Histone Deacetylase Inhibitors: Assessing Their Potential for Clinical Use in Neurodegenerative Disorders

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Clinical trials with HDAC inhibitors (non-cancer)

- Phase I – Phenylbutyrate in Huntington’s disease. 60 patients; 20 weeks.
- Phase I – Phenylbutyrate in Amyotrophic Lateral Sclerosis (ALS). 26 patients; 20 weeks.
- Phase I – Valproate in Spinal Muscular Atrophy (SMA). 33 patients; 6 months.
- Phase I/IIa – Valproate and Phenylbutyrate in Spinal Muscular Atrophy (SMA). 10 patients; 14 weeks.
- Phase I/II – Vorinostat (SAHA) in Niemann-Pick Disease. 15 patients; 3 months.
- Phase IIa - FRM-0334 in Frontotemporal Dementia. 30 patients; 28 days.
- Phase II – Valproate in Rubinstein-Taybi Syndrome. 60 children; 1 year.
- Phase I – 109 in Friedreich’s ataxia. 20 patients; 29 days.
Goals

• Understanding the role(s) of epigenetic modifications in disease mechanisms and response to therapies.
  - HDAC inhibitors in Huntington’s disease; preclinical studies from mouse models.

• Discover how epigenetic analysis can be applied in clinical trials to identify markers of response.
  - HDAC inhibitors in Friedreich’s ataxia; clinical data from patients.
HDAC inhibitors activate gene expression by changing chromatin structure

Condensed chromatin

Increased transcription

Open chromatin

Decreased transcription

* Several neurodegenerative disorders are associated with histone hypoacetylation and altered gene expression, including Huntington’s disease.
Huntington's disease (HD)

- Caused by CAG repeat expansion in exon 1 of the HD gene, resulting in a translated huntingtin protein with an expanded polyQ tract.
- Autosomal dominant; affects ~1 in 10,000 people.
- Obvious symptoms are random, uncontrollable movements called chorea, lack of coordination, unsteady gait. Other cognitive and psychiatric symptoms are often present.
- Hallmark feature of disease is the formation of huntingtin aggregates in the brain.
- Largely adult-onset. Typically, patients live 15 years after diagnosis.
- No cure; no good therapies.
Abnormal gene regulation

(Steffan et al., 2001; McCampbell et al., 2001; Cong et al., 2005; Ferrante et al., 2003; Stack et al. 2007; Sadri-Vakili et al., 2007; McFarland et al., 2012)

Abnormal gene expression

(Luthi-Carter, 2000; Luthi-Carter, 2002; Chan et al. 2002; Desplats et al., 2006; Hodges et al., 2006; Kuhn et al., 2007; Friedrich et al., 2012)
Huntington’s disease is associated with a range of chromatin/gene expression abnormalities

New targets for drug treatment are aimed at correcting faulty transcription: “histone deacetylase (HDAC) inhibitors”
Novel benzamidine-type HDAC inhibitors show low toxicity

Table 1. Activities and IC50 values of HDAC inhibitors. Structures for each compound (numbers in bold) are shown with corresponding transcriptional change in frataxin mRNA in the FRDA lymphoid cell line and IC50 for inhibition of histone deacetylation activity in a HeLa extract.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fold-change (IC50)</th>
<th>Compound</th>
<th>Fold-change (IC50)</th>
<th>Compound</th>
<th>Fold-change (IC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>1.4 ± 0.06 (238 µM)</td>
<td>7b</td>
<td>2.6 ± 0.14 (123 µM)</td>
<td>10b</td>
<td>2.5 ± 0.17 (438 µM)</td>
</tr>
<tr>
<td>4b</td>
<td>2.5 ± 0.24 (78 µM)</td>
<td>7c</td>
<td>2.0 ± 0.08 (186 µM)</td>
<td>11b</td>
<td>3.0 ± 0.17 (17 µM)</td>
</tr>
<tr>
<td>4c</td>
<td>1.4 ± 0.06 (87 µM)</td>
<td>8b</td>
<td>2.6 ± 0.14 (140 µM)</td>
<td>12b</td>
<td>2.5 ± 0.17 (84 µM)</td>
</tr>
<tr>
<td>5b</td>
<td>1.4 ± 0.15 (204 µM)</td>
<td>8c</td>
<td>2.0 ± 0.08 (99 µM)</td>
<td>13b</td>
<td>2.4 ± 0.10 (91 µM)</td>
</tr>
<tr>
<td>6b</td>
<td>1.5 ± 0.13 (500 µM)</td>
<td>9b</td>
<td>2.3 ± 0.11 (54 µM)</td>
<td>14b</td>
<td>1.8 ± 0.12 (&gt;1 mM)</td>
</tr>
<tr>
<td>6c</td>
<td>2.1 ± 0.15 (65 µM)</td>
<td>9c</td>
<td>1.8 ± 0.07 (470 µM)</td>
<td>15b</td>
<td>1.5 ± 0.06 (387 µM)</td>
</tr>
</tbody>
</table>

1 Fold-change of frataxin mRNA in affected GM15850 cells, normalized to GAPDH mRNA, were determined in triplicate by real-time quantitative RT-PCR after incubation with each compound at 5 µM for 96 h. Values are relative to untreated control cells.

2 IC50 values (in parenthesis below fold-change values) were determined by total histone deacetylation inhibition in a HeLa nuclear extract.
**Benzamide-type HDAC inhibitors preferentially target HDAC1 and/or HDAC3**

Table 1. HDAC subtype selectivity profiles for the HDAC inhibitors tested in qPCR analysis (plus SAHA as a reference).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>IC_{50} for Class I HDAC enzymes:</th>
<th>IC_{50} for Class II HDAC enzymes:</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HDAC1</td>
<td>HDAC2</td>
<td>HDAC3</td>
</tr>
<tr>
<td>4b</td>
<td><img src="image1" alt="Structure" /></td>
<td>199 nM</td>
<td>1.59 μM</td>
<td>69 nM</td>
</tr>
<tr>
<td>109</td>
<td><img src="image2" alt="Structure" /></td>
<td>300 nM</td>
<td>1.28 μM</td>
<td>63 nM</td>
</tr>
<tr>
<td>136</td>
<td><img src="image3" alt="Structure" /></td>
<td>5.2 μM</td>
<td>3.0 μM</td>
<td>400 nM</td>
</tr>
<tr>
<td>228</td>
<td><img src="image4" alt="Structure" /></td>
<td>61 nM</td>
<td>314 nM</td>
<td>3.33 μM</td>
</tr>
<tr>
<td>SAHA*</td>
<td><img src="image5" alt="Structure" /></td>
<td>171 nM</td>
<td>389 nM</td>
<td>200 nM</td>
</tr>
</tbody>
</table>

HDACs 6, 9, and 10 were not tested.

* IC_{50} values for SAHA taken from Reaction Biology Corp (www.reactionbiology.com)

NS, non-selective. ND, not determined.
Pipeline for screening novel HDAC1/3-targeting inhibitors for Huntington's disease

Library of ~100 novel HDAC inhibitors

Compounds tested for pharmacokinetics, cell permeability, metabolic stability, receptor cross-reactivity, cytotoxic properties, etc.

Investigational New Drug (IND) Application

Mouse models (R6/2, N171-82Q, CAG140 KI)
Selective HDAC1/3 inhibitors ameliorate disease phenotypes in Huntington’s disease model systems

The HDAC inhibitor 4b ameliorates the disease phenotype and transcriptional abnormalities in Huntington’s disease transgenic mice

Histone deacetylase (HDAC) inhibitors targeting HDAC3 and HDAC1 ameliorate polyglutamine-elicited phenotypes in model systems of Huntington’s disease

Selective histone deacetylase (HDAC) inhibition imparts beneficial effects in Huntington’s disease mice: implications for the ubiquitin–proteasomal and autophagy systems
Gene expression signatures associated with HDACi 4b treatment

Thomas et al., PNAS USA 105:15564-9 (2008)
Histone modifications

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Acetylation  Me Methylation  P Phosphorylation  Ub Ubiquitination
Benzamide-type HDAC inhibitors increase histone acetylation at specific sites

Western blot of SHSY5Y neuroblastoma cells treated with HDAC inhibitors

Unpublished
HDACi 4b treatment reverses expression downregulation coincident with increased histone H3K9 acetylation at promoters of key genes

qPCR validation:
HDACi 4b treatment reverses expression downregulation coincident with increased histone H3K9 acetylation at promoters of key genes.

qPCR validation:

Chromatin immunoprecipitation (ChIP) for acetylated H3 (AcH3K9):
# HDAC1/3 inhibition alters the expression of DNA methylation-related genes in WT and HD mouse brain

Table 1. qPCR validation of DNA methylation related genes altered by HDACi 4b treatment in cortex, striatum and muscle.

<table>
<thead>
<tr>
<th>N171-82Q</th>
<th>Cortex</th>
<th>Striatum</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symbol:</strong> Dnmt1</td>
<td>DNA (cytosine-5-)-methyltransferase 1</td>
<td>0.91</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Dnmt3a</td>
<td>DNA (cytosine-5-)-methyltransferase 3 alpha</td>
<td>0.94</td>
<td>1.40*</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Gadd45b</td>
<td>Growth arrest and DNA-damage-inducible 45 beta</td>
<td>1.44*</td>
<td>0.049</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Hdac1</td>
<td>histone deacetylase 1</td>
<td>0.81*</td>
<td>0.040</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Hdac2</td>
<td>histone deacetylase 2</td>
<td>0.80</td>
<td>0.210</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Hdac3</td>
<td>histone deacetylase 3</td>
<td>0.88</td>
<td>0.310</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Mbd3</td>
<td>methyl-CpG binding domain protein 3</td>
<td>1.57**</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Symbol:</strong> MeCP2</td>
<td>methyl CpG binding protein 2 (Rett syndrome)</td>
<td>0.97</td>
<td>0.890</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Parp1</td>
<td>Poly (ADP-ribose) polymerase family, member 1</td>
<td>1.41*</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Rnf4</td>
<td>RING finger protein 4</td>
<td>1.11*</td>
<td>0.030</td>
</tr>
</tbody>
</table>

**WT**

<table>
<thead>
<tr>
<th>N171-82Q</th>
<th>Cortex</th>
<th>Striatum</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symbol:</strong> Dnmt1</td>
<td>DNA (cytosine-5-)-methyltransferase 1</td>
<td>1.00</td>
<td>0.48</td>
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<tr>
<td><strong>Symbol:</strong> Dnmt3A</td>
<td>DNA (cytosine-5-)-methyltransferase 3 alpha</td>
<td>0.78</td>
<td>0.16</td>
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<tr>
<td><strong>Symbol:</strong> Gadd45b</td>
<td>Growth arrest and DNA-damage-inducible 45 beta</td>
<td>1.11</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Hdac1</td>
<td>histone deacetylase 1</td>
<td>0.90</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Hdac2</td>
<td>histone deacetylase 2</td>
<td>0.98</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Hdac3</td>
<td>histone deacetylase 3</td>
<td>0.90</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Mbd3</td>
<td>methyl-CpG binding domain protein 3</td>
<td>0.74**</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Symbol:</strong> MeCP2</td>
<td>methyl CpG binding protein 2 (Rett syndrome)</td>
<td>0.85</td>
<td>0.17</td>
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<tr>
<td><strong>Symbol:</strong> Parp1</td>
<td>Poly (ADP-ribose) polymerase family, member 1</td>
<td>1.25*</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Symbol:</strong> Rnf4</td>
<td>RING finger protein 4</td>
<td>1.07</td>
<td>0.20</td>
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</table>

Bold font indicates fold-change (FC) that was significantly different, as determined by Student's t test (unpaired; two-tailed).
**HDACi 4b elicits DNA methylation changes in human fibroblasts** - (Infinium HumanMethylation450 BeadChip)

*KDM5D*: Lysine (K)-specific demethylase 5D

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<table>
<thead>
<tr>
<th>Probe ID</th>
<th>Chr. Position</th>
<th>REFGENE ID</th>
<th>WT1-Veh</th>
<th>WT2-Veh</th>
<th>WT3-Veh</th>
<th>WT4-4b</th>
<th>WT5-4b</th>
<th>WT6-4b</th>
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<th>HD4-4b</th>
<th>HD5-4b</th>
<th>HD6-4b</th>
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</tr>
</tbody>
</table>

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52 DMPs

- **KDM5D**
- **SYNPR**
- **SRY**
- **TTSY14**
- **TSPY4**
- **EIF1AY**
- **KDM5D**

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Jia et al., PNAS, 112(1):E56-64 2015
**HDACi 4b elicits increased methylation at several sites at the Kdm5d locus**

MeDIP RT-PCR analysis

![Graph showing increased methylation at several sites at the Kdm5d locus]

Kdm5d locus:

Jia et al., PNAS, 112(1):E56-64 2015
Epigenetic markers of HDAC inhibition

HDAC inhibitors

Increased expression of DNA methylation-related genes

Increased histone acetylation marks – H3K9

Altered DNA methylation – KDM5D
Goals

• Understanding the role(s) of epigenetic modifications in disease mechanisms and response to therapies.
  - HDAC inhibitors in Huntington’s disease; preclinical studies from mouse models.

• Discover how epigenetic analysis can be applied in clinical trials to identify markers of response.
  - HDAC inhibitors in Friedreich’s ataxia; clinical data from patients.
Friedreich’s ataxia (FRDA)

- Caused by an expansion of a GAA triplet repeat in the first intron of the FXN gene, which encodes the essential mitochondrial protein, frataxin.

- Autosomal recessive, progressive neurological disease.

- Most common form of hereditary ataxia, affecting about 1 in every 50,000 people in the United States.

- Main symptom is impaired muscle coordination (ataxia); it can also lead to scoliosis, heart disease and diabetes, but does not appreciably affect cognitive function.

- Symptoms typically begin between the ages of 5 and 15 years. Generally, within 10 to 20 years after the appearance of the first symptoms, the person is confined to a wheelchair.

- No therapies that address pathology.
Friedreich’s ataxia (FRDA)

Normal FRDA Patient

FRDA gene

**GAA**\textsubscript{20}

\[ \text{FRDA gene} \]

\[ \begin{align*}
\text{exon 1} & \quad \text{exon 2} \\
\text{RNA} & \\
\text{Frataxin protein} & \\
\end{align*} \]

**GAA**\textsubscript{500}

\[ \text{FRDA gene} \]

\[ \begin{align*}
\text{exon 1} & \quad \text{exon 2} \\
\text{RNA} & \\
\text{Frataxin protein} & \\
\end{align*} \]
Novel HDACi 4b increases frataxin mRNA and protein in FRDA patient lymphocytes/lymphoblastoid cell lines

qPCR for frataxin mRNA

Western blot for frataxin protein

Reversal of the transcription defect to at least carrier status

ChIP at the FXN locus

HDAC inhibitor effect on gene expression profiles in cultured peripheral blood mononuclear cells (PBMCs)

77 gene biomarker panel

Dose-dependent increases in frataxin gene expression:

Chromatin immunoprecipitation experiments identify key residues for FXN activation.

ChIP experiments demonstrate that H3K9 and H4K8 are critical residues for FXN gene activation. These could be used as a biomarker in FRDA patient trials.
**RG2833 (109): First in patient clinical study**

- San Luigi Gonzaga Hospital, University of Turin, Italy.
- 22 patients (split into 4 cohorts receiving different doses: 30-180 mg).
- Biomarker measures:
  - Frataxin mRNA and protein in blood, PBMCs and buccal cells
  - HDAC activity in PBMCs
  - ChIP for H3K9 acetylation in PBMCs

**TABLE 1**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
</tr>
<tr>
<td>Age, yr$^2$</td>
<td>30.0±8.1</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>9 (40.9)</td>
</tr>
<tr>
<td>F</td>
<td>13 (59.1)</td>
</tr>
<tr>
<td><strong>Disease data</strong></td>
<td></td>
</tr>
<tr>
<td>GAA·TTC triplet expansion on shortest allele$^a$</td>
<td>1.084 ±/784.5</td>
</tr>
<tr>
<td>Age of onset, yr$^2$</td>
<td>10.7±4.6</td>
</tr>
<tr>
<td>FARS$^b$ score at screening$^a$</td>
<td>59.7±23.2</td>
</tr>
<tr>
<td>Cardiac function, ejection fraction %$^a$</td>
<td>63.0±9.9</td>
</tr>
</tbody>
</table>

$^a$ Data are shown as the mean±standard deviation.

$^b$ See Beconi et al. 

F=female; FARS=Friedreich Ataxia Rating Scale; M=male.
Frataxin protein and mRNA levels strongly correlated in blood, PBMCs and buccal cells

FXN mRNA in adult Friedreich ataxia patients after oral administration of RG2833/109

Increases in FXN mRNA in PBMCs from 5 patients after a single dose (180 mg) of RG2833

Increases in FXN mRNA observed in 9/10 patients overall
Increases in histone H3K9 acetylation in patient PBMCs after a single dose (180 mg) of RG2833
Increases in FXN mRNA and histone H3K9 acetylation in patient PBMCs after two doses (120 mg) of RG2833

Summary

Preclinical studies from mice:
- Show good efficacy of HDAC1/3-targeting inhibitors in HD mouse models.
- HDACi 4b treatment is associated with a reversal of histone hypoacetylation at H3K9 at the promoter of several candidate genes.
- DNA methylation may also prove useful as a marker of drug response.

In-patient clinical studies:
- Treatment with RG2833 was safe and well tolerated (at 180 mg or 120 mg twice a day) and no drug related adverse effects were reported.
- Frataxin mRNA was increased in 9/10 patients and expression levels in different cell types were correlated.
- ChIP promoter histone acetylation was increased in patient PBMCs after single and multiple doses. H3K9 acetylation is a useful epigenetic biomarker for drug response.

Second generation compounds with improved brain penetration and metabolic stability have been generated. A clinical candidate from the new generation of compounds will be taken forward for IND filing for a second round of clinical trials.
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Biomarin®
Microarray gene expression analysis

Illumina RefSeq-8 Beadchip Expression v. 1.0 arrays
Using gene expression data for biomarker identification in PBMCs

Good biomarkers

Bad biomarkers

Unpublished