Table 1 Disease Modifying Experimental Therapies for HTT

Strategy	Pro and Con	Citations
Humanized synthetic ZFN-KRAB repressors	+ no risk of DSBs - off target effects - triggers innate immune responses - temporary effects depending on protein turnover	Garriga-Canut, M. *et al.* (2012) Synthetic zinc finger repressors reduce mutant huntingtin expression in the brain of R6/2 mice. *Proc. Natl Acad. Sci.* [54].
CRISPR knockout of mHTT	+ permanent - too large to fit in AAV - requires PAM site near PolyQ tract - bacterial origin of Cas9 elicits innate immune response - CAG repeats within sgRNA form secondary structure, limiting efficiency	Malkki H. (2016) Selective deactivation of Huntington disease mutant allele by CRISPR–Cas9 gene editing. *Nature Reviews Neurology*.
Intrabodies	- immunogenic when injected as naked protein - Nucleic acid delivery requires a large vector such as lentivirus, which integrates genomically and can cause cancer	Cardinale, A *et al.* (2008). The potential of intracellular antibodies for therapeutic targeting of protein-misfolding diseases. Trends in Molecular Medicine [55].
siRNA/miRNA and Antisense Oligonucleotides (ASOs)	+ drug-like properties, more suited to regulation than gene therapy requiring viral vectors + can be easily customized for allele specificity + symptoms can improve for longer than the period of mRNA knockdown ("Huntingtin Holiday") See Note 1. - short acting effect, requires long-term continuous dosing - renal and hepatic toxicity, non-trivial off target effects - inflammatory when recognized by extracellular toll-like receptors	Kordasiewicz, H. B. *et al.* Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. *Neuron*. Rao et al. (2009) siRNA vs. shRNA: Similarities and differences. J. Advanced Drug Delivery Reviews [56].
shRNA-based RNAi	+ longer lasting but not permanent (months to years in primates) + can fit inside an AAV, episomal plasmid in nucleus + shRNA is virally encapsulated and elicits less inflammation from toll-like receptors + constructs can be inserted into an artificial miRNA scaffold to mitigate neurotoxicity specifically - overdose due to excessively strong promoters is common - off target effects can occur	Davidson, B (2008). Artificial miRNAs mitigate shRNA-mediated toxicity in the brain: Implications for the therapeutic development of RNAi. *PNAS* [57]. See also [58, 59].

Note 1: Symptoms are reversed for longer than the period of HTT knockdown [60], known as a 'Huntingtin Holiday,' theoretically enabling cellular repair to occur [61]
Note 2: Designer RNAi possible based on SNPs in loci nearby to the polyQ tract, to prevent theoretical problems associated with WT HTT silencing

a greater knockdown by shRNA and fewer off-target effects (For CDKN1A, 470 transcripts downregulated by siRNA versus 19 by shRNA – of which two were shared). When lentivirus-transduced or expressed from a stable inducible cell line, shRNA showed considerably less OTEs than transfected siRNA with the same 19-mer core sequence. Titrating lower doses of siRNA could not achieve the same signal to noise ratio as shRNA. The authors used a single promoter (H1) – attempts using promoters of varying strength may yield more control over the system.

Comparing large scale RNAi screens, two using shRNA [24, 25] and the other using siRNA [26], the shRNA screens showed minimal OTEs whereas the siRNA screen found OTEs to "dominate" the results.

Another group found shRNA to be significantly more potent than siRNA on a molar basis [27]. The differential potency and accuracy of shRNA versus siRNA may be explained by a few factors: shRNA is treated more like endogenously occurring pri-miRNA hairpins – an shRNA transcript driven by a promoter such as RNA polymerase II (instead of the typically used RNA PolIII promoter) is polyadenylated, processed by Drosha in the nucleus, subject to normal nuclear export, and loaded onto the RISC complex in the cytoplasm [28].

siRNA does not undergo such processing, requires higher concentrations and frequent dosing to achieve comparable knockdown. Unprotected siRNA in the cytoplasm may be vulnerable to degradation and modifications that reduce on-target binding.

shRNA can be further optimized in the form of artificial pri-miRNA transcripts. This is achieved by by embedding the shRNA sequence into a miRNA context