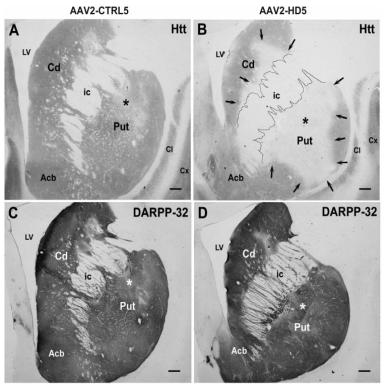
Efficacy of shRNA-mediated non-allele-specific partial suppression of huntingtin in patient-derived iPS cells

Bill Kaemmerer[a], Mike Kaytor[a], Eric Burright[a], Jack Keimel[a], Julia Kaye[b], Ashkan Javaherian[b] and Steven Finkbeiner[b]

[a] *CGTA Research Group, Eagan, MN, a 501(c)3 public charity*
[b] *Gladstone Institute of Neurological Disease, San Francisco, CA*

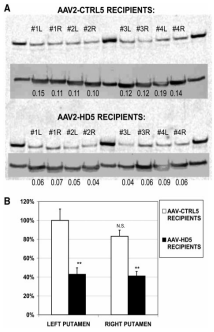
Background: shRNA

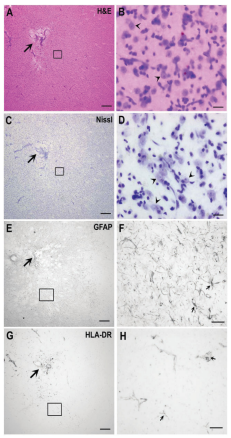
We have previously reported on the development and testing in primates of a short-hairpin RNA (shRNA) targeting the human and rhesus huntingtin (Htt) gene (Grondin, *et al.,* 2012, *Brain*). Bilateral delivery of this shRNA to the caudate and putamen of rhesus monkeys via AAV2 achieved sustained expression of the shRNA transcript and concomitant reduction in Htt mRNA and protein for six months without causing any detectable adverse reactions or evidence of neuropathology.



Htt immunostaining showing substantial reduction in htt protein in the putamen of monkeys receiving the active anti-htt shRNA (AAV2-HD5) but not monkeys receiving a control shRNA (AAV2-CTRL5) comprised of the same shRNA but with the middle 11 nucleotides in scrambled order.

Western blot for htt (top bands) and tubulin (lower bands) in total protein isolated from brain tissue punches from monkeys 6 months post-delivery of AAV2-HD5 or AAV-CTRL5, showing ~ 45% average reduction in htt protein in the putamen of recipients of AAV2-HD5.





No signs of neuropathology in monkeys receiving AAV2-HD5 were seen in life, nor by blinded analysis of tissue sections by a qualified pathologist.

Background: i-neurons

Finkbeiner and others have shown that skin fibroblasts from HD and control donors can be reprogrammed to become iPS cells, then further treated to become cells of a neuronal lineage. Further treatments induce development and maturation of neuronal cells ("i-neurons") including cells that have a medium spiny neuron-like phenotype.

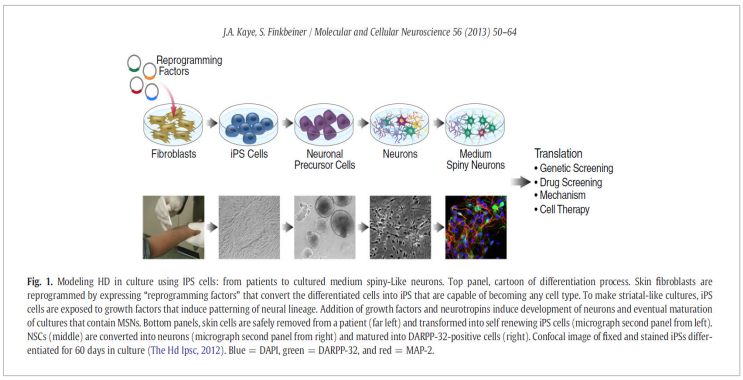
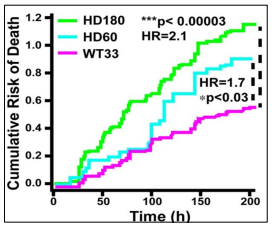
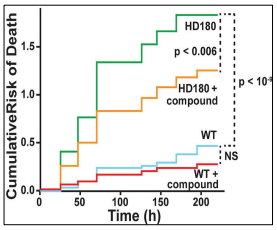


Fig. 1. Modeling HD in culture using IPS cells: from patients to cultured medium spiny-Like neurons. Top panel, cartoon of differentiation process. Skin fibroblasts are reprogrammed by expressing "reprogramming factors" that convert the differentiated cells into iPS that are capable of becoming any cell type. To make striatal-like cultures, iPS cells are exposed to growth factors that induce patterning of neural lineage. Addition of growth factors and neurotropins induce development of neurons and eventual maturation of cultures that contain MSNs. Bottom panels, skin cells are safely removed from a patient (far left) and transformed into self renewing iPS cells (micrograph second panel from left). NSCs (middle) are converted into neurons (micrograph second panel from right) and matured into DARPP-32-positive cells (right). Confocal image of fixed and stained iPSs differentiated for 60 days in culture (The Hd Ipsc, 2012). Blue = DAPI, green = DARPP-32, and red = MAP-2.

Induced pluripotent stem cells from patients with HD show CAG-repeat-expansion-associated phenotypes (HD iPS Consortium, 2012, *Cell Stem Cell*, 11:264-278). Compared to i-neurons from donors with wildtype HD alleles, i-neurons from HD patients with 180 or 60 CAG repeats have a higher cumulative risk of death over time (middle panel, below). The cumulative risk of death can be used as an outcome measure indicative of the effects of treatments, such as application of compound that induces autophagy (right panel, below).





Objective:

"Does non-allele-specific suppression of htt by HD5 enhance the survival of i-neurons derived from HD patients?"

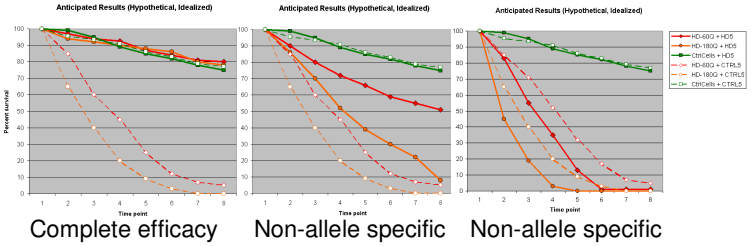
Plan:

Blinded cell culture study, 2 by 3 factorial design

i-neurons: Treatments = transfection with:	HD-60Q	HD-180Q	Control (HD33)
HD5-GFP plasmid	2x	2x	2x
CTRL5-GFP plasmid	2x	2x	2x

Outcome measures: neurite morphology, cell survival by robotic microscopy

Possible outcomes (idealized)



Complete efficacy

Non-allele specific suppression only partially effective

Non-allele specific suppression is toxic

Significance:

- establish whether an agent yielding ~45% suppression *in vivo* is sufficient for phenotypic improvement in neurons, and
- whether non-allele-specific suppression is safe not only in wild type cells, but also in cells expressing both normal and mutant huntingtin.