COMPOUND <sup>1</sup>		ACTIVE	
STRUCTURE <sup>2</sup>	MODEL <sup>3</sup>	CONC. <sup>4</sup>	<b>OBSERVATION</b> <sup>5</sup>
Ligand 1	MBNL1:r(CUG)4/12	~50uM	CUG-MBNL1 complex disruption
H <sub>2</sub> N T N T NH <sub>2</sub>			
N N			
NH OCH3			
CI LINIJ			
Ligand 3	MBNL1:r(CUG)12	~10uM	CUG-MBNL1 complex disruption
	DM1 HeLa cell	50-100uM	Foci reduction, minigene mis-splicing
			rescue
N NH NH2	DM1 Drosophila	200-400uM	Rescued a glossy eye phenotype
H <sub>2</sub> N <sup>L</sup> N <sup>L</sup> N <sup>NH</sup>			
(New) Ligand 9	(CTG·CAG) <sub>74</sub>	50-10011M	Transcription inhibition
	r(CUG)16	5-100uM	Cleaved hairpin structure
	DM1 HeLa cell	25-150uM	Foci reduction, minigene mis-splicing
$ \begin{array}{c} \uparrow & \uparrow $	Diffi field ten	20 100000	rescue, reduced r(CUG) <sub>exp</sub> levels
	DM1 Drosophila	100-400uM	Rescued a glossy eve and larval
n n	F		locomotor phenotypes, reduced
			r(CUG) <sub>exp</sub> levels
Ligand 4	(CTG·CAG)74	0.05-0.4uM	Transcription inhibition
NH <sub>2</sub>	r(CUG)16		Cooperative binding
	DM1 HeLa cell	0.2-4uM	Foci reduction, minigene mis-splicing
			rescue, reduced r(CUG)exp levels
HN' CLINH	DM1 patient-derived cell	0.2uM	Foci reduction
	DM1 Drosophila	20-80uM	Rescued a progressive climbing defect
N y N	DM1 liver-specific mouse	2mg/kg	Foci reduction, mis-splicing rescue,
NH2			reduced r(CUG)exp levels
DAP	r(CUG)9 or r(CAG)9	25uM	Higher binding affinity for r(CUG)9
	d(CUG)20 luciferase		Decrease luciferase expression
	reporter		
R-N N-R			
n n			
DDAP	r(CUG)9 or r(CAG)9	30-120nM	Higher binding affinity for r(CUG)9
	MBNL1:r(CUG)20	10-200nM	CUG-MBNL1 complex disruption
	DM1 C2C12 cell	10-40uM	Mis-splicing rescue
*-N, N-V-NH	DM1 HSALR mouse	20 or 50mg/kg	Mis-splicing rescue
*= (CH <sub>2</sub> ) <sub>3</sub> NH <sub>2</sub>			
2K-4 and 3K-4	MBNL1:r(CCUG)24	8-50nM	CCUG-MBNL1 complex disruption
*N.	DM2 C2C12 cell	5-20uM	Minigene mis-splicing rescue
LIN NH HOLO			
NH2 NNN OH			
2K-4 Dimer *			
*			
O N-N N-N			
3K-4 Trimer * *			

TABLE 1. Small molecules that target the toxic RNA through rational design.

Ligands 6, 8, 10, 11	MBNL1:r(CCUG)8	15-75uM	CCUG-MBNL1 complex disruption
$\begin{array}{c} H_2N \\ & N \\ & N \\ & N \\ & NH_2 \end{array} \xrightarrow{H_2} H_2 \\ & NH_2 \\ & NH_2 \end{array} \xrightarrow{H_2} H_2 \\ & H_2N \\ & NH_2 \\$	DM2 HeLa cell	100uM	Foci reduction
$\begin{array}{c} \begin{array}{c} & & & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ $			
<i>Bis</i> -benzimidazole	MBNL1:r(CUG)109	110uM	CUG-MBNL1 complex disruption
2H-4	MBNL1:r(CUG)109	11uM	CUG-MBNL1 complex disruption
	DM1 HeLa cell	5-25uM	Minigene mis-splicing rescue, foci reduction
2H-K4NMeS	MBNL1:r(CUG)12		CUG-MBNL1 complex disruption
	DM1 patient-derived cell	10nM	Foci reduction, mis-splicing rescue
Cugamycin	DM1 patient-derived cell	1-4uM	Foci reduction, mis-splicing rescue
	DMI HSA <sup>L®</sup> mouse	10mg/kg	Foci reduction, mis-splicing rescue, myotonia rescue
$\begin{array}{c} \begin{array}{c} & & \\ $			
1 Norma of commenced			

<sup>1</sup> Name of compound.<sup>2</sup> Chemical structure of compound.

<sup>3</sup> In vitro or in vivo model used.

<sup>4</sup> Concentrations/ranges or doses where effect was observed in DM model (micromolar concentrations or mg/kg of body weight doses).

COMPOUND <sup>1</sup>		ACTIVE	
STRUCTURE <sup>2</sup>	MODEL <sup>3</sup>	CONC. <sup>4</sup>	OBSERVATION <sup>5</sup>
Compound 4	MBNL1;r(CUG)109	1-100uM	CUG-MBNL1 complex disruption
	DM1 HSA <sup>LR</sup> mouse	40mg/kg	Mis-splicing rescue
H2N NH HN KN KN			
Compound 11	MBNL1:r(CUG)109	1-100uM	CUG-MBNL1 complex disruption
HANNE HANN	DMI HSA <sup>LK</sup> mouse	40mg/kg	Mis-splicing rescue
NH NH2 HN CN CN			
Pentamidine	DM1 HeLa cell	10-75uM	Foci reduction, minigene mis-splicing
HN	(CTG·CAG)54	10-100uM	Transcription inhibition
NH <sub>2</sub> NH <sub>2</sub>	DM1 HSA <sup>LR</sup> mouse	25-40mg/kg	Mis-splicing rescue
Heptamidine	DM1 HeLa cell	5-15uM	Minigene mis-splicing rescue
HN	DM1 HSA <sup>LR</sup> mouse	25-40mg/kg	Mis-splicing rescue, reduced r(CUG) <sub>exp</sub> levels, myotonia rescue
NH2NH2	MBNL1:r(CUG)4	10-125µM	CUG-MBNL1 complex disruption
	DM1 HeLa cell	20-80uM	Foci reduction, mis-splicing rescue
H <sub>2</sub> N O NH <sub>2</sub>	DM1 patient-derived cell	0.25-4uM	Foci reduction, mis-splicing rescue,
			rescued gene expression, increase
	DM1 HSALR mouse	10.30 mg/kg	MBNL1/2 protein levels
	DWITTISA IIIOUSE	10-3011g/Kg	expression, reduced r(CUG) <sub>exp</sub> levels
Neomycin	MBNL1:r(CUG)100	4-64uM	CUG-MBNL1 complex disruption
оно-Сон			
ŇH <sub>2</sub>			
Erythromycin	MBNL1:r(CUG)100	4-64uM	CUG-MBNL1 complex disruption
, ×Ĭ, , ,	DM1 C2C12 cell	50uM	Foci reduction, mis-splicing rescue
HO OH OH OH	DM1 patient-derived cell DM1 HSA <sup>LR</sup> mouse	25-100uM 150-900mg/kg	Mis-splicing rescue, myotonia rescue
OF CONTRACTOR		<i>o</i> , o	
o H <sub>2</sub> O			
HO I			
I and four size		0.01.1	CUC MINI 1 arms 1 - 1' - 1'
Lomotungin	MBNL1:r(CUG)12 DM1 fluc800 cell	0.01-1uM 1011M	CUG-MBNL1 complex disruption
J. N. J.		100101	levels
CHN COH	DM1 C2C12 cell	10uM	Mis-splicing rescue
Ro 31-8220	DM1 patient-derived cell	5-10uM	Foci reduction, mis-splicing rescue,
o Neo			aownregulation of CELFI levels, increased cytoplasmic levels of MBNI
S_NH2	DM2 patient-derived cell	5-10uM	Foci reduction, increased cytoplasmic
N N NH			levels of MBNL
-	DM1 Zebrafish	5uM	Body length phenotype rescue

## TABLE 2. Small molecules identified via screens that target toxic CUG RNA.

	DM1 heart-specific mouse	6mg/kg	Improved cardiac conduction and contractile abnormalities, mis-splicing rescue
APB1 peptide Ac-ppyawe-NH2*	DM1 Drosophila	40-250uM	Increased muscle area, rescued eye and muscle degeneration, foci reduction
	DM1 HSA <sup>LR</sup> mouse	0.5 or 10ug	Mis-splicing rescue, increased Clcn1 expression, reduced central nuclei
Daunorubicin	MBNL1:r(CUG)26 DM1 patient-derived cell DM1 Drosophila	10uM 3-10uM 1uM	CUG-MBNL1 complex disruption Foci reduction Rescued cardiac dysfunction, increased survival, foci reduction, mis- splicing rescue
Chromomycin A3	DM1 patient-derived cell DM1 patient-derived cell	0.04-4uM 0.04-4uM	Foci reduction, mis-splicing rescue, downregulation of CELF1 levels, increased cytoplasmic levels of MBNL Foci reduction, increased cytoplasmic levels of MBNL
И ОН СТАТОН			

 $^{\scriptscriptstyle 1}$  Name of compound.

<sup>2</sup> Chemical structure of compound.

<sup>3</sup> *In vitro* or *in vivo* model used.

<sup>4</sup> Concentrations/ranges or doses where effect was observed in DM model (micromolar concentrations or mg/kg of body weight doses).

 $^{\scriptscriptstyle 5}$  Observations of compound effect on DM molecular pathogenesis or phenotypes in DM model used.

\*Represents the lower case one-letter code for D-amino acids.

TABLE 3.	Small molecules the	at upregulate N	<b>ABNL protein</b>	levels.

COMPOUND <sup>1</sup>		ACTIVE	
STRUCTURE <sup>2</sup>	MODEL <sup>3</sup>	CONC. <sup>4</sup>	OBSERVATION <sup>5</sup>
ISOX	MBNL-tag HeLa cell	1-6uM	Increased MBNL1 protein levels
	DM1 patient-derived cell	5uM	Increased MBNL1 protein levels, mis- splicing rescue
N J '' OH			
Vorinostat	MBNL-tag HeLa cell	1-6uM	Increased MBNL1 protein levels
С Н С С С С С С С С С С С С С С С С С С	DM1 patient-derived cell	5uM	Increased MBNL1 protein levels, mis- splicing rescue
Phenylbutazone (PBZ)	C2C12 cell	50-950uM	Increased Mbnl1 expression
	DM1 HSA <sup>LR</sup> mouse	16.7mg/kg	Increased Mbnl1 protein levels, mis- splicing rescue, improved grip strength, reduction of central nuclei

<sup>1</sup> Name of compound. <sup>2</sup> Chemical structure of compound.

<sup>3</sup> In vitro or in vivo model used.

<sup>4</sup> Concentrations/ranges or doses where effect was observed in DM model (micromolar concentrations or mg/kg of body weight doses).

COMPOUND <sup>1</sup>		ACTIVE	
STRUCTURE <sup>2</sup>	MODEL <sup>3</sup>	CONC.4	OBSERVATION <sup>5</sup>
Manumycin A	DM1 C2C12 cell	10-40uM	Mis-splicing rescue
	DM1 HSALR mouse		Mis-splicing rescue
ACH O			
Lithium	DM1 HSALR mouse	0.24% diet	Improved grip strength, reduced
Li <sup>+</sup>			central nuclei, myotonia rescue
TDZD-8	DM1 HSALR mouse	10mg/kg	Improved grip strength, myotonia
			rescue
O'S'N-			
Tideglusib	Phase II clinical trials	400mg, 1000mg	Safety and efficiency
She she she			
Actinomycin D	DM1 HeLa cell	0.005-0.02uM	Foci reduction, reduction of r(CUG)exp
NO ON			levels
	DM1 patient-derived cell	5-10nM	Reduced r(CUG) <sub>exp</sub> levels
	DM1 HSA <sup>LK</sup> mouse	1.25-2.5mg/kg	Mis-splicing rescue
$\rightarrow$			
4 Jol Jo			
C-16	DM1 patient-derived cell	1uM	Downregulation of CELF1, foci
	1		reduction, mis-splicing rescue
N NH			
l → o N			
C-51	DM1 patient-derived cell	30uM	Downregulation of CELF1, foci
СТА Н			reduction, mis-splicing rescue
<sup>1</sup> Name of compound.			
<sup>2</sup> Chemical structure of compound.			
<sup>3</sup> In vitro or in vivo model used.			

TABLE 4.	Small molecules that affect downstr	ream signaling pathways o	or restore CUGBP1 activity.
	Sman morecures mat arreet aomist	cum orginaling pathways c	

<sup>4</sup> Concentrations/ranges or doses where effect was observed in DM model (micromolar concentrations or mg/kg of body weight doses).

TABLE 5. Small molecules that block transcription of the CTG expansion.

COMPOUND <sup>1</sup>		ACTIVE	
STRUCTURE <sup>2</sup>	MODEL <sup>3</sup>	CONC. <sup>4</sup>	OBSERVATION <sup>5</sup>
Actinomycin D	DM1 HeLa cell	0.005-0.02uM	Foci reduction, reduction of r(CUG)exp
			levels
	DM1 patient-derived cell	5-10nM	Reduced r(CUG)exp levels
	DM1 HSALR mouse	1.25-2.5mg/kg	Mis-splicing rescue
/ >-o HN -o o- NH O /			
N NH2			
- Colto			

<sup>1</sup> Name of compound.

<sup>2</sup> Chemical structure of compound.

<sup>3</sup> In vitro or in vivo model used.

<sup>4</sup> Concentrations/ranges or doses where effect was observed in DM model (micromolar concentrations or mg/kg of body weight doses).