BIOGRAPHICAL SKETCH

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NAME: **SAMULSKI, RICHARD JUDE**

eRA COMMONS USER NAME (credential, e.g., agency login): **rjsamulski**

POSITION TITLE: **Professor, Gene Therapy Center**

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Clemson University - Clemson, SC	BS	05/76	Microbiology
University of Florida – Gainesville, FL	PhD	05/82	Molecular Biology
SUNY - Stony Brook, NK	Postdoctoral	05/84	Microbiology
Princeton University - Princeton, NJ	Postdoctoral	05/86	Molecular Biology

A. Personal Statement

I have the expertise, leadership, and motivation necessary to successfully carry out the proposed research project, “Integrative profiling of humoral immune response of AAV-mediated gene therapy and host genetic variation influence with Collaborative Cross mouse model”. I have a broad background in gene therapy related to adeno-associated virus (AAV) vector, with specific expertise in optimization of AAV vector for clinical applications. Since our contribution of the original cloning of an infectious AAV genome, my lab has focused over 30 years on development of this unique virus as a gene delivery system. These efforts have led to the first long term gene delivery in muscle, brain and other target tissue. Development of novel AAV variants as alternative vectors and improvements in AAV production have allowed the lab to support the first clinical trial for gene delivery to brain and develop the first chimeric AAV for gene delivery for muscular dystrophy. One of my current goals is to continue to derive delivery systems for safe and efficient use in human gene therapy with the ultimate goal of facilitating the progression and translation of gene therapy research from the laboratory bench into Phase I clinical trials for the treatment of human disease. I have actively participated in studies in AAV biology and pre-clinical/clinical trials in the gene therapy community by collaboration or providing support, especially in three clinical trials including Duchenne's muscular dystrophy, canavan disease and hemophilia. In this collaboration, I will provide my expertise and knowledge in the field of AAV biology and gene therapy for supervising AAV vector construction and production. I welcome the opportunity to add PKAN to the list of diseases we can successfully treat with AAV.

a) Mendell JR, Campbell K, Rodino-Klapac L, Sahenk Z, Shilling C, Lewis S, Bowles D, Gray S, Li C, Galloway G, Malik V, Coley B, Clark KR, Li J, Xiao X, Samulski J, McPhee SW, **Samulski RJ**, Walker CM. Dystrophin immunity in Duchenne's muscular dystrophy. N Engl J Med. 2010 Oct 7;363(15):1429-37.

b) Leone P, Shera D, McPhee SW, Francis JS, Kolodny EH, Bilaniuk LT, Wang DJ, Assadi M, Goldfarb O, Goldman HW, Freese A, Young D, During MJ, **Samulski RJ**, Janson CG. Long-term follow-up after gene therapy for canavan disease. Sci Transl Med. 2012 Dec 19;4(165):165ra163.

c) Ishikawa K, Fish KM, Tilemann L, Rapti K, Aguero J, Santos-Gallego CG, Lee A, Karakikes I, Xie C, Akar FG, Shimada YJ, Gwathmey JK, Asokan A, McPhee S, Samulski J, **Samulski RJ**, Sigg DC, Weber T, Kranias EG, Hajjar RJ. Cardiac I-1c overexpression with reengineered AAV improves cardiac function in swine ischemic heart failure. Mol Ther. 2014 Dec;22(12):2038-45.

d) Ling C, Wang Y, Lu Y, Wang L, Jayandharan GR, Aslanidi GV, Li B, Cheng B, Ma W, Lentz T, Ling C, Xiao X, **Samulski RJ**, Muzyczka N, Srivastava A. Enhanced transgene expression from recombinant single-stranded D-sequence-substituted adeno-associated virus vectors in human cell lines in vitro and in murine hepatocytes in vivo. J Virol. 2015 Jan 15;89(2):952-61.

B. Position and Honors

Positions and Employment

1986-1992 Assistant Professor, Department of Biological Sciences, University of Pittsburgh, Pittsburgh PA
1992-1993 Associate Professor, Department of Biological Sciences, University of Pittsburgh, Pittsburgh PA
1993-1999 Associate Professor, Department of Pharmacology, University of North Carolina, Chapel Hill
1994-1997 Member, Recombinant DNA Advisory Committee, NIH
1993-present Director, Gene Therapy Center, University of North Carolina, Chapel Hill, NC
1999-present Professor, Department of Pharmacology, University of North Carolina, Chapel Hill, NC
2003–present Founder, Acting Chief Scientific Officer, Asklepios BioPharmaceuticals, Inc., Chapel Hill, NC
2009-2010 Vice President, American Society of Gene & Cell Therapy (ASGCT).
2010-2011 President-Elect, American Society of Gene & Cell Therapy (ASGCT).
2011-2012 President, American Society of Gene & Cell Therapy (ASGCT).

Ad hoc reviewer for National Science Foundation grant applications
Ad hoc reviewer for March of Dimes grant applications
Member, International Advisory Committee for the Xth International Congress of Virology

Honors and Awards

1990 Outstanding Young Men of America Award
1991 University of Pittsburgh, President's Distinguished Research Award
2008 ASGCT Outstanding Achievement Award

C. Contribution to Science

1. AAV biology: AAV transduction involves many steps including AAV binding to the receptors or co-receptors on the cell surface, endocytosis, escape from the endosomes, trafficking to the perinuclear space, nucleus entrance, uncoating and transgene expression. A strong focus of my lab is basic understanding of AAV biology which has led to the first discovery of an AAV receptor (heparan sulfate) and co-recptor ($\alpha_v\beta5$), mechanism of viral trafficking and genome persistence. As pioneers, we have identified several primary receptors and co-receptors as well as elucidated the mechanism of AAV trafficking intracellularly, and maintain a robust effort studying AAV biology with both natural and chimeric AAV capsids.

a. Summerford C, **Samulski RJ**. Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. J Virol. 1998 Feb;72(2):1438-45.
b. Summerford C, Bartlett JS, **Samulski RJ**. AlphaVbeta5 integrin: a co-receptor for adeno-associated virus type 2 infection. Nat Med. 1999 Jan;5(1):78-82.
c. Xiao PJ, **Samulski RJ**. Cytoplasmic trafficking, endosomal escape, and perinuclear accumulation of adeno-associated virus type 2 particles are facilitated by microtubule network. J Virol. 2012 Oct;86(19):10462-73.
d. Nicolson SC, **Samulski RJ**. Recombinant adeno-associated virus utilizes host cell nuclear import machinery to enter the nucleus. J Virol. 2014 Apr;88(8):4132-44.

2. AAV capsid engineering: Reengineering of adeno-associated virus (AAV) isolates may yield variants with improved properties for clinical applications. We have performed the first clinical trial in patients with Duchenne muscular dystrophy using a chimeric adeno-associated virus (AAV) capsid variant (designated AAV2.5) derived from a rational design strategy. The novel chimeric vector has the improved muscle transduction capacity of AAV1 with reduced antigenic cross-reactivity against both parental serotypes, while keeping the AAV2 receptor binding. Also, we observed that reengineering AAV receptors change AAV tropism and enhance transduction. Using an AAV evolution approach, AAV mutants can be isolated with specific tissue tropism and the ability to evade neutralizing antibodies. This is a major focus of the lab.

a. Li W, Asokan A, Wu Z, Van Dyke T, DiPrimio N, Johnson JS, Govindaswamy L, Agbandje-McKenna M, Leichtle S, Redmond DE Jr, McCown TJ, Petermann KB, Sharpless NE, **Samulski RJ**. Engineering and

selection of shuffled AAV genomes: a new strategy for producing targeted biological nanoparticles. Mol Ther. 2008 Jul;16(7):1252-60.

b. Asokan A, Conway JC, Phillips JL, Li C, Hegge J, Sinnott R, Yadav S, DiPrimio N, Nam HJ, Agbandje-McKenna M, McPhee S, Wolff J, **Samulski RJ**. Reengineering a receptor footprint of adeno-associated virus enables selective and systemic gene transfer to muscle. Nat Biotechnol. 2010 Jan;28(1):79-82.

c. Bowles DE, McPhee SW, Li C, Gray SJ, Samulski JJ, Camp AS, Li J, Wang B, Monahan PE, Rabinowitz JE, Grieger JC, Govindasamy L, Agbandje-McKenna M, Xiao X, **Samulski RJ**. Phase 1 gene therapy for Duchenne muscular dystrophy using a translational optimized AAV vector. Mol Ther. 2012 Feb;20(2):443-55.

d. Shen S, Horowitz ED, Troupes AN, Brown SM, Pulicherla N, **Samulski RJ**, Agbandje-McKenna M, Asokan A. Engraftment of a galactose receptor footprint onto adeno-associated viral capsids improves transduction efficiency. J Biol Chem. 2013 Oct 4;288(40):28814-23.

3. AAV Neutralizing antibody: While AAV gene therapy continues to yield clinical results supportive of the hope for eventual treatment of many diseases, the presence of patient neutralizing antibodies (NAbs) remains a challenge. NAb-mediated elimination of AAV vectors has become a rate-limiting step in advancing the field and a determinant for repeat administration of AAV gene transfer. More than 90% of the population has been exposed to natural AAV2 infection, and half of those infected carry NAbs in their blood, highlighting the significance of this problem. The study of interaction of neutralizing antibody with AAV virion allows us to develop effective strategies to evade NAb activity in patients with positive NAb or for re-administration.

a. Li C, Narkbunnam N, **Samulski RJ**, Asokan A, Hu G, Jacobson LJ, Manco-Johnson MJ, Monahan PE; Joint Outcome Study Investigators. Neutralizing antibodies against adeno-associated virus examined prospectively in pediatric patients with hemophilia. Gene Ther. 2012 Mar;19(3):288-94

b. Li C, Diprimio N, Bowles DE, Hirsch ML, Monahan PE, Asokan A, Rabinowitz J, Agbandje-McKenna M, **Samulski RJ**. Single amino acid modification of adeno-associated virus capsid changes transduction and humoral immune profiles. J Virol. 2012 Aug;86(15):7752-9.

c. Wang M, Crosby A, Hastie E, Samulski JJ, McPhee S, Joshua G, **Samulski RJ**, Li C. Prediction of adeno-associated virus neutralizing antibody activity for clinical application. Gene Ther. 2015 Dec;22(12):984-92.

d. Li C, Wu S, Albright B, Hirsch M, Li W, Tseng YS, Agbandje-McKenna M, McPhee S, Asokan A, **Samulski RJ.** Development of Patient-specific AAV Vectors After Neutralizing Antibody Selection for Enhanced Muscle Gene Transfer. Mol Ther. 2016 Feb;24(1):53-65.

4. Optimization of transgene cassette: AAV is a single stranded virus; after uncoating in the nucleus, the single stranded AAV genome must be converted into double-stranded DNA intermediate for transcription. The requirement of second strand synthesis has led to the development of a new class of AAV vectors called "self complementary" (scAAV), which induces much higher and faster transgene expression. To further optimize scAAV vector for clinical trial, we have explored different elements in transgene cassettes for controlling tissue specific transgene expression, as well as optimization of coding and noncoding sequences that impact transgene expression, including the inverted terminal repeats (ITR).

a. McCarty DM, Monahan PE, **Samulski RJ**. Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. Gene Ther. 2001 Aug;8(16):1248-54.

b. Wu Z, Sun J, Zhang T, Yin C, Yin F, Van Dyke T, **Samulski RJ**, Monahan PE. Optimization of self-complementary AAV vectors for liver-directed expression results in sustained correction of hemophilia B at low vector dose. Mol Ther. 2008 Feb;16(2):280-9.

c. Li C, Hirsch M, Carter P, Asokan A, Zhou X, Wu Z, **Samulski RJ**. A small regulatory element from chromosome 19 enhances liver-specific gene expression. Gene Ther. 2009 Jan;16(1):43-51.

d. Gray SJ, Foti SB, Schwartz JW, Bachaboina L, Taylor-Blake B, Coleman J, Ehlers MD, Zylka MJ, McCown TJ, **Samulski RJ**. Optimizing promoters for recombinant adeno-associated virus-mediated gene expression in the peripheral and central nervous system using self-complementary vectors. Hum Gene Ther. 2011 Sep;22(9):1143-53.

e) AAV capsid antigen presentation: Recent clinical trials in patients with hemophilia B have demonstrated that an AAV capsid -specific CTL response eliminates AAV transduced hepatocytes resulting in therapeutic failure. To better understand this "rate-limiting" step, we observed and published that capsid antigen presentation in AAV transduced cells is dependent on proteasome mediated AAV capsid degradation as well as determined that capsid antigen presentation is dose-dependent.

a. Li C, Hirsch M, DiPrimio N, Asokan A, Goudy K, Tisch R, **Samulski RJ**. Cytotoxic-T-lymphocyte-mediated elimination of target cells transduced with engineered AAV type 2 vector in vivo. J Virol. 2009 Jul;83(13):6817-24.
b. Li C, He Y, Nicolson S, Hirsch M, Weinberg MS, Zhang P, Kafri T, **Samulski RJ**. Adeno-associated virus capsid antigen presentation is dependent on endosomal escape. J Clin Invest. 2013 Mar 1;123(3):1390-401
c. He Y, Weinberg MS, Hirsch M, Johnson MC, Tisch R, **Samulski** RJ, Li C. Kinetics of adeno-associated virus serotype 2 (AAV2) and AAV8 capsid antigen presentation in vivo are identical. Hum Gene Ther. 2013 May;24(5):545-53.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/40585640

D. Research Support

R01HL151348 Samulski RJ (PI) 09/01/2020-08/31/2024
NHLBI
Development of AAV vectors for CF therapy

Completed Research Support

R01 EY005951 Campochiaro P (PI), Samulski RJ (Sub PI) 05/01/2012-04/30/2017
NIH/National Eye Institute – Subcontract to Johns Hopkins University
Oxidative damage and cone cell death in RP
The long-term objective of this collaborative effort is to evaluate protective gene therapy for the treatment and prevention of oxidative damage and cone cell death in human RP.

1P01HL112761 Asokan (PI) 02/08/2013-01/31/2018
NIH/NHLBI
NEUTRALIZING ANTIBODY & AAV FIX GENE THERAPY
Project 1-FIX Gene Therapy and Role of AAV Nab
This proposal will investigate the relationship of HLA class II phenotype with AAV neutralizing antibody against different serotypes, and explore the novel approach to evade neutralizing antibody activity by using Nab specific aptamers.

R01 AI072176 Hirsch 05/15/2013-04/30/2018
NIH/NIAID
Rational and Combinatorial Engineering of AAV Vectors
The primary goal is to develop more efficient safe AAV vectors that overcome rate-limiting properties associated with current AAV vector transduction and provide a more desirable delivery reagent for future clinical trials.

R01 AR 064369 Hirsch 09/17/2013-08/31/2018
NIH/NIAMS
Overcoming our clinical complications: AAV vector design for the treatment of DMD
Explore the peptides from viruses to inhibit antigen presentation from transgene minidystrophin after AAV muscular delivery in canine model.

R01 AI117408 Li (PI), Samulski RJ (Sub PI) 03/01/2016-02/28/2021
NIH/NIAID
Enhanced AAV liver transduction with capsid immune evasion

The primary objective of this proposal is to develop AAV capsid with ability to target human hepatocytes and insufficient capsid antigen presentation.