Safety Studies for Cell-Based Gene Therapy (TargetAMD project): In Vivo Exclusion of Tumorigenicity and Proof of Cell Product Quality

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Purpose
Personalized medicine demands customized control strategies to ensure patient safety. TargetAMD aims at completion of a clinical trial for the personalized treatment of neovascular age-related macular degeneration (nAMD). Within one surgical intervention, autologous iris pigment epithelial (IPE) cells will be transfected with the gene for pigment epithelium-derived factor (PDE) and transplanted subretinally in the same patient to suppress choroidal neovascularization by decreasing the expression of vascular endothelial growth factor.

Methods
Rabbit IPE cells were transfected with the PEDF or the Venus reporter gene using the SB100XpSFAR4-miRNAplasmid technology and transfection efficiency determined by imaged-based cytometry. To demonstrate the feasibility of isolating, transfecting and transplanting IPE cells in one hour, cells were isolated, transfected and transplanted in 4 rabbits. Biodistribution was examined by transplanting subretinally in rabbits 20’000 PEDF-transfected homologous IPE cells and from 1 to 90 days, 43 organs were analyzed macroscopically and histologically for abnormalities and by qRT-PCR for the presence of the vector or PEDF.

Results
Table 1 lists controlled organs and taken biopsies. During follow-up welfare of the animals was documented including weight control (Fig. 1) and intra ocular pressure (IOP) measurements (Fig. 2). Transfection efficiency measured by imaged-based cytometry and anti-Venus staining revealed a reproducible, high efficacy of 72.4±29.7% (rbRPE) and 45.3±15.7% (rbIPE), respectively (Fig. 3). Weight of core organs showed no abnormalities (Fig. 4). A validation run proved the feasibility of the approach and follow funduscopy confirmed tolerability of the transplant (Fig. 5). Cell isolation time was 30 minutes (+/-5 min of trypsination, unnecessary for human cell isolation), transplantation plus delivery to operating room was 20 minutes and transplantation 10 minutes, for a total of ~60 minutes. No systemic trafficking of transplanted cells was observed by qRT-PCR so far, but Venus-transfected cells could be retrieved 1h, 7 and 90 days post-transplantation (Fig. 6). No gross or histological abnormalities were found after transplantation of transfected IPE cells.

Conclusions
It is evident from the results above that cell isolation, transfection and transplantation within one hour is feasible and safe, since organ integrity is not affected and transplanted cells are not trafficking systemically.

Figure 1. Weight control. Weekly weighing during follow-up confirmed well-being of the treated rabbits by gain of weight from 3.5±0.54 kg to 4.06±0.42 kg.

Figure 2. IOP determination. IOP was measured weekly during follow-up and remained mostly constant from 13.0±2.1 to 14.5±0.1 mmHg (treated eyes) and from 11.9±2.1 to 12.0±0.1 mmHg (control eyes), respectively, with no differences between the treated and the control eyes.

Figure 3. Organ weights. Measured were the weights of the core organs brain, heart, lung, liver, spleen and kidney. None of the organs showed morphological abnormalities and all weights were in the normal range.

Figure 4. Subretinal transplantation, validation run and funduscopy control. [A] Representative image of a subretinal bed after transplantation. [B-C] Normal fundus at day 7 and 90. [D-E] Steps of the validation run including indentation, vitreotomy and subretinal transplantation.

Figure 5. Subretinal transplantation, validation run and funduscopy control. [A] Representative image of a subretinal bed after transplantation. [B-C] Normal fundus at day 7 and 90. [D] Steps of the validation run including indentation, vitreotomy and subretinal transplantation.

Figure 6. Detection of Venus’ cells in treated retina at 2h, day 7 and 90 post-transplantation. To unambiguously distinguish auto-fluorescence or artefacts from Venus-positive signals samples were stained with an anti-Venus antibody followed by an AlexaFluor™ 488 conjugated secondary antibody. After 2h, 7 and 90 days of follow-up cells could be retrieved only in the area of the subretinal bleb lying as a “string of pearls” above the photoreceptor layer.