to stimulate the activity of peroxisome proliferator-activated receptor gamma co-activator 1 α (PGC-1 α). As described by LaSpada, PGC-1 α relieves oxidative stress by inducing the transcription of stress-related genes, including those involved in the reactive oxygen species (ROS) defense system. Summarizing how his team identified PGC-1 α as a potential HD target, LaSpada said the researchers first noticed that HD mice (N171-82Q) become tremulous and their body temperatures drop as their disease advances. They also noticed that warming the animals extended their lifespan.

Tracking down this thermoregulation deficiency, LaSpada and co-workers found that the expression of uncoupling protein 1 (UCP-1)—a protein that helps generate heat by uncoupling oxidative phosphorylation in brown fat—is abnormal in HD mice. UCP-1 expression is regulated by PGC-1 α and when the researchers performed microarray analyses of the HD mice, they observed a downregulation of 24 PGC-1 α -regulated genes. The team then created a PGC-1 α -inducible system to test the effects of over-expressing PGC-1 α in HD mice. When the inducible construct, under the regulation of the ubiquitous promoter Rosa 26, was turned on, PGC-1 α expression increased 2-3 times in the cortex, striatum and muscle. This upregulation correlated with significant improvements in several motor and behavioral tasks, the elimination of aggregates, and increased neuronal numbers. The researchers also observed a normalization of the expression of mitochondrial and oxidative phosphorylation genes. However, the team did not observe an extension of survival, probably because the inducible construct did not alter PGC-1 α expression significantly in brown fat.

Experimenting with cultured cells, LaSpada and colleagues have found that PGC-1 α appears to mediate its effects by relieving oxidative stress. Furthermore, PGC-1 α 's effects on aggregation reduction appear to be dependent on both proteasomes and autophagy.

Consistent with these findings, Yang has observed increased markers of oxidative stress in BAC-HD mice which correlate with increased aggregate formation and cell death. In addition, Rosas has recorded low body temperatures in HD patients and observed increases in several markers of oxidative stress. Rosas added that there are several indicators of oxidative stress that can be monitored in blood and should be very useful as biomarkers for clinical trials. Rectal body temperature could also serve as a biomarker. To determine which of these candidates will be most useful, however, it will be important to elucidate the time course of each of the alterations.

Participants asked several questions about the role of PGC-1 α , its effects, and regulation. For example, Frydman asked if PGC-1 α is involved in cells' responses to other types of stress, such as heat shock. And Rosas wondered about a relationship between PGC-1 α and hypothyroidism, which she sometimes observes in HD patients. Moreover, Young and Yamamoto asked about the effects of downregulating or upregulating PGC-1 α in wildtype animals. As noted by LaSpada, mice that underexpress PGC-1 α are thin, hyperactive, and suffer from degeneration in the brain, not specific to the striatum. Overexpressing the protein, on the other hand, results in cardiomyopathy.

LaSpada's future plans include investigating the mechanism of PGC-1 α dysregulation in HD and other neurodegenerative diseases. A recent meta-analysis of 17 studies on Parkinson's disease using Gene Set Enrichment Analysis (GSEA), for example, revealed a coordinated decrease in the expression of hundreds of genes regulated by PGC-1 α . It is known that PGC-1 α is a very dynamic protein, but the mechanistic details of its regulation are not well understood.

Moreover, LaSpada described ongoing efforts that may yield therapeutic opportunities. For example, several research groups are developing drugs to target the protein that works with PGC-1 α to regulate transcription, peroxisome proliferator-activated receptor gamma (PPAR- γ). Much effort and resources are being invested in these projects because the activities of PPAR- γ and PGC-1 α are relevant to the pathology of several diseases, including diabetes. One of these drugs, noted LaSpada, has already been tested in HD by Jenny Morton. Although the results of this particular test were negative, other drugs might prove effective. For example, LaSpada is interested in targeting PPAR- δ , a relative of PPAR- γ which is specifically expressed in the brain. To pursue this candidate, LaSpada has set up a collaboration with a company that recently developed a PPAR- δ activator.

Stem Cell Transplants

Another therapeutic strategy discussed by participants was the delivery of neuroprotective molecules to vulnerable brain regions using stem cell transplants. As explained by Leslie Thompson, stem cells can serve as vectors for delivering trophic factors and anti-inflammatory cytokines. As discussed at the HD2010 meeting, several recent studies indicate that stem cell transplants in HD help alleviate pathology mostly through nursing effects. The transplantation of mesenchymal stem cells (MSC) derived from bone marrow or adipose tissue has yielded encouraging results. Studies in R6/2 mice, for example, have shown that stem cell transplants can have beneficial effects on behavior and synaptic function.

Thompson is now setting up to test the effects of transplanting human stem cells into mice, with the support of a grant from the California Institute for Regenerative Medicine. Thompson will test several kinds of clinical-grade stem cells—including mixed progenitors, astrocyte precursors, and neural precursors derived from human embryonic cells—to help assess their relative therapeutic potentials.

Participants discussed some of the challenges associated with this approach. For example, Johnson noted that the transplants in mice will be heterologous (across species) and will thus elicit rejection reactions. Thompson explained that her team will use standard immunosuppression treatments to address this problem. She acknowledged that this will limit the length of their studies, but considered it was not worthwhile to invest much effort in developing new ways to ameliorate rejection given that the ultimate goal in humans will be to perform autologous transplantations. Nevertheless, Thompson noted that initial studies in humans may be non-autologous, in which case it will be necessary to address this problem more directly. Aronin pointed out that

transplants using tissues from family members can often reduce the problem of rejection so that only mild immunosuppression is necessary.

Another safety issue, noted by Khoshnan, is the possibility of the transplanted stem cells overgrowing in the host tissue. Thompson said this risk has not been well characterized, but it is clear that transplanted cells can spread to distant regions within the brain. Cells injected into the striatum, for example, have been later seen in the cortex. One other safety concern discussed was the possibility of the transplanted cells “catching” HD, as described by Young and Diamond. Although the cause of HD is a genetic mutation, studies in other diseases with a genetic basis have revealed that transplanted cells can acquire at least some of the characteristics of their host’s altered phenotype, perhaps through disrupted cell-cell and/or cell-environment interactions. Thompson acknowledged this possibility and said she was planning to test for it.

Understanding huntingtin’s normal function: Can it help accelerate the search for HD treatments?

Although the workshop focused on promising therapeutic avenues in HD research, participants also noted the importance of studies addressing the basic biology of huntingtin protein. For example, as previously noted, normal huntingtin’s role in autophagy may prove important for designing therapies that seek to reduce abnormal huntingtin protein. Setting the stage for a discussion of additional proposed functions for wildtype huntingtin, DiFiglia explained that the protein localizes to endosomes, the plasma membrane, the trans-Golgi, and synaptic vesicles, and several functions consistent with these locations have been proposed.

For example, DiFiglia described studies by Xueyi Li from her lab indicating that huntingtin is important for the activation of Rab11, a GTPase involved in endosomal recycling. When huntingtin is mutated, this function is disrupted leading to deficits in vesicle formation. DiFiglia and colleagues initially observed that vesicles labeled with transferrin were unusually tubular in HD fibroblasts, probably resulting from a problem in vesicle budding. Examining other cargoes, the researchers have now observed HD-associated defects in the recycling of neuron-specific transporters that are key to neuronal function. For example, recycling of the EAAC1 glutamate and cysteine transporter, as well as of the glucose transporter 3 (Glut-3) appear to be impaired in HD cultured neurons. Overexpressing Rab11, however, normalized the activities of these two proteins. As noted by DiFiglia, these deficits are detectable very early in the disease process, in presymptomatic mice, and are probably highly cell selective. DiFiglia speculated that another cargo that might be affected by the alteration of Rab11-huntingtin interactions, is cholesterol, which has been shown to be reduced in HD.

DiFiglia’s team has several plans to extend these findings. Li is currently investigating how huntingtin interacts with Rab11 and cell membranes using reconstitution experiments. In addition, the team is generating transgenic mice expressing a dominant-active Rab11 protein. If the mice are generally healthy, they will be crossed with a knock-in mouse model of HD to assess whether enhancing Rab11 activity can ameliorate the HD phenotype.

As noted by DiFiglia, there is also evidence for huntingtin playing a role in synaptic function. Bob Hughes, for example, has identified several synaptic proteins, including syntaxin, SNAP-25, and voltage-sensitive calcium channels, in screens for huntingtin associated proteins. Yang noted that his in vivo studies of huntingtin binding partners are very consistent with these findings. Kennedy cautioned, however, that pull-down experiments are noisy and mass spectrometry is very sensitive which can result in many false **POSITIVES**. Acknowledging these limitations, Yang explained that his team has used semi-quantitative methods, spiking the immunoprecipitated proteins with a label and checking his results for inter-sample variability. He has also used a computer program to assess the context of his findings in terms of established protein networks. In addition to yielding biological insights, the analysis helps validate the data obtained for individual proteins. Moreover, Thompson and DiFiglia noted that approximately 80% of the proteins identified by Hughes emerged as modifiers of HD neurodegeneration in independent screens conducted by Juan Botas's group in *Drosophila*. In addition, Hughes has used various in vitro assays to validate his findings, including the administration of miRNAs and siRNAs against these genes to assess their roles in a slice model of HD.

Understanding the full extent of abnormal huntingtin's effects on synaptic function, however, will be complicated. As noted by Levine, alterations in neurotransmitter release, for example, change over the course of HD, and affect various cell types differentially. In addition, both primary and compensatory effects contribute to the alterations and can be difficult to tease apart.

Huntingtin has also been implicated in other cellular functions. For example, as noted by DiFiglia, Frédéric Saudou and Sandrine Humbert have described a role for huntingtin in vesicular transport, including the transport of brain-derived neurotrophic factor (BDNF). Moreover, Kennedy pointed out that Eduardo Marcora recently showed a slowing down of the transport of the transcription complex NF- κ B in dendritic spines of huntingtin knockout mice. Similar results were observed in a knock-in model of HD. Thus, it is possible that huntingtin is involved in the transport of NF- κ B from neuronal processes to the nucleus, and this function is disrupted by polyglutamine expansion. This is of particular interest given NF- κ B's proposed role in synaptic plasticity.

Despite the obvious importance of the above mentioned studies, as well as of equivalent studies in other neurological disorders, Kennedy noted that the National Institute of Mental Health (NIMH) has been lacking a study section devoted to molecular pathophysiology. Kennedy informed participants that the new director of the NIMH is planning to set up such a section, which should include molecular synaptic physiology. She urged participants to support this new committee and be willing to serve on it. She also stressed the need for improved mechanisms within the NIH to keep study sections aligned with current research needs.

Bridging the Gaps Between Cell Studies, Animal Studies, and Human Studies

Participants identified two general challenges on the road to finding treatments for HD that require linking studies at different levels. One challenge is to find relevant outcome measures for cell-based studies—i.e., ways to bridge the gap between the information gleaned from cells and the disease process in whole animals. The other challenge is to link animal studies to human studies more effectively. In particular, participants noted the need for additional and better biomarkers that can be used in both animals and humans.

Bridging the gap between cell and animal studies

As noted by Chesselet, many of the powerful models to study the cell biology of HD do not have toxic endpoints. Furthermore, as noted by Yamamoto, it is not clear whether, or how much, cellular toxicity is relevant to the disease process. Diamond suggested analyzing past studies to find relevant outcome measures, seeking to identify cellular assays which are predictive of whole animal responses. One difficulty, however, as noted by Thompson, is the great degree of variability between the experimental procedures and results of different laboratories. Another consideration, pointed out by Kennedy, is that huntingtin's multi-functionality means that a single readout won't be sufficient to fully characterize the effects of a particular candidate treatment. Indeed, a combination treatment is likely to be the ultimate "cure" for HD.

Participants discussed how induced pluripotent stem cells (iPSCs) promise to help address the challenge of identifying relevant cell-based outcome measures. As explained by Thompson and Chesselet, iPSC lines derived from HD patients should help reveal which cellular readouts correlate with different aspects of HD. For example, Thompson's group is planning to correlate age-of-onset (and CAG repeat length) with various cellular activities—including electrophysiological responses, gene expression profiles, calcium dynamics, cell vulnerability, aggregate formation, and repeat instability—in partially differentiated iPS cell lines. Thompson estimated there are currently 50 such lines available for such experiments.

Another tool, currently under development in Housman's lab, provides a way to perform cellular assays within the context of a whole living animal. As described at the HD2010 meeting, the assay relies on crossing a line of GENSAT mice expressing GFP driven by the dopamine D2 receptor (D2-R) promoter with mouse models of HD. Because D2-R expression declines over the course of HD, it is a potentially good output measure for HD pathology. To test the effects of perturbing particular cellular components, a pool of shRNAs (e.g., a collection of shRNAs against regulators of synaptic function, or chaperone proteins such as CCT1) is delivered into the striatum using AAV at a density such that individual cells will likely be transfected by, at most, a single virus. After the silencing effects have developed over the course of a few weeks or months, the animal is sacrificed and D2-R expression quantified using a fluorescence-activated cell sorter (FACS). The cells with higher expression levels than untreated controls represent cells expressing shRNAs with potential therapeutic value. To validate the system, the researchers will deliver shRNAs against abnormal huntingtin exon 1, as well as constructs encoding intrabodies against the abnormal protein. As noted by Housman, this system could complement the above mentioned iPSC studies.

Participants were positive about this new system, but debated whether expression of the D2 receptor is a good indicator of HD pathology. As noted by Yang, who has performed numerous microarray studies in HD mice, the change in D2-R expression levels is not one of the most pronounced, nor earliest, transcriptional changes associated with HD. Chesselet added that, in her experience, D2-R expression does not correlate well with disease progression. Another complication pointed out by Levine, is that a recent study found that GENSAT GFP-D2 mice appear to overexpress the D2 receptor, as assessed by measurements of both mRNA and protein levels. Levine added that a recent study by his group, in BAC and YAC mice, revealed that D1-expressing cells appear to be more functionally affected by HD than D2-expressing cells.

Yang proposed that Housman use a panel of indicators, rather than D2-R expression alone, to monitor HD pathology. Housman argued that, as long as D2-R expression decreases with HD, the system should work, even if D2-R expression is not as closely tied to HD pathology as the expression of other genes. Housman also noted that his team plans to validate the system, as described above, and do a full set of characterizations, using a parallel cell culture system.

Bridging the gap between animal and human studies

Participants also discussed the importance of finding new ways of translating animal findings to humans and, in particular, of linking animal pre-clinical studies with human clinical trials. For example, as noted by Bennett, one of the rate-limiting factors for moving gene silencing treatments into the clinic, is the availability of robust, quantitative biomarkers that can be followed in both animals and humans. The standard markers used to monitor the disease process in animals are different from those used in humans, and the relationships between the two sets remain unclear.

Discussing behavioral outcome measures used in mice, Kordasiewicz noted her group uses rotarod, open-field activity, and elevated maze assays, and is now planning to add a tail-hang assay, a social conditioning test, and possibly a forced-swim test. Chesselet added that the running wheel provides a sensitive means for detecting behavioral improvement, and is particularly useful for HD models with milder symptoms. As noted by Yang, monitoring the circadian rhythmicity of various behaviors may also provide good behavioral biomarkers.

In humans, on the other hand, some of the most promising biomarkers are emerging from imaging studies. And as noted by Chesselet, these have not yet been linked to animal studies. Yang noted, however, that longitudinal MRI studies in HD mice are being performed by at least one group. In addition, Rosas pointed out that there are other efforts to link animal and human biomarkers. For example, Wayne Matson is analyzing the metabolomic profiles of HD animal models, as well as of humans afflicted with HD, and correlating the two.

Participants also discussed potential new ways to monitor treatments. Fischbeck proposed searching for biomarkers to monitor the symptoms that are most burdensome to patients. Unfortunately, noted Young, cognitive symptoms are probably the most bothersome for most patients, but they are hard to correlate with

animal behaviors and difficult to measure reliably. Cha agreed, adding that the cognitive phenotype is variable and noisy. To address the problem of HD phenotype variability, in general, Patterson suggested administering treatments that require intracranial delivery, such as gene silencing agents, to only one side of the brain. Cha agreed with this proposal, adding that unilateral delivery would help reduce potential risks.

Labeled ligands were also discussed as possible biomarkers. Bordelon noted her group has conducted studies using a positron emission tomography (PET) ligand that binds protein aggregates which assume an amyloid or amyloid-like structure, known as [F18]FDDNP. However, the ligand is not specific for HD and, as noted by Patterson, the resolution provided by PET is not as great as that provided by MRI.

Cha and Fischbeck also noted the opportunity to learn from Alzheimer's disease trials. In particular, Fischbeck pointed to ongoing studies at Novartis, in which multiple factors are being monitored in a very systematic way. Wexler agreed that this learning opportunity should not be wasted, but urged the HD research community to be creative and avoid limiting itself to following the lead of others.

Concluding thoughts

Participants converged on the realization that a greater coordination of efforts is needed to move promising therapeutic candidates into the clinic. Young stressed the importance of creating a group that can help organize and facilitate communications between research groups to guide the development of new clinical trials. She added that each candidate intervention will need a specific committee because of their different needs. Participants also agreed that such a committee is needed with particular urgency for the coordination of gene silencing interventions. Chesselet and Aronin noted that the CHDI Foundation had organized a meeting to discuss gene silencing as a candidate therapy, but there were no follow-up meetings and the organization of clinical trials was not strongly addressed.

Several ideas were put forth to assemble effective committees. Participants agreed that committees will need to include researchers who can guide the selection of robust and reliable biomarkers. Rosas noted that the selection should focus on markers whose value as indicators of disease progression is based on longitudinal data. Her team, for example, has developed and worked with several imaging markers, blood markers and metabolites that have been studied longitudinally. Aronin added that statisticians, both frequentist and Bayesian, should be included in the committees. Moreover, Cha emphasized the need to involve the FDA at an early stage. Also, representatives from industry, noted Young, should be asked to participate. Although companies will have their own agendas, hopefully they will benefit from being involved in the process. (LaSpada considered that, at least Isis, is likely to be receptive to this idea. Kordasiewicz agreed, but noted that Isis is being funded by the CHDI, so they will have to be mindful of that foundation's agenda as well.)

Wexler considered it possible for the HDF to spearhead the organization of at least some of these committees. LaSpada noted that involving Fischbeck, who is Chief of the Neurogenetics Branch of the NINDS, and Walter Koroshetz, who is Deputy Director of the NINDS, would be advantageous.