Questions & Answers, September 11, 2020

** Questions asked by the audience and answered by the panel members in real-time.**

- **Question**

What is the molecular loss of function of mHTT in ERK activation? (How does HTT actvativate ERK?)

Answer

ERK is activated in response to BDNF acting on their receptors TrkB. The vesicles containing these TrkB-containing vesicles activated by BDNF are called TrkB signaling endosomes. They travel from the striatal postsynatic site to the soma and then activate ERK. This trafficking also depends on HTT and is altered in HD neurons. consequently, lower level of BDNF released at synapse and lower level of TrkB activation and trasport leads to reduced activation of ERK in the soma of striatal neurons.

- **Question**

Do you see a decrease in vesicle trafficking if the mutation is within the CAG repeat length seen somatically in HD patients? If not, is there a threshold CAG repeat length at which this effect is seen, and what is it?

Answer

So far the CAG range we have looked at are around 46-60 in human ES cells or IPSC-derived human neurons, or up to 140 CAG in neurons from heterozygous mice. In both cases, we do see strong decrease in trafficking. we have not looked at higher CAG.

- **Question**

Any idea of the time scale at which the effects of reduced BDNF may be observable e.g. via something like cell death or axonal degeneration?

Answer

In vitro, this is very difficult to see consequences such as cell death or axon degeneration. the read out we have are differential activation of survival kinases or calcium synchrony within striatal neurons.

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- **Question**

Have you checked any other markers for analyzing the axonal transport in pre-synaptic neuron inside HD on-a-chip? (for example, lysosome, mitochondria)

Answer

Yes we have looked at TrkB-containing signaling endosomes, APP containing vesicles and synaptic vesicles. Their trafficking depend on HTT and is altered in HD neurons. In contrast, the trafficking of mitochondria does not depend on HTT because the complex trafficking modulating the transport of mitochondria does not require HTT (and HTT does not associate with the motors transporting mitochondria). Our work show that trafficking of mitochondria is altered later as a consequence of neuronal dysfunction but this is not a direct effect of mutant HTT.

- **Question**

Is there any evidence for somatic contraction in brain cells?

Answer

Yes, but expanions are much more frequent (Swami, M., Hendricks, A. E., Gillis, T., Massood, T., Mysore, J., Myers, R. H., & Wheeler, V. C. (2009). Somatic expansion of the Huntington's disease CAG repeat in the brain is associated with an earlier age of disease onset. Human Molecular Genetics, 18(16), 3039–3047. http://doi.org/10.1093/hmg/ddp242)

- **Question**

Does mutant Htt also affects the ciliary traficking in other tissues?

Answer

We also looked at epithelial cells and primary neurons that are monociliated. We also found differences in their size when containing the HD mutation.

- **Question**

My question is that since gene therapy is coming for HD, is there still significance for exploring the modification factors for age of onset?

Answer

Yes. There are very encouraging signs that the HTT lowering ASOs may be beneficial, but we do not how effective they will be in absolutey stopping disease and we must continue to explore all possible ways to tackle the disease. AS for gene therapy by gene editing, very primoising, but we are yaesr away from any sort of trial to edit the HTT gene in vivo with sfaftey and delivery remaiing as major challenges. In lloknig for new targets, there is a lot of evidence that drug targets that have human genetics support have a much higher likelihood of providing effective therapies, with a diret way of asessing likley therapeutic impacts by comparison to naturally occuring variants. Thus lots of reasson why we shoudl continue to identify genetic [and environmental] modifiers of the disease pathway.

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** Questions asked by the audience and answered by the panel members in real-time.**

- **Question**

Might PCM1 and MSH3 be scaffolded for autophagic degradation by HTT, impaired by polyQ expansion, resulting in accumulation of PCM1 and MSH3 impacting ciliogenesis and somatic expansion?

Answer

That is a possibility indeed.

- **Question**

Primary cilia are important during embryonic development - is there any indication of a neurodevelopmental component in HD (which might change the way we look at the disease)?

Answer

I mentioned in my talk the work of Sandrine Humbert that has been recenty published in Science. Indeed, they showed that ciliogenesis is altered early in human (at gestation week in huma HD fetuses obtained from medical abortion)

- **Question**

So targeting mhtt trough tbk1 inearly stages of disease as it acts on soluble form or monomer form og mhtt.

Answer

Indeed, our findings suggest that TBK1 activation would be more effective during early stages of the disease, possibly once genetic diagnosis is made. Personally , I believe that lowering Htt may also not be sufficient in advanced stages of the disease. We will need combination therapiess aimed at lowering Htt levels and removing seeding competent aggregates that are already there in the brain. Of course, this is based on the assumption that Htt aggregation plays central role in the pathogenesis of HD.

- **Question**

How does TBK1 differentiate between the mutant and WT HTT? Because TBK1 phosphorylates monomers of HTT and also enhaces the HTTs autophagy, is it tagging the WT HTT for autophagy, as the mHTT is forming aggregates to which TBK1 cannot phosphorylate?

Answer

TBK1 phosphorylates both WT and mutant HTT monomers, but not the fibrillar forms of both proteins

- **Question**

Is there a TBK1 KO mouse and what is the phenoytpe?

Answer

TThe Tbk1Δ/Δ homozygous mutation is lethal. In our work (EMBO paper), we used a mouse model of Tbk1−/− on the Tnf-α−/− background (https://pubmed.ncbi.nlm.nih.gov/15210742/)

Questions & Answers, September 11, 2020

** Questions asked by the audience and answered by the panel members in real-time.**

- **Question**

Just personal curiosity : How can we utilize the role of TBK1's activity in therapeutical way? Many small molecule drugs are likely to be enzymatic inhibitor rather than enzymatic activator. Is the upsteam regulator of TBK1?Correction : Is there upsteam regulator of TBK1?

Answer

Very little is known, but a couple Ca and CK2-dependent mechanisms have been proposed. More work is needed to map upstream TBK1 activation signaling cascades, especially in neurons.

- **Question**

Are you concerned that activation of TBK1 after age-induced lysosomal impairment might induce cell death?

Answer

Yes. Given that TBK1 has multiple functions, one has to be careful about which approaches are used to upregulate or activate the proteins. This is why I emphasized that we need to know more the mechanisms of TBK1 activation. TBK1 activation is usually driven by recruitment to discrete signaling complexes. It would be interesting to explore mechanisms of TBK1 activation in the brain and whether there neuron or cell-type-specific TBK1 activation signaling pathways that could be exploited for selective upregulation of TBK1.

- **Question**

Growing out from the centriole, cells normally only have one primary cilium, are the cilia shown to be extended all primary cilia?

Answer

Yes cells with primary cilia have also longer cilia.

- **Question**

Can you speculate whether HTT phosphorylation levels could become a predictive biomarker, e.g. high phosphorylation levels indicative of slow progression?

Answer

All our in vitro studies and cellular data suggest that we should see lower levels of phosphorylated HTT in HD patients. Whether it could be a predictive biomarker or not is not yet clear. We still need more reliable tools and assay to measure phosphroylated HTT levels in biological fluids. We desperately need such assays.

A couple years ago, we reported in a study with our colleagues at IRBM that “Phosphorylation of huntingtin at residue T3 is decreased in Huntington's disease and modulates mutant huntingtin protein conformation” https://pubmed.ncbi.nlm.nih.gov/29162692/

Questions & Answers, September 11, 2020

** Questions asked by the audience and answered by the panel members in real-time.**

- **Question**

Do you know whether Htt pays a role in vescicle traficking in heart muscles?

Answer

We never tested this. but HTT is present in muscle so that's a possibility.

- **Question**

How spefic for HTT will such an oral splicing modifyer be?

Answer

Unlikely to be absolutely specific to HTT alone but precedent for other splcing modulators in the literature is that surprisingly few genes may be affected - selectivity not disclosed yet for PTC program - look out for their publication in the coming months.

- **Question**

Would the pseudo exon approach work for other genetic disorders (or has it been used already?), where degradation of mRNA would prevent a toxic gan of function?

Answer

In principle splicng modulators may be found for other disease relevant genes - Pfizer have published splicing modulators for tau for example and PTC/Roch for SMN2. It depends on the gene structures of the targets and whether they have some unusual combinations of pseuoexons (for lowering) or alternatively splcied transcripts for increasing expression/changing isoforms.

- **Question**

It's wonderful to know that oral drug for HD is having bioavailability 100%.

Answer

The compound i showed is not necesarily representative of the clinical candidates' profile but certainly shows it can be done in mice at least.

- **Question**

Whether this specifically targets mutant htt alone?

Answer

I mostly answered this in the panel - unlikely to be absolutely specific to HTT alone but precedent for other splcing modulators in the literature is that relatively few genes may be affected not disclosed yet for PTC program - look out for their publication in the coming months.

See answer to Q20(note this approach is not mHTT allele selective)

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** Questions asked by the audience and answered by the panel members in real-time.**

- **Question**

Can Darren and Fred comment upon the effects upon the cell cycle and mitosis in early development?

Answer

Not really! There is evdience for some very early repeat length mutations, but these are pretty rare for smaller expansions (40-50). Thus I think any neurodevelopmental effects are likely to be largely independent of somatic expansion

Hello, Tissues from HD fetuses show mislocalization of mutant huntingtin and junctional complex proteins, defects in neuroprogenitor cell polarity and differentiation, abnormal ciliogenesis, and changes in mitosis and cell cycle progression. Altogether this results in fewer proliferating cells and more neural progenitors prematurely entering lineage specification. Same phenomena is observed in several HD mouse models. As said by D. Monckton, whether this can be linked to somatic expansion is unknown so far … as far as I know, we don't known about somatic expansion/contraction in fetuses.

- **Question**

Do you think the large repeat mosaicism (which we observe in the pediatric cases and is substantially and significantly larger than in adults) may interfere with the brain development in HD children?

Answer

It may and is definitely worth to further investigate. We should discuss!!

- **Question**

PRECISION-HD: why is NfL not goig down?

Answer

We can only speculate but maybe a visible effect on Nfl cannot be seen because of the short time period (5 months). We know that after a head trauma NFL normalizes within 3 months, so this might be the quickest time period in which an effect might be seen with an efficient neuroprotective drug.

Second, the analysed data concern the lower dose groups (2,4,8,16 mg) and reduction of 12,4% of mHTT was observed for those groups. Maybe this limited reduction of only 12% is not enough to inverse the natural increase of NFL? It will be interesting to see the 34 mg group data.

- **Question**

What is the allele specificity and efficiency in preclinical cell and animal models? Does the ASO only downregulate the mutant protein? Only 1 nucleotide difference between the mutant and wild type target.Answer

Answer

In vitro studies showed selective knockdown of mHTT compared to wtHTT. In studies with fibroblasts derived from patients, heterozygous for either SNP1 or SNP2, WVE-120101 ou 120102 , respectively, resulted in knockdown for mHTT without meaningful wtHTT reduction in the heterozygous fibroblast, suggesting target selectivity by differentiation between the mutant and wild-type HTT transcripts despite only 1 bp difference in nucleotide

sequence. Further, in an in vitro reporter assay system, WVE-120101 and WVE-120102 were shown to differentiate between mutant and wild-type transcripts as compared with stereorandom compounds of the same sequence which were found to be non-selective

There were no animal studies done to evaluate the allele specifity or ability to lower mHTT because of the lack of an appropriate animal model. Tissue distribution and toxicity was evaluated in mice, rats and monkeys to support clinical dosing.

- **Question**

I think I missed this, was the 12% Htt reduction measuring total Htt or did you have a specific assay for the mutant?

Answer

The 12% reduction concerns mHTT. Total Huntingtin has also been measured and there was no difference between the two groups.

- **Question**

What would be the effect of targeting DDR genes on cancer?

Answer

There have been a few questions about the cancer risk - this is a very important point with respect to targeting DDR pathways. The proposed Triplet therapeutic would be given intrathecally so should access the CNS but not other body tissues, which woud very much reduce the risk.

Anne makes a very relevant point in terms of targeted delievry to brain tissue. In addition, there are marked differences between ddr genes. For some genes LoF mutations cause cancer, such as Lynch Syndrome for MSH2. For others genes, human and animal genetics show normal life span and no impact on tumor incidence with LoF variants.

Small reductions of expression of some DDR genes is known to reduce the occurance of triplet repeat expansions without increasing the risk of cancer. For example the MSH3 variant described in the presentation is known to be associated with lower levels of somatic expansion of the HTT and the DM1 (Myotonic Dystrophy type 1, caused by a CTG expansion) repeat but this variant is also known not to be associated with an increased risk of cancer.

Very large decrease in MSH3 expression are needed to increase the risk of cancer.

Not all the DDR genes are good target

- **Question**

Are some participant inGeneration HD 1 suffers from nfections?

Answer

The trial is ongoing, there is no data on this.

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- **Question**

How can we become involved in and follow The Heated Project?

Answer

Thanks for showing an interest in the HEATED project. I think there are some things you can do and some things the HEATED group can do!

Firstly, whoever you are in the HD community, you can start by raising awareness about this issue and finding out about how things are locally.

You may have a specific research interest you want to follow in this area. If that's the case then the HEATED group can help you to pursue that.

You may have some specific local or general knowledge that you could share with the HEATED group.

You could ask to make sure that the HEATED issue is raised in the sorts of meetings you attend.

I'm happy to come and talk to meetings about this issue and help people decide what to do.

As I said in the talk, this is a problem that the whole community needs to get involved with at many levels. Anything you can do to get that going would be useful.

- **Question**

With the definition of HD you have used (i.e. all HD mutation carriers) the prevalence of HD would be around 50 per 100,000 (e.g. Kay et al. 2016). Much higher than the disease prevalence (as defined by clinical manifestation). How much do you expect the cost of the drugs to decrease if we consider/present HD like that?

Answer

It's a really good question. I guess you wouldn't give it to people whose brains have not started to deteriorate (I think), so that brings the number down a little bit. Even if you take that into account I really don't know how much it will change the cost. My guess is that it won't change things much!