National Human Genome Research Institute

Anc80L65 and SVP-Rapamycin: A novel approach to AAV gene therapy for methylmalonic acidemia

Randy J. Chandler[1], Lina Li[1], Eva Andres-Mateos[2], Ru Xiao[2], Petr Ilyinskii[3], Christopher J.Roy[3], Alicia M. Michaud[3], Takashi Kei Kishimoto[3], Luk H. Vandenberghe[2], Charles P. Venditti[1]

[1] NHGRI, NIH, Bethesda, MD. [2]Grousbeck Gene Therapy Center, MEEI, Harvard Medical School, Boston, MA. [3]Selecta Biosciences, Inc. Watertown, MA

Abstract

Methylmalonic acidemia (MMA) is a severe organic acidemia frequently caused by mutations in methylmalonyl-CoA mutase (MUT). Severely affected patients can benefit from liver transplantation and may require kidney transplantation due to renal failure. Our aim is to develop AAV gene therapy for MUT MMA patients. We plan to use synthetic vaccine particles containing rapamycin (SVP-R) to mitigate the immunogenicity of the AAV capsid, with the goal of retaining the possibility to re-dose patients later in life. The use of a synthetic Anc80L65 capsid has the additional advantage of reducing the chance that naturally occurring anti-AAV antibodies would impact Anc80-mediated gene therapy. We have therefore generated a series of Anc80L65 and AAV8 vectors to deliver the MUT gene. Anc80L65 vectors that used either a liver-specific promoter or constitutive promoter in wild-type mice showed similar levels of hepatic MUT expression, while Anc80L65 vector with a constitutive promoter also showed significant expression in the kidney. After treatment with Anc80L65 or AAV8 vectors at doses between 5e10^11 and 6e10^12 GC/kg, a hypomorphic murine model of MMA displayed improved growth, reduced levels of circulating metabolites, and increased MUT activity. The effects of AAV gene therapy with all vectors was apparent by 12 days, and has persisted for as long as one year. Furthermore, Anc80L65 vectors administered to newborn mice (1e10^13 GC/kg) were effective in rescuing a murine model of MMA with a neonatal-lethal phenotype. Addition of SVP-R to Anc80L65 inhibited the formation of anti-Anc80 IgG antibodies. SVP-R also inhibited the T cell response to AAV. Our results suggest Anc80L65 MUT vectors are potent and, in combination with SVP-R, has the potential to comprise a treatment for MMA that could delay or eliminate the need for transplantation, and enable vector re-administration.

Background

Methylmalonic Acidemia (MMA)

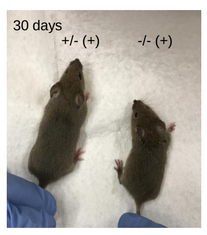
- MMA is a group of recessive genetic disorders with a prevalence of ~1:50,000 at birth.
- The majority of patients with MMA have mutations in the methylmalonyl-CoA mutase (MUT) gene.
- The loss of MUT activity hinders the conversion of propionyl-CoA to L-methylmalonyl-CoA and results in elevation of methylmalonic acid in the plasma and urine.
- Clinically this disorder is characterized by metabolic instability, poor growth, increased morbidity and early mortality.
- Severely affected patients can benefit from elective liver transplantation.
- Gene therapy, systemic and/or liver-directed, in young patients has the potential to correct the major complications of the disease.
- The ability to re-administer gene therapy as the expression of the therapeutic transgene wanes over time is an important consideration for this life long affliction.
- Currently, vector re-administration is limited by the formation of neutralizing antibodies against the AAV capsid.

Murine Models of MMA

Neonatal Lethal Model of MMA (*Mut* $^{-/-}$)

This murine model was made by knocking out the 3rd exon of the *Mut* gene. *Mut*$^{-/-}$ mice have no detectable *Mut* mRNA or Mut protein expression. *Mut*$^{-/-}$ mice have elevations of methylmalonic acid and display a lethal phenotype with most pups dying a few days after birth

Severe Hypomorphic Model of MMA (*Mut* $^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$)



Muscle Specific *Mut* Rescue

Tg$^{INS\text{-}MCK\text{-}Mut}$ rescue construct

This murine model is rescued from lethality by *Mut* transgene expression in the skeletal and cardiac muscle but displays a severe MMA phenotype . *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice have elevated methylmalonic acid and exhibit delayed growth. This model of MMA is well suited to measure MUT correction in the liver.

Anc80L65 AAV Capsid

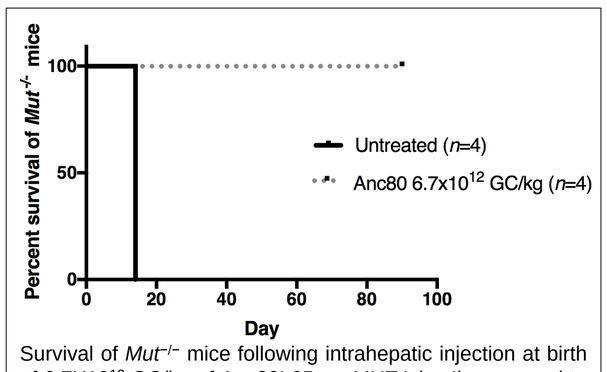
- In silico-designed synthetic AAV capsid
- A putative ancestor of natural AAV serotypes AAV2, AAV8 and AAV9
- Reduced cross-reactivity with neutralizing antibodies against wild type AAV serotypes

SVP-Rapamycin

- Biodegradable synthetic vaccine particles encapsulating rapamycin, an mTOR inhibitor
- Mitigates immunogenicity of biologic drugs
- Currently in Phase 2 clinical trials in combination with a pegylated uricase enzyme for the treatment of severe chronic gout

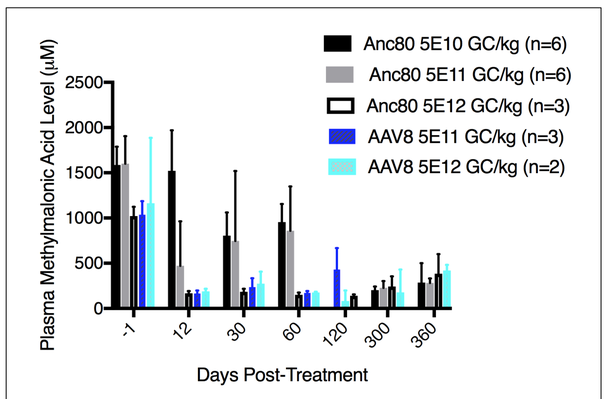
Results

Mut$^{-/-}$ neonatal rescue with Anc80L65



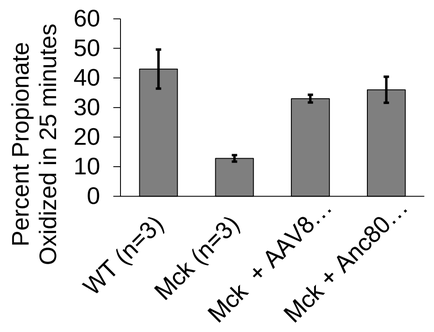
Survival of *Mut*$^{-/-}$ mice following intrahepatic injection at birth of 6.7X10^{12} GC/kg of Anc80L65-synMUT4 (*n*=4) compared to untreated *Mut*$^{-/-}$ mice (*n*=4). *Mut*$^{-/-}$ mice used for this comparison were derived from the same breeding unit. (*P*<0.001).

Plasma methylmalonic acid levels decrease after Anc80L65 or AAV8 gene therapy



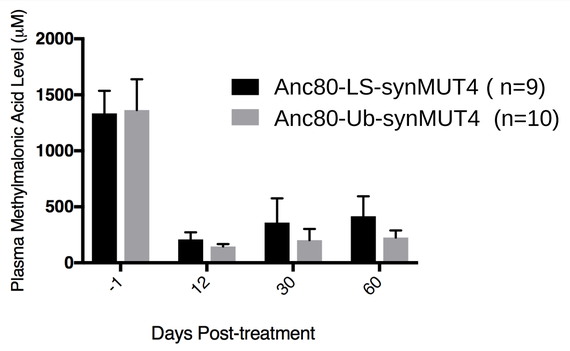
Plasma methylmalonic acid levels of adult (2-8 months of age) *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice following retro-orbital injection of dose ranging from 5X10^{10} to 5X10^{12} GC/kg of Anc80L65-LS-synMUT4 or AAV8-LS-synMUT4. Wild-type mice have plasma methylmalonic acid levels of 5-10 μM (not depicted). LS = liver-specific promoter

Increased MUT activity after Anc80L65 or AAV8 gene delivery



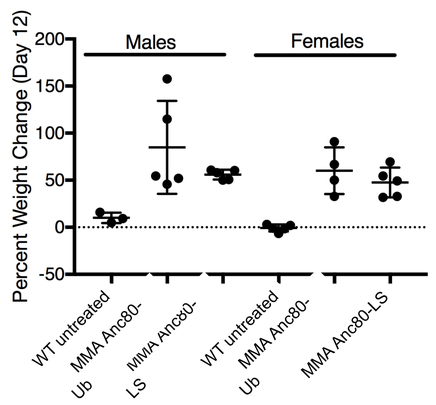
1-^{13}C propionate oxidation was measured in adult (2-8 months of age) *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ (Mck) mice 12 days after retro-orbital injection of a dose of 5X10^{12} GC/kg of Anc80L65-LS-synMUT4 or AAV8-LS-synMUT4 in comparison to untreated wild-type (WT) mice.

Plasma methylmalonic acid levels decrease after Anc80L65 gene delivery liver-specific or ubiquitous promoter



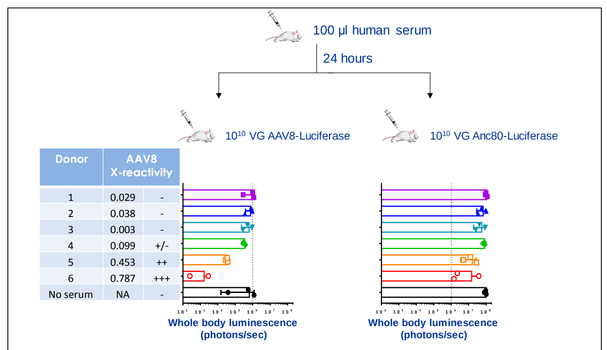
Plasma methylmalonic acid levels of adult (2-4 months of age) *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice following retro-orbital injection of a dose of 6X10^{12} GC/kg of Anc80L65-synMUT4 with either a liver-specific (LS) or a ubiquitous (Ub) promoter.

Increased growth after Anc80L65 gene delivery using liver-specific or ubiquitous promoter



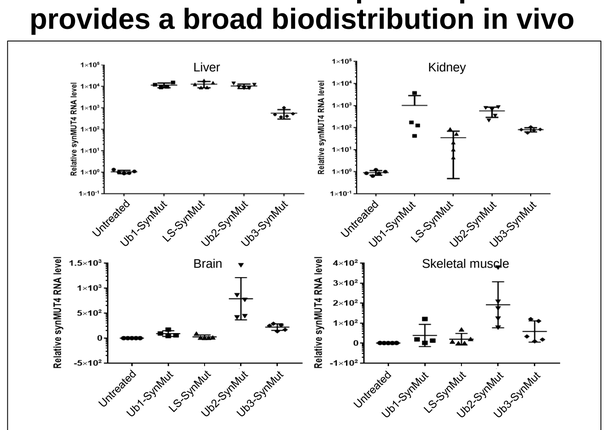
Percent weight change of adult (2-4 months of age) *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice following retro-orbital injection of a dose of 6X10^{12} GC/kg of Anc80L65 with either a liver-specific (Anc80-LS) or ubiquitous (Anc80-Ub) promoter expressing synMUT4 in comparison to untreated wild-type (WT) mice.

Anc80L65 shows reduced neutralization by human sera containing naturally occurring antibodies to AAV8



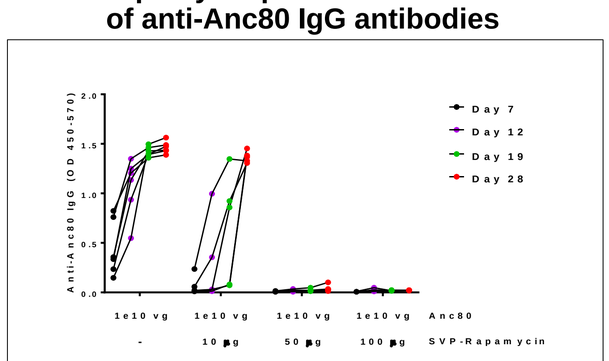
Mice were injected with 100 μl of human serum (i.v.) 1d prior to AAV8-Luc or Anc80L65-Luc administration (tail vein, 5X10^{11} GC/kg). Luciferase expression was measured on day 28.

Anc80L65-MUT with ubiquitous promoter provides a broad biodistribution in vivo



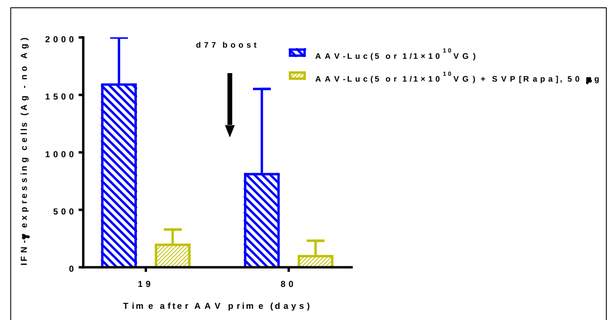
Anc80L65-MUT with a liver-specific (LS) or various ubiquitous (Ub1-Ub3) promoters were administered I.V. and synMut4 expression in various tissues was assessed by PCR

SVP-Rapamycin prevents the formation of anti-Anc80 IgG antibodies



C57Bl/6 mice (n=6/group) were given Anc80 CMV-Luc (1e10 GC/mouse) without or with 10, 50, or 100 μg SVP-Rapamycin at doses. Anti-Anc80 IgG are shown for individual animals in each cohort at various time points as indicated.

SVP-Rapamycin inhibits T cell responses to AAV



C57Bl/6 mice (n=4/group) were given AAV8-Luc (5e10 GC/mouse once or 1e10 GC/mouse twice) without or with 50 μg SVP-Rapamycin. Spleens were taken at times indicated and stimulated in vitro for 7 days with an AAV8 capsid peptide library (125,000 cells/well). Background subtracted.

Conclusions

- These studies show that the Anc80L65 vector is functionally equivalent to AAV8 for the correction of *Mut* deficiency in mouse models of MMA.
- Anc80L65 has the added advantage of reduced neutralization by human serum containing naturally occurring antiAAV8 antibodies
- Anc80L65 with a ubiquitous promoter provided broad tissue distribution of MUT expression, including the kidney
- The addition of SVP-Rapamycin was effective in inhibiting both IgG and T cell responses against the capsid
- Our findings support the clinical development of a combined Anc80L65-Mut/SVP-Rapamycin gene therapy for MUT MMA that has the potential to be broadly expressed, enable enrollment of patients with pre-existing anti-AAV antibodies, reduce the need for steroid use by mitigating T cell responses, and allow for the possibility of re-dosing

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