Gene Therapy Strategies for the treatment of Huntington’s disease

Challenges for Improved Delivery

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Can be termed ‘aggregation disorders’ – all have intracellular inclusions

Emphasis on Genetics and Pathology have driven field

- Genetics support pathology findings (APP, Tau, HTT, α-synuclein)
- Still unresolved: ubiquitin inclusions
Cellular aspects: conserved phenomena

- Axonopathies & synaptic dysfunction
- Inflammatory responses – microgliosis, astrocytosis
- Energy homeostatic imbalances/Oxidative Stress

Systems dysfunction

- Specific populations degenerate– unknown aetiology
- Selected circuits are differentially affected
- Clinical management dependent on affected circuitry
- Decreased glucose utilization observed in PET studies
- Degeneration precedes symptomatology by many years
Huntington’s disease is an autosomal dominant disorder.

- **Prevalence**, 1 in 10,000 worldwide
  - USA 30,000 Cases, >100K at risk
- **Exclusively genetic (monogenic)**
- **Dominantly inherited**
  - Risk for children of affected parent: 50%
- **Displays anticipation via paternal lineage**
  - Earlier onset due to instability of repeats
The mutation that causes Huntington’s disease

The Huntingtin (Htt) gene

- Exons
  - 1
  - 2
  - 3
  - 66
  - 67

180 kb DNA

CAG CAG CAG….

≤ 35 CAG – Unaffected

≤ 36 - 39 CAG – High probability of developing HD

≥ 40 CAG – 100 % penetrant. Will develop adult HD

≥ 65 CAG – Juvenile HD
The *Htt* gene contains a polymorphic CAG repeat

<table>
<thead>
<tr>
<th>Normal alleles</th>
<th>HD alleles</th>
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- J. Gusella, Harvard/MGH
HD is not only a disease of the brain

Photos courtesy of Bernhard Landwehrmeyer

1985

1998

Van der Burg, Lancet Neurology 2009
### Classic neurological symptoms
- Motor – chorea, dyskinesias
- Cognitive – basal ganglia
- Psychiatric & behavioral

### Other clinical features
- Weight loss
- Skeletal muscle atrophy
- Sleep disturbances
- Autonomic disturbances

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![Brain imaging](image)

**Control** | **Pre-Diagnosis** | **HD**
---|---|---

TRACK-HD Study. Courtesy of Dr. Tabrizi
Volumetric and functional changes in the HD brain

Change in white matter

Baseline groups
- PreHD-A
- PreHD-B
- HD1
- HD2

T score
- 0
- 5
- 10
- 15
- 20

Eidelberg HDRP: network analysis using FDG-PET

Track-HD: white matter changes in 24 months
Dr. Tabrizi (TRACK-HD)

C¹¹-Raclopride (Karolinska/CHDI)
Progressive atrophy in the brains of HD subjects

* $p<0.05$
** $p<0.01$
*** $p<0.001$

Normal decline
5-15 years prior to onset
1-5 years prior to onset
Clinically symptomatic
Pre-symptomatic stage
Early stage
Late stage

Clinical Stage

Time

Natural decline (normal)
Decline desired after intervention
HD decline

Early stages= Stages 1-2 (5-6 years after clinical (motor) diagnosis)
Late stages= Stages 3-4 (6-15+ years after diagnosis)

CHDI Foundation’s Mission – Halting Progression
CHDI Foundation – who are we?

Not-for-profit biomedical R&D organization
Solely dedicated to Huntington’s disease

Resources not a limiting factor
USD 140m+ year
Funded by private donors

Research through global partnership networks
- 80+ internal employees (FTEs)
- >500 FTEs worldwide (at CROs)
- 100 academic collaborations
- Multiple pharma & biotech collaborations
- Internal drug discovery campaigns (CHDI-owned)
- Extensive clinical infrastructure (Enroll-HD)
CHDI’s focused areas for therapeutic development

1. Modulate mutant *Huntingtin* expression (gene therapy or small molecules)

2. Modulate HTT *structure-function* to decrease toxicity
Characterize systems dysfunction in HD models
- Identify key neuronal, glial and circuitry alterations

Validate assumptions in patients
- Imaging technologies (PET, qEEG, fMRI)
- Deep brain stimulation (DBS) trials - recordings

Test therapeutics based on BG alterations
- Pharma drugs – apathy, motor, cognition
  - Pfizer PDE10 inhibitor - Phase 2 (Pfizer)
  - M4 PAM, H3 antagonist, PDE4 inhibitors, etc.
- Neuroprotective strategies
  - TrkB/p75/RhoK signaling
  - MLK/JNK signaling
- Glial-directed strategies
  - EAAT2/Glt1 transporter, Kir K-channels
  - Glial progenitor grafts (impact of human glia)

Targeted therapies will address symptoms but will they be disease modifying?
New clinical trials in HD - ongoing and planned

New clinical studies
- PDE10 inhibition (Pfizer, Amaryllis trial) – Phase 2, ongoing
- DBS trial (Germany, academic) – ongoing
- Laquinimod (Teva, Legato-HD trial) – Phase 2, ongoing
- VX15 (Vaccinex, Signal trial) – Phase 2 planned 2016
- Glial stem cell transplantation (academic) – IND estimated 2018
- Others in late preclinical development stages

Gene therapy programs targeting HTT
- Roche/ISIS (ASO) – ongoing
- Shire/Sangamo (ZFP) – IND estimated 2017
- Genzyme (miRNA) – IND estimated 2018
- UniQure (miRNA) – IND estimated 2017
- Wave (ASO) – IND estimated 2017
- Biomarin (AS) – IND estimated 2017
Gene Silencing as a Therapeutic Strategy for HD

ZFP repressor (allele-selective for mHTT)
Sangamo/Shire

ISIS/Roche – antisense (both alleles)
Genzyme/Sanofi/Voyager, Uniqure, Spark - miRNA

CHDI internal R&D
Pharma companies
Small molecules
Sustained beneficial effects of suppressing HTT expression

Reversal of Neuropathology and Motor Dysfunction in a Conditional Model of Huntington's Disease

Neuron Article
Sustained Therapeutic Reversal of Huntington's Disease by Transient Repression of Huntingtin Synthesis

Genetically
Pharmacologically
Sangamo ZFPs stop pathology \textit{in vivo} in rodent models of HD

\begin{itemize}
  \item Sangamo ZFPs administered via direct AAV injection into striatum
\end{itemize}
Sustained elimination of HTT pathology in mice

Treatment: 2-4 months of age (Q175 knock-in mice)

Number of nuclear inclusions

Extranuclear inclusions

Density of inclusions

[ # per µm²]

Control AAV  ZFP 3074

Treatment: 6-10 months of age (Q175 knock-in mice)

Number of nuclear inclusions

Nuclear inclusions:

[ # per GFP+ DARPP32+ cells]

Control AAV  ZFP 30640  ZFP ΔDBD

Number of extranuclear inclusions

Density of inclusions

[ # per µm²]

Control AAV  ZFP 30640  ZFP ΔDBD

n.s.

***
The challenge of gene delivery to the brain
Current gene therapy strategies lack adequate distribution

**Oligonucleotides**

**siRNAs**

**AAV-Cargo**
- Grondin, et al., Brain, 2012

**Companies**
- Medtronic
- Ionis-Roche (IT)
- Wave (?)
- Genzyme-Voyager
- Biomarin (ICV)
- Sparks
- Sangamo-Shire
- Uniqure
Which animal would be best to most closely mimic humans?

- Rhesus macaque (Macaca mulatta)
- Common marmoset (Callithrix jacchus)
- Domestic Pig (Sus scrofa domesticus)
- Domestic Sheep (Ovis aries domestica)

Key considerations

- Which will develop most human-like HD pathology?
- Which has the most human-like brain anatomy?
- Which species is most suitable for long-term experiments?
- When using viruses – is tropism conserved?
- Brain size suitable for catheter placement – regulatory approval
- Which model best mimics the human immune system?
Delivery strategies to increase brain exposure

**Approaches**
- Direct delivery of nucleic acids
  - Chemical modifications
  - Coupling to BBB targeting antibodies
- Nucleic acid encapsulation
  - Exosomes
  - Liposome formulations
- Viruses as delivery vehicles

**In vitro BBB models**
- Fresh porcine preparations
- Human ES-derived preparations
  - Exploration of transcytosis mechanisms
    - Lipid-mediated uptake (Msfd2a)
    - Protein-protein mediated uptake (Transferrin-R, Insulin-R)
  - Antibody and viral-engineering to cross the BBB
Hydrophobically-Modified Small RNAs to Improve Stability

Chemically modified nucleic acid therapeutics (Ionis, Biomarin, Wave, etc)
- Stable in serum
- Currently delivered via direct ICV or IT dosing
- Efficient cellular uptake
  - No need for a delivery vehicle
- Challenges for brain targeting
  - Efficient transfection after systemic administration
  - Pharmacodynamic effect in brain cells (BBB crossing)
  - Immune-system trigger

_Exosomes — naturally secreted membrane vesicles_
- Efficient exosome-mediated transfer of siRNA _in vitro_ and _in vivo_

Intrathecal ASO Drug Delivery to the CNS: ISIS-HTT$_{Rx}$

- Ionis-HTT$_{Rx}$ is a human HTT-targeted antisense oligonucleotide (ASO)
  - 5-10-5 Gen 2.0 MOE gapmer mixed backbone (PO/PS) through RNase H
- Lumbar intrathecal bolus administration of Ionis HTT$_{Rx}$ into the spinal cord
  - Onset of action~4-6 weeks (maximal protein suppression)
  - Expected duration of action ~4 months
  - ASOs have long half-lives (several months) in CNS tissue, with even longer duration of action, so enables infrequent dosing.
  - For more frequent dosing, there are implantable devices that can also be used
  - Precedence for IT drug delivery with anesthetics, pain medications, and chemotherapeutics
- Targeting a 50% reduction in the cortex, little distribution to basal ganglia expected
- Ionis-HTT$_{Rx}$ is well tolerated in mice rats, dogs and non-human primates
- Phase 1/2a study in progress – results expected Q1 2017

![monkey spinal cord](image1)
![monkey brain](image2)

Courtesy of Dr. Tabrizi, PI for the study
The blood-brain barrier works very well

Omidi et al., 2012
Natural mechanisms to cross the BBB

Omidi et al., 2012
The insulin receptor (IR) and Transferrin receptors (TfR) are the main current transcytosis targets using antibodies.

- Fusion proteins aim to increase access of therapeutics to the brain
- Optimization of fusion proteins dependent on mechanisms being targeted
Bivalent antibodies for the treatment of Alzheimer’s

Boosting Brain Uptake of a Therapeutic Antibody by Reducing Its Affinity for a Transcytosis Target

Y. Joy Yu,¹* Yin Zhang,²* Margaret Kenrick,³ Kwame Hoyte,⁴ Wilman Luk,⁴ Yanmei Lu,⁴ Jasvinder Atwal,¹ J. Michael Elliott,⁵ Saileta Prabhu,⁵ Ryan J. Watts,¹† Mark S. Dennis²†

![Diagram of antibody interactions with brain tissues showing a comparison between high-affinity and low-affinity antibodies.](image)

**Graph B:**

- **controlled IgG**
- **Anti-BACE1**
- **Anti-TfR/BACE1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control IgG</td>
<td>2000</td>
<td>1500</td>
</tr>
<tr>
<td>Anti-BACE1</td>
<td>1800</td>
<td>1200</td>
</tr>
<tr>
<td>Anti-TfR/BACE1</td>
<td>1600</td>
<td>1000</td>
</tr>
</tbody>
</table>

Significance levels:
- **P < 0.001**
- **P < 0.01**
- **P < 0.05**
- **P < 0.001**
- **P < 0.001**

Legend:
- **Black**: 50 mg/kg
- **Gray**:

**High-affinity antibody (anti-TfR⁶):** Trace dosing = more uptake

**Low-affinity antibody (anti-TfR⁰):** Trace dosing = less uptake
Viral engineering to increase distribution

- Exploit natural variations in viral tropism (serotypes)
  - Many AAV serotypes with very different tropisms and properties
  - Unclear mechanism of entry for AAVs—however receptor now identified (Pillay et al., 2016)

- Engineer viruses to enhance BBB entry
  - Engineer viruses to express envelope proteins targeting transcytosis mechanisms
    - Transferrin & Insulin receptors, Msfd2a receptor (lipid receptor)
  - Retarget viruses to avoid peripheral sinks (change the ratio)

- Explore other viruses beyond AAV with better distribution properties
  - Paramyxoviruses, Herpes, etc
Viral distribution differs after systemic administration.
Engineering viruses to transverse the BBB

making do with what is available

natural AAV variants

engineered AAVs

customized

efficiency

reduced immunity

specificity

Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain

Benjamin E Deverman¹, Piers L Pravdo¹, Bryan P Simpson¹, Sripriya Ravendra Kumar¹, Ken Y Chan¹, Abhik Banerjee¹, Wei-Li Wu¹, Bin Yang¹, Nina Huber², Sergiu P Pasca² & Viviana Gradinaru¹
System AAV9 in the adult provides widespread but inefficient transduction of the CNS

AAV9

GFP immunostaining

3 weeks post IV inj. ssAAV9:CAG-GFP

$1 \times 10^{12} \text{VG}$
The AAV-PHP.B variant transduces the adult mouse brain with high efficiency.

AAV-PHP.B  
GFP immunostaining

3 weeks post IV inj. ssAAV-PHP.B:CAG-GFP  
$1 \times 10^{12} \text{VG}$
New AAV-PHP.B variant distribution in mice

Biodistribution ($1 \times 10^{11}$ vg)

Courtesy of Dr. Deverman
AAV-PHP.B efficiently transduces astrocytes, neurons and oligodendrocyte lineage cells
Issues to consider for systemic delivery

- Titers & manufacturing
  - \( \sim 10^{10} - 10^{12} \) viral genomes/kg
  - Tropism depends on method for production (AAVs) and age of animal

- Safety considerations
  - Presence of pre-existing antibodies (e.g., AAV, AdV)
  - Immune responses to viral proteins and cargos
  - Single or multiple injections required?

- Easily manipulated, to address safety and distribution issues
  - Liver & other peripheral sinks
  - Retargeting to transcytosis mechanisms (Transferrin and insulin receptors)
  - Enhance tropism for neurons/glia (choice of promoters)

- Expression: transient vs long-term
  - Integrating or not
  - Kill-switches
Conclusions

Gene therapy strategies are entering clinical stages for HD

- First gene therapy for HD is being tested in patients (HTT-Rx – Ionis/Roche)
- Several viral (AAV)-based therapies in late stage preclinical development
  - Safety shown for AAV2 (10+ years evaluation in PD patients with AAV2-GDNF/neurturin)

Challenges remain

- Increase distribution to entire brain
  - Exploit axonal transport mechanisms to enhance brain region uptake
  - Modifications in nucleic acid chemistry

- Enhance tropism
  - Neurons versus glia expression

- Enhance systemic delivery mechanisms
  - Exploit targeting mechanisms to cross the BBB
  - Explore different viral systems
  - Decrease peripheral sinks

- Address safety issues
  - Immunogenicity
  - Immune response triggers
  - Unforseen safety liabilities upon systemic expression
Implications of HD clinical trials for other indications

- **HD is monogenic and we can track progression very well**
  - Define optimal time for intervention - staging of progression and clinical domains
    - Gene therapy trials will in the future start prior to clinical diagnosis
  - Identify predictive measurements for ‘clinical conversion’
  - Identify biomarkers
    - Non-invasive – imaging based, to monitor degeneration and loss of compensatory mechanisms
    - CSF or blood-based (eg HTT levels)

- **HD cellular and systems mechanistic principles are generalizable**
  - Connections - degeneration, circuit alterations & symptoms (cognition, apathy, motor)
    - White matter changes as indicators of subcircuit alterations – correlation with symptoms
    - Establish impact of potential therapies in circuitry and functional domains (eg cognition)
  - Generation of large animal models will improve predictive power
    - Primate models around the corner
    - Minipig, sheep and rodent models available
  - For many current hypotheses in neuroscience, HD is an ideal indication for testing them
    - Aggregation (toxic species)
    - Oxidative stress (Nrf2/keap1)
    - Axonopathies – axonal degeneration mechanisms
    - Complement activation (C3/C1q)
    - Reconstituting circuitry via glial grafts
THANK YOU!

- Organizers – Dr. Mark Rose
- Drs. Tabrizi, Deverman, Lee for slides
- Sangamo/Shire, Ionis/Roche, Evotec
- CHDI Colleagues

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