Development of a novel adeno-associated viral vector in combination with tolerogenic nanoparticles for the treatment of Ornithine Transcarbamylase Deficiency

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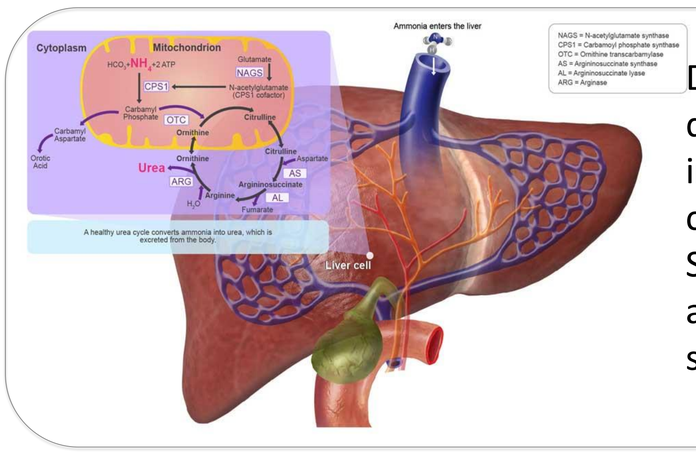

Despite dietary protein restriction and ammonia scavenging drugs, children with ornithine transcarbamylase deficiency (OTCd), a urea cycle disorder, develop neurological damage caused by hyperammonemia, and many die in the first two decades of life. Our aim is to develop AAV vectors encoding the hOTC gene for administration in combination with biodegradable synthetic vaccine particles containing rapamycin (SVP-R). In pre-clinical studies SVP-R blocked humoral and cellular immune responses to AAV, which in OTCd would have two major benefits: 1) ability to treat patients at an early age, while maintaining the possibility to re-dose, and 2) minimize use of steroids, which may trigger metabolic crisis.

Optimization of the transgene cassette expressing human OTC transgene under the transcriptional control of a liver-specific promoter

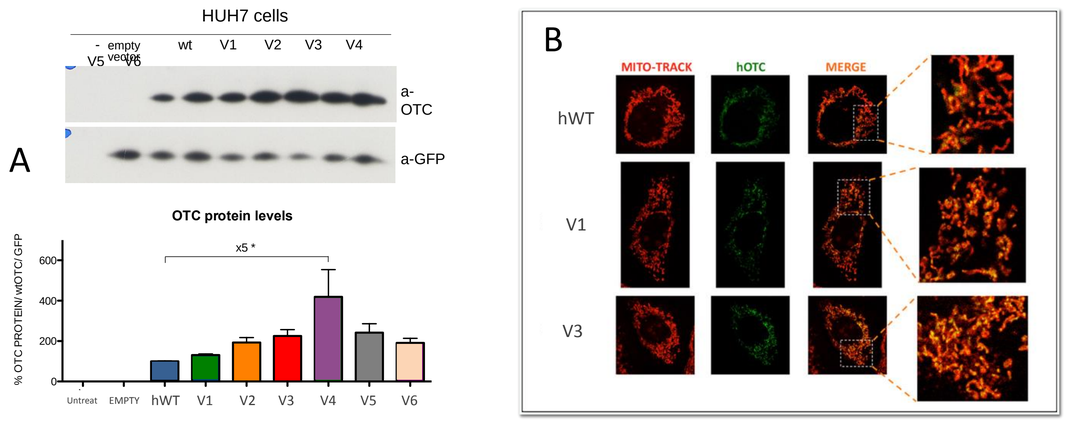

Figure 1. wt-hOTC and 6 codon-optimized version of hOTC were transfected into HUH7 cells. (**A**) WB analysis of OTC protein in total liver lysate and band quantification. (**B**) Sub-cellular study of wt-hOTC and 2 representative co-hOTCs into mitochondria by co-immunofluorescence with anti-OTC and mitochondrial marker.

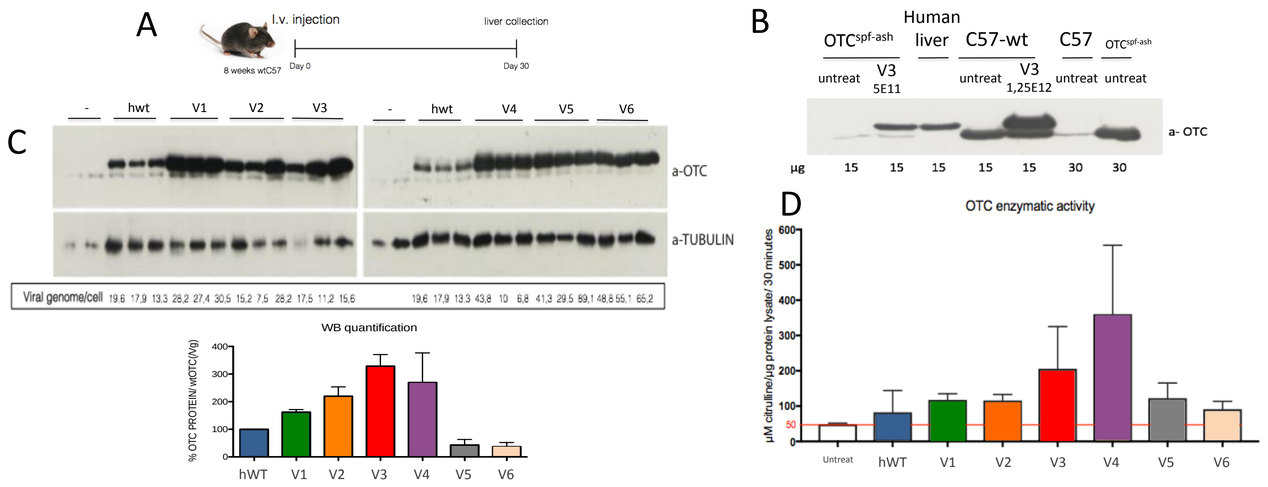

Figure 2. AAV8-WT-hOTC and co-OTC viruses were used to infect WT C57Bl/6 (5E12 vgp/kg) to compare vector efficiency in liver transduction. (**A**) 8 weeks-old mice were i.v. injected with viruses and sacrificed 1 month-post injection. (**B**) WB analysis of human liver extract and mouse liver extract. (**C**) WB analysis of injected animals and band quantification. (**D**) *In vitro* enzymatic OTC activity in liver extracts of injected animals.

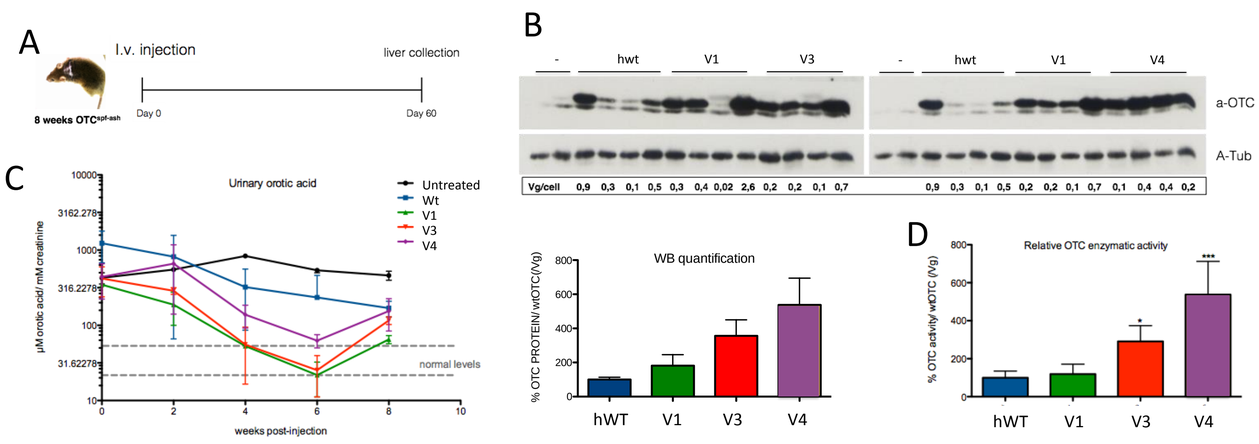

Figure 3. AAV8-wt-hOTC and the 3 best codon-optimized version of hOTC were used to transduce OTC$^{spf\text{-}ash}$ mice (5E11 vgp/kg) to test their efficacy to correct the phenotype. 8/10 weeks-old mice were i.v. injected with viruses and sacrificed 2 month-post injection. (**B**) WB analysis of liver extracts and band quantification. (**C**) Urinary Orotic acid measurement by Mass spectrometry . (**D**) *In vitro* enzymatic OTC activity in liver extracts of injected animals, results are represented as a fold change respect to wt construct.

Selection of a AAV capsid with the ability to target the liver with high efficiency in nonhuman primates

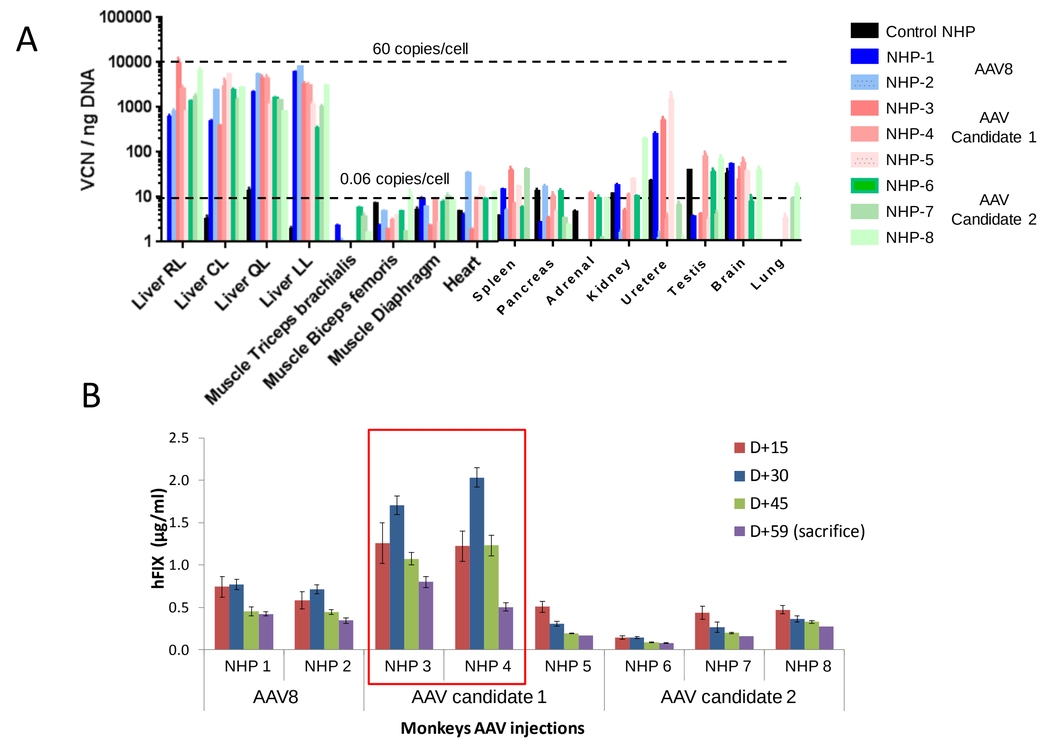

Figure 4. Male *macaca fascicularis* monkeys were dosed with 5E12 vg/kg of AAV8 or two candidate AAV vectors expressing human coagulation factor IX (hFIX). A) Biodistribution of AAV was assessed at day 60 after vector dosing. B) Human FIX expression in serum was assessed by ELISA at different time points as indicated.

SVP-Rapamycin inhibits T and B cell responses to AAV and enables successful vector re-administration

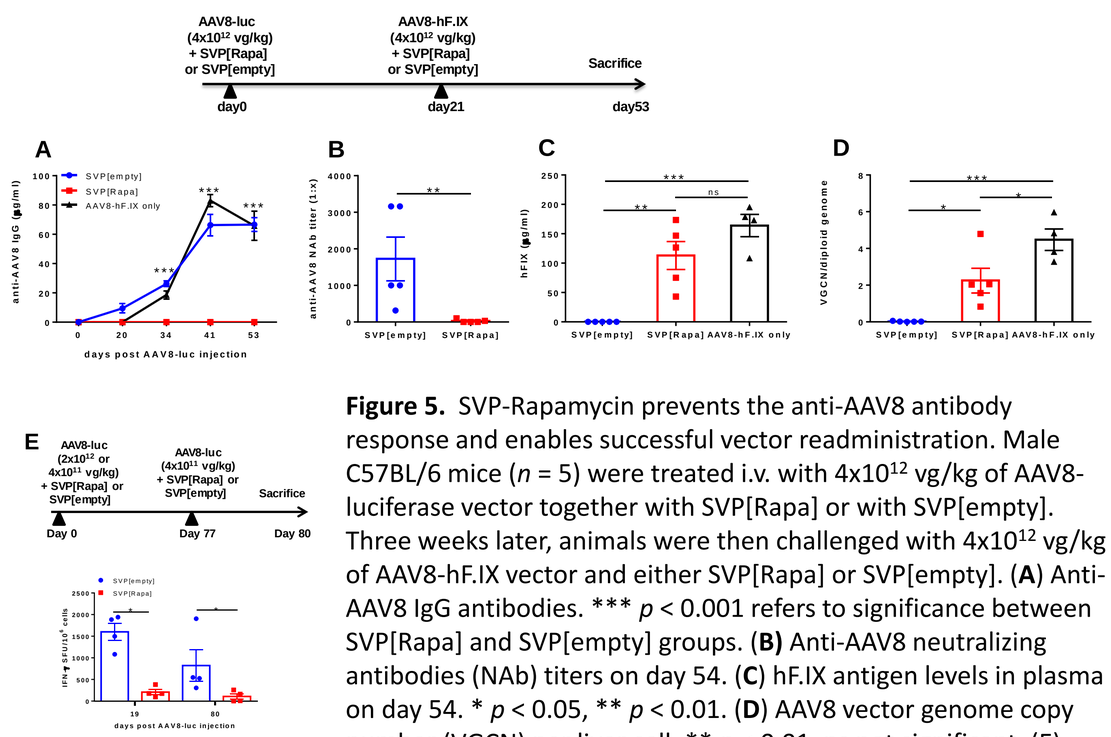

Figure 5. SVP-Rapamycin prevents the anti-AAV8 antibody response and enables successful vector readministration. Male C57BL/6 mice (n = 5) were treated i.v. with 4x10^{12} vg/kg of AAV8-luciferase vector together with SVP[Rapa] or with SVP[empty]. Three weeks later, animals were then challenged with 4x10^{12} vg/kg of AAV8-hF.IX vector and either SVP[Rapa] or SVP[empty]. (**A**) Anti-AAV8 IgG antibodies. *** $p < 0.001$ refers to significance between SVP[Rapa] and SVP[empty] groups. (**B**) Anti-AAV8 neutralizing antibodies (NAb) titers on day 54. (**C**) hF.IX antigen levels in plasma on day 54. * $p < 0.05$, ** $p < 0.01$. (**D**) AAV8 vector genome copy number (VGCN) per liver cell. ** $p < 0.01$, ns not significant. (E) SVP-Rapamycin inhibits T cell responses. IFN-γ EliSpot responses after overnight stimulation with AAV8 peptides pool.