Restoring Vision - Optogenetic Gene Therapy Targeted at Human ON-Bipolar Cells

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Questions & Answers from the Presentation

If AAV cargo size is limiting, for your chosen gene replacement, have you tried alternative carriers (e.g., nanoparticles)?

> Every vector/carrier has advantages and disadvantages. For the eye, AAV is currently the vector of choice. We designed our promoters accordingly. We did, however, use lentiviral vectors in early experiments (where we could not yet keep explants in culture for long), not because of the packaging size, but because of the fast onset of transgene expression.

Have you also considered using promoters other than the one of GRM6 as templates for promoter design?

> Yes, we identified promising endogenous promoters to use as templates for promoter design and chose three additional promoters to test, based on the TNNT1, TRNP1 and TRPM1 promoters. None produced the desired ON-bipolar cell-specific expression.

Have you tried mGLUR6 expression in other cells than retinal cells (CNS neurons)?

> No. We were specifically interested in expression in the retina.

Did you treat both eyes, or did you use the contralateral eye as a mock-injected control?

> We always treated both eyes.

Did you also check the promoter described by Macé E et al 2015?

> If I am not mistaken, the promoter used in **Macés paper** is the original promoter designed by **Kim et al. 2008**. It is constructed of the SV40 basal promoter and the 200 bp murine enhancer. We only made experiments with its "upgraded" version, the 4xGRM6-SV40 promoter with the quadruple tandem repeat of the 200 bp enhancer (**Cronin et al. 2014**).

What is the max durability of expression you observed (i.e. how many months post injection)?

> Up to approx. 1 year in wild type mice. We did not quantify changes in transgene expression levels, however.

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Can the explant cultures be maintained for longer to check for off-target expression in cells which may have reduced transduction efficiency and delayed expression? (can you re-mention the time that you kept the explants in culture?)

> Yes, they could. In the experiments for the publication, we kept the explants in culture for 7 days. We improved the culturing however and could have cultured them for extended periods of time (multiple weeks).

Did you assess any other functional measures in your treated vs untreated rd1 mice (e.g.ERG)? What about functional measures in the human retinal explants (e.g. patch clamp experiments)?

> No, not for this publication. The Kleinlogel lab is however establishing eclectrophysiological experiments for AAV-treated human retinal explants, and are doing patch clamp, MEA and V1-recording experiments for treated mice in the meantime.

Did you see any contralateral eye rescue? Often with gene therapy via AAV we see a contralateral eye rescue? either via IHC or functional assessment?

> No. We could not since we did bilateral injections.

Did you see any toxicity in your promoter? Some promoters, including CMV, were shown to be toxic in the retina.

> No, but we also did not assess toxicity. However, I expect the toxicity from CMV to be mostly due to its strong (excessive?) expression strength, its unspecific expression and/or its viral origin. None of those are factors concerning our promoter.

Do you plan on evaluating the long promoter in vivo in Non-human primates by IVT injection?

> This is confidential information of Arctos Medical AG. For any inquiries, please contact Sonja Kleinlogel.

In the human explant experiment, only Grm6-GFP is expressed. Did you try Grm6-optogenetic tool-GFP?

> No, not at that time.

Have you considered the genetic sequence of non-human primates during promotor design? I am interested in conversation across different species and wonder whether this construct would be usable in a in vivo primate study for translational purposes.

> No, I did not. Since the human genome-based promoter is effective in both the human and the mouse retina, I would expect it to be effective in non-human primates retina as well.

Did you consider doing MEA recording with the human explant?

> Yes. We however needed to improve the longevity of our explant cultures first. The Kleinlogel lab have done or are planning those experiments now.

You still have some space left for additional DNA in your AAVs - have you or will you try a multimerized version of your P(L) enhancer element to try and achieve higher levels of expression?

> I did not and I think we will not. The enhancer is the larger part of the promoter so tandem repeats will eat up space fast and we showed very promising results in our rd1 mice. This indicates that expression levels are adequate. Too strong expression can lead to a burden for the cells and increased off-target expression.

Were your injections in the mouse in vivo targeted to particular regions, e.g. central vs periphery? If so, do you see different levels and cell type selectivity of viral transduction for injections directed to different retinal regions?

> We injected intravitreally. In general, we saw a somewhat higher transduction efficacy in non-central regions. We did not see noticeable differences in cell-type specificity in different regions.

Can you elaborate a little more on the parameters used for the optokinetics? Was the study done with 100% contrast, any other parameters tested?

> The brightness of the screens was 1013 photons cm-2 s-1 and the contrast was set to maximum.

Were the human donor retinae checked for mutations linked to a human retinal disease?

> No.

Why don't you need all of the components of the visual cycle to maintain the rhodopsin function?

> As evidenced by our experiments using OPN1mw in rd1 mice as well as by other researchers using rhodopsin or channelrhodopsin, it appears that the degenerated retina easily provides/regenerates enough 11-cis-retinal for the optogenetic tools to be functional.

How does the OPN1mw work in bipolar cell?

> Vertebrate Opsin is a G-protein coupled receptor which, in photoreceptors, activates transducin. It has been **shown as early as 2005** that ectopically expressed opsins can couple to and activate Gi/o pathways in other neurons as well. In ON bipolar cells, opsin can activate the natural intracellular G-protein coupled signalling cascade, taking advantage of the internal mechanisms of signal amplification and adaptation.

Regarding the OptoDrum did you test different spatial frequencies (cycles/degree)?

> Yes, that is how we measured the visual acuity.

How is the vector delivered?

> In human retinal explants by placing the virus directly onto the explant (which on day 0 of culturing was not submerged in medium). In mice by intravitreal injection.