PI: **Li, Chengwen**	Title: Enhance AAV Liver Transduction with Capsid Immune Evasion	
Received: 06/25/2015	FOA: PA13-302	Council: 01/2016
Competition ID: FORMS-C	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
1 R01 AI117408-01A1	Dual: DK,HL	Accession Number: 3838759
IPF: 578206	Organization: UNIV OF NORTH CAROLINA CHAPEL HILL	
Former Number:	Department: Pediatrics	
IRG/SRG: GDD	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: Year 2: Year 3: Year 4: Year 5:	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N Year -30re f 220.92 656 6

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier [redacted]

b. Agency Routing Number

2. DATE SUBMITTED 2015-06-25

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: University of North Carolina at Chapel Hill
Department: Office of Sponsored Research
Division: Research
Street1*: 104 Airport Drive, CB 1350
Street2: Suite 2200
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 27599-1350

Person to be contacted on matters involving this application
Prefix: First Name*: Pamela Middle Name: P Last Name*: Bordsen Suffix:
Position/Title: Grants Analyst/Reviewer
Street1*: CB:9525 1140-C Bioinformatics , 130 Mason Farm Road
Street2:
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 27599-9525
Phone Number*: ([redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Enhance AAV Liver Transduction with Capsid Immune Evasion

12. PROPOSED PROJECT
Start Date* 04/01/2016
Ending Date* 03/31/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
NC-004

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Chengwen Middle Name: Last Name*: Li Suffix:
Position/Title: Research Assistant Professor
Organization Name*: University of North Carolina at Chapel Hill
Department: Pediatrics
Division:
Street1*: 7113 Thurston Bowles;CB:7352
Street2:
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 27599-7352
Phone Number*: [redacted] Fax Number: (919) 966-0907 Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	[redacted]
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	[redacted]
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: Barbara Middle Name: Last Name*: Entwisle Suffix: Ph.D.
Position/Title*: Vice Chancellor for Research
Organization Name*: University of North Carolina at Chapel Hill
Department: Office of Sponsored Research
Division: Research
Street1*: 104 Airport Drive, Suite 2200
Street2: CB 1350
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 27599-1350
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative*
Barbara Entwisle

Date Signed*
06/25/2015

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:CoverLetter1022072232.pdf

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The University of North Carolina at Chapel Hill
Duns Number:	[redacted]
Street1*:	104 Airport Drive, CB 1350
Street2:	Suite 2200
City*:	Chapel Hill
County:	Orange
State*:	NC: North Carolina
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	27599-1350
Project/Performance Site Congressional District*:	NC-004

File Name

Additional Location(s)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number [redacted]

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract1022072214.pdf
8. Project Narrative*	Narrative1022072216.pdf
9. Bibliography & References Cited	Bibliography1022072258.pdf
10.Facilities & Other Resources	FacilitiesAndResources1022072218.pdf
11.Equipment	Equipment1022072219.pdf

ABSTRACT

Aim 1

Aim 2

Aim 3 *in vivo*

NARRATIVE

FACILITIES AND RESOURCES

Laboratory:

centrifuge. The PI also has access to all common equipment in the Gene Therapy Center's

Animals:

Computers:

Office:

Other:

FACILITIES AND RESOURCES

Laboratory:

WE also have access to all common equipment in the Gene Therapy Center's 2,345 square foot core facility,

Clinical

Animal:

Computer:

Office

Other:

MAJOR EQUIPMENT

MAJOR EQUIPMENT

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Chengwen Middle Name Last Name*: Li Suffix:

Position/Title*: Research Assistant Professor
Organization Name*: University of North Carolina at Chapel Hill
Department: Pediatrics
Division:
Street1*: 7113 Thurston Bowles;CB:7352
Street2:
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7352

Phone Number*: [redacted] Fax Number: [redacted] E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: MD/PhD Degree Year: 1995

	File Name
Attach Biographical Sketch*:	Li_Biosketch1022072247.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

Prefix:	First Name*: Richard	Middle Name J	Last Name*: Samulski	Suffix:

Position/Title*:	Dir, Gene Therapy Center, Prof
Organization Name*:	University of North Carolina at Chapel Hill
Department:	Pharmacology
Division:	
Street1*:	7119 Thurston-Bowles;CB:7352
Street2:	
City*:	Chapel Hill
County:	Orange
State*:	NC: North Carolina
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	27599-7352

Phone Number*: [redacted] Fax Number: [redacted] E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI	Other Project Role Category:
Degree Type: PhD	Degree Year: 1982

	File Name
Attach Biographical Sketch*:	Samulski_Biosketch1022072231.pdf
Attach Current & Pending Support:	

BIOGRAPHICAL SKETCH

DO NOT EXCEED FIVE PAGES.

LI, CHENGWEN

[redacted]

RESEARCH ASSISTANT PROFESSOR

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

	(if applicable)		

A. Personal Statement

My lab's research has focused on the study of the immune response *in vitro*

1. Li C,

2. Li C,

3. Li C

4. Li C.

B. Position and Honors

Positions and Employment

Professional Societies

C. Contribution to Science

1. AAV Neutralizing antibody:

Li C

Li C

Li C

2. Immune response to transgene:

Li C

Li C

3. Optimization of transgene cassette:

Complete List of Published Work in MyBibliography:

D. Research Support

Ongoing Research Support

R01DK084033-03 (Li/Samulski MPI)

P01 HL112761 (Samulski, PI) –

BIOGRAPHICAL SKETCH

DO NOT EXCEED FIVE PAGES.

SAMULSKI, RICHARD JUDE

[redacted]

Director, Gene Therapy Center **Professor**

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

	(if applicable)		
–			

A. Personal Statement

better understand this “rate limiting” step

hypothesis of “evolving AAV capsid to avoid immune response in humanized liver model”.

1. **Samulski RJ**

2. **Samulski RJ**

3. **Samulski**

[redacted]

5. Broad collaboration in gene therapy community:

Complete List of Published Work in MyBibliography:

D. Research Support

<u>Ongoing Research Support</u>

R01DK084033 Li/Samulski (MPI)

R01 EY005951 Campochiaro P (PI), Samulski RJ(Sub PI) –
–

1P01HL112761 Samulski, RJ (PI)

R01 AI072176 Hirsch/Samulski (MPI) –

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: Chengwen
Middle Name:
Last Name*: Li
Suffix:

2. Human Subjects

Clinical Trial? ❍ No ❍ Yes
Agency-Defined Phase III Clinical Trial?* ❍ No ❍ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ❍ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ❍ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Modular Budget

OMB Number: 0925-0001

Budget Period: 1

Start Date: 04/01/2016 End Date: 03/31/2017

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	[redacted]
Consortium F&A	0.00
Total Direct Costs*	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	Organized Research_On Campus	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Darryl Mayes, 202-401-2808

Indirect Cost Rate Agreement Date: 05/16/2012

Total Indirect Costs: [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

PHS 398 Modular Budget

Budget Period: 2	
Start Date: 04/01/2017 End Date: 03/31/2018	

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	[redacted]
Consortium F&A	0.00
Total Direct Costs*	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	Organized Research_On Campus	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Darryl Mayes, 202-401-2808

Indirect Cost Rate Agreement Date: 05/16/2012

Total Indirect Costs: [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

PHS 398 Modular Budget

Budget Period: 3	
Start Date: 04/01/2018 End Date: 03/31/2019	

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	[redacted]
Consortium F&A	0.00
Total Direct Costs*	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	Organized Research_On Campus	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Darryl Mayes, 202-401-2808

Indirect Cost Rate Agreement Date: 05/16/2012

Total Indirect Costs: [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

PHS 398 Modular Budget

Budget Period: 4

Start Date: 04/01/2019 End Date: 03/31/2020

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	[redacted]
Consortium F&A	0.00
Total Direct Costs*	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	Organized Research_On Campus	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Darryl Mayes, 202-401-2808

Indirect Cost Rate Agreement Date: 05/16/2012

Total Indirect Costs: [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

PHS 398 Modular Budget

Budget Period: 5

Start Date: 04/01/2020 End Date: 03/31/2021

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	[redacted]
Consortium F&A	0.00
Total Direct Costs*	[redacted]

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	Organized Research_On Campus	[redacted]	[redacted]	[redacted]
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Darryl Mayes, 202-401-2808

Indirect Cost Rate Agreement Date: 05/16/2012

Total Indirect Costs: [redacted]

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): [redacted]

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium F&A for Entire Project Period ($)	[redacted]
Section A, Total Consortium F&A for Entire Project Period ($)	0.00
Section A, Total Direct Costs for Entire Project Period ($)	[redacted]
Section B, Total Indirect Costs for Entire Project Period ($)	[redacted]
Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period ($)	[redacted]

2. Budget Justifications

Personnel Justification	PersonnelJustification1022072246.pdf
Consortium Justification	
Additional Narrative Justification	

PERSONNEL JUSTIFICATION

Chengwen Li, Ph.D., Principal Investigator, (3.6 CM Years 1-5)

Richard J. Samulski, Ph.D., Principal Investigator (0.6 CM Years 1-5)

Maxim Salganik, Post-doctoral Fellow, (6 CM Years 1-5)

in vitro *in vivo*

Karen Hogan, Research Specialist, (6 CM Years 1-5)

Chen Xiaojing, Research Assistant, (9 CM Years 1-2, 8.4 CM Year 3, 7.8 CM Year 4, 6 CM Year 5)

Fringe benefits:
Fringes for PI's and Research Tech are calculated at [redacted]

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	Introduction1022072221.pdf
2. Specific Aims	SpecificAims1022072222.pdf
3. Research Strategy*	ResearchStrategy1022072259.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	VertebrateAnimals1022072224.pdf
9. Select Agent Research	SelectAgentResearch1022072227.pdf
10. Multiple PD/PI Leadership Plan	MultiplePI_LeadershipPlan1022072225.pdf
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	ResourceSharingPlan1022072245.pdf
Appendix (if applicable)	
14. Appendix	

INTRODUCTION

“ ”. We would like to thank the reviewers for their **(22 percentile)** the submission as “*highly significant because it addresses the issue of antigen cross-presentation, thought to be a critical barrier to the use of AAV gene therapy for hemophilia B”, “the logical plan for inhibiting the CTL response to the capsid and the outstanding investigators deemed to be leaders in the field*”.

1)

2

3

Point 1)

Point 2)

Point 3)

in vitro *in vivo*

1. “*It is not clear how the observed data, particularly those from mutants whose transduction mechanisms have not been clearly understood, can be generalized or exploited to generate novel capsids that can avoid the CTL-mediated destruction of AAV transduced cells in Aim 3*”

’s

“ section”.

2. “*It is not clearly stated how the ultimate goal of the project "enhance AAV liver transduction with capsid immune evasion" will be achieved using the study outcomes*”

3. “*HepG2/H2-Kb/TAP-/- xenograft model may not be the best model for this project*”

4.

“*Insertion of the OVA SIINFEKL peptide into the HI loop of various AAV2 and AAV8 mutants might negatively affect the ability to transduce cells in vitro and in vivo”*

It completely addresses the reviewer’s concern. **5.**

“*Biosafety consideration in the use of AAV and adenoviral vectors is not described in the application*”

SPECIFIC AIMS

in vitro *in vivo*

Aim 1a and 1b

in vitro[10]

in vitro

in vivo

in vivo

Aim 1c

in vivo **Aim 2a**

in vitro

in vivo

Aim 2b and 2c

Aim 3

in vivo *human*

1. Study the effect of AAV empty particles on AAV capsid antigen cross-presentation *in vivo*.

a *in vivo*

b. *in vivo*

c

2. Investigate AAV capsid antigen presentation following administration of AAV mutants and/or proteasome inhibitors for enhanced liver transduction *in vivo*.

a

b *in vivo*

c *in vivo*

3 Isolate AAV chimeric capsids with human hepatocyte tropism and the capacity for CTL evasion.

a

b

c

RESEARCH STRATEGY
A. SIGNIFICANCE

in vitro

in vivo

in vitro

in vitro

in vivo

in vivo

in vivo **Aim 1**

Aim 2a

in vitro

in vivo **Aim 2b and c**

in vivo [1,2,43,44]

Aim 3

1

in vitro[12] **2**

in vivo[11], **3**

in vitro

Fig. 1 4

Fig. 5 6 5

in vivo **(Fig. 7 6**

7

B. INNOVATION

C. APPROACH

in vivo,

C1. Study the effect of AAV empty particles on AAV capsid antigen cross-presentation *in vivo*. *Rationale*.

in vivo

in vitro

in vivo

in vitro

in vivo

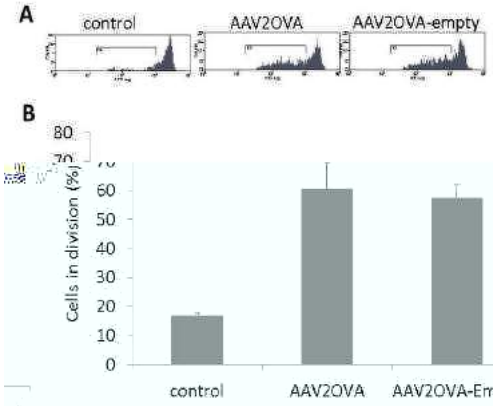

Fig. 1. Capsid antigen presentation after systemic administration of AAV2 empty virions. A.

B.

Fig. 1

in vitro

in vivo

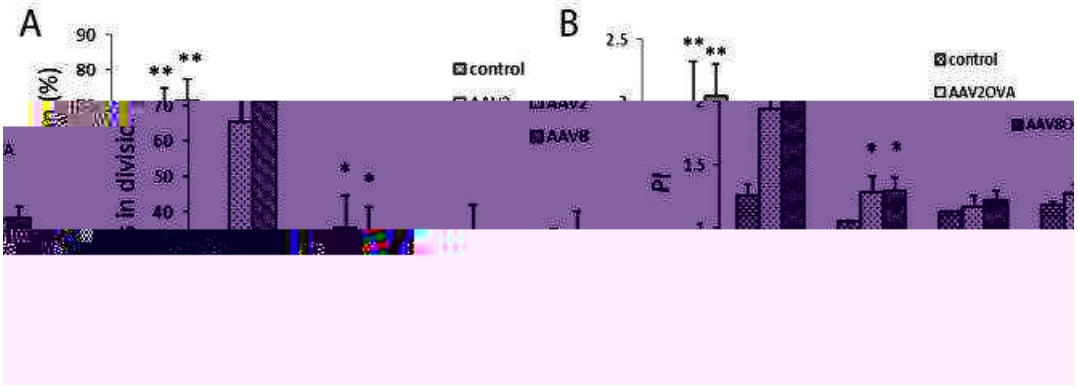

Fig. 2. The kinetics of capsid antigen presentation after AAV8OVA transduction in mice.

(A)

(B)

p *p*

Fig. 2

in vivo,

Fig. 3

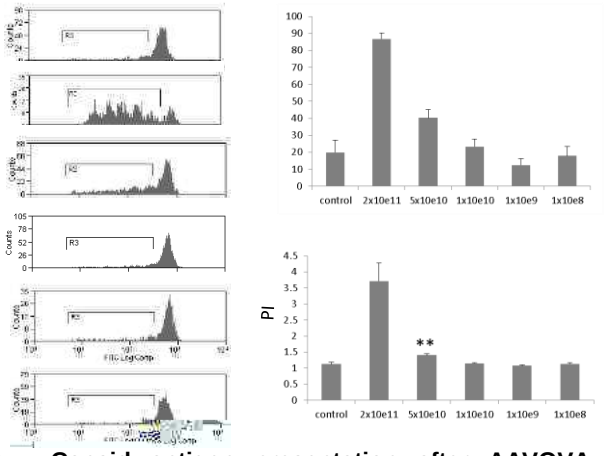

FIG. 3. Capsid antigen presentation after AAVOVA transduction is dose responsive *in vivo*

(A) **(B)**

(C)

p *p*

C1.1

C1.2

Fig. 4A

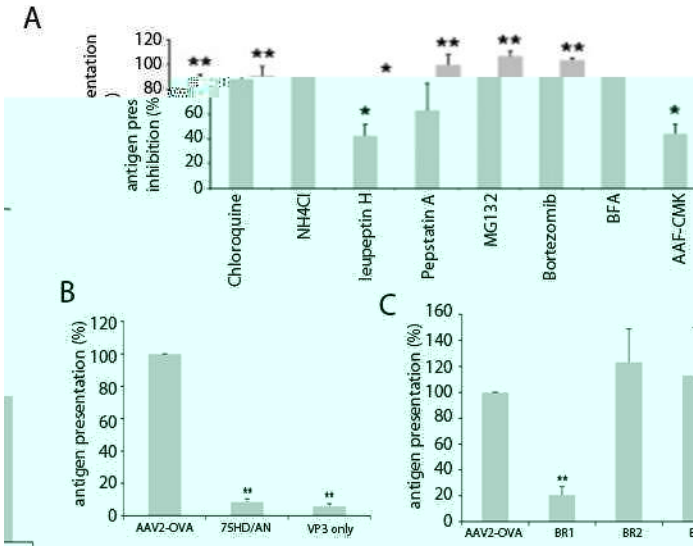

Fig. 4A

Fig. 4. Mechanism of capsid antigen cross-presentation in HepG2/H-2Kb cells. A

B

C

P *P*

Fig. 4A

Fig. 4B

Fig. 4B

Fig. 4B

Fig. 4C

Fig. 4C

in vitro

in vivo

Aim1.3

C1.1. The kinetics and dose-response of AAV capsid antigen presentation from AAV empty virions *in vivo.*

Empty AAV production

Animal experiments for kinetics of antigen presentation from AAV empty capsids.

Animal experiments for dose-response of AAV capsid antigen presentation from AAV empty capsids.

Statistics analysis:

C1.2. The effect of empty particles on AAV capsid antigen cross presentation from full particle AAV transduction *in vivo*.

in vivo

in vivo

Animal experiment for transgene production.

Animal experiment for capsid antigen presentation.

in vivo

C1.3. The mechanism of empty capsid antigen presentation *in vivo*.

in vivo

Transgene expression analysis.

In vivo T cell proliferation assay.

Anticipated results, potential pitfalls and alternative approaches

in vivo

30

in vivo

in vivo

inhibitors, the drugs' short half

Fig. 8

, we don't foresee any problems in carrying out these

C2. Investigate effect of AAV mutants and proteasome inhibitors on AAV capsid antigen presentation.
***Rationale*.**

Fig. 3

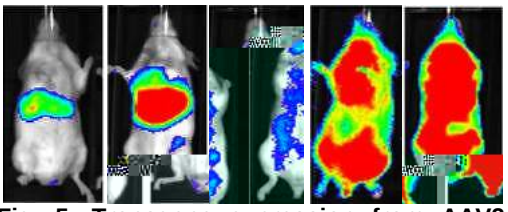

Fig. 5. Transgene expression from AAV2 mutants with modification of primary receptors

Fig. 5

Fig. 6

Fig. 6

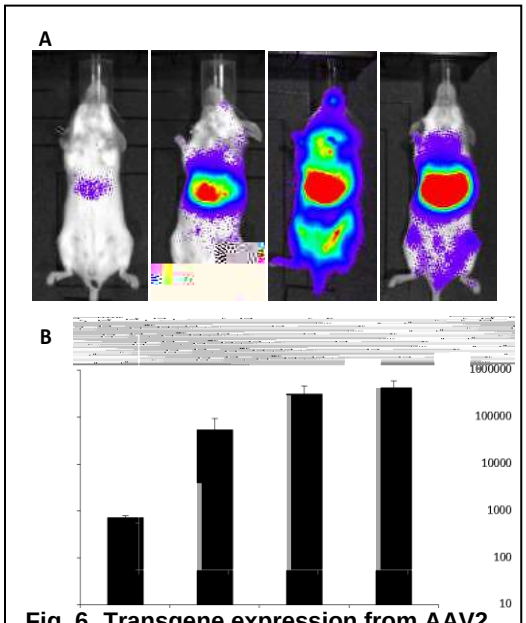

Fig. 6. Transgene expression from AAV2 mutants. A

B

in vivo

Aim 2.1

in vivo

Fig. 7

Figs. 4 7

Fig. 8

in vitro

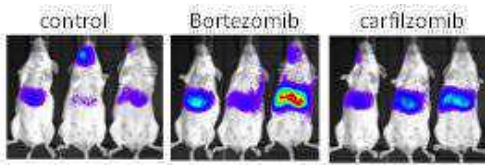

Fig. 7. The effect of proteasome inhibitors on AAV2 transduction.

enhanced,

in vivo

Aim 2.2

in vitro

Aim 2.3

C2.1. Study capsid antigen cross-presentation from AAV mutants.

in vitro *in vivo*

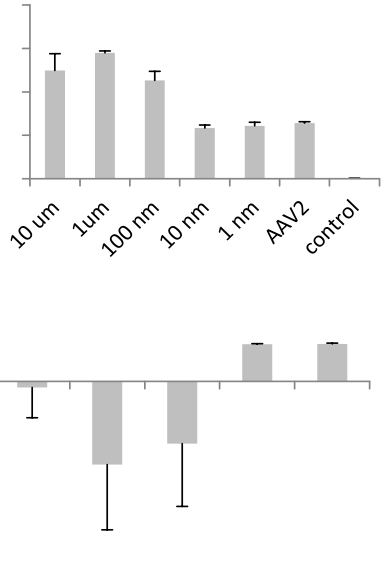

Fig. 8. The effect of bortezomib on capsid antigen presentation. A.
B.

Clone of AAV mutants.

Ubiquitin conjugation assay and immunoblotting.

–

at 37°C for 4 hr. Equal volumes of sodium dodecyl

–

Luciferase imaging in mice.

Antigen presentation in mice:

C2.2. The effect of proteasome inhibitors on AAV capsid antigen presentation *in vivo*.

Antigen presentation in mice.

C2.3. The effect of proteasome inhibitors on antigen presentation *in vivo* using liver enhanced AAV mutants.

C1.1 C1.2

Mouse experiments:

Anticipated results, potential pitfalls and alternative approaches

in vitro

,

Fig. 8

in vitro In vivo,

in vitro **Fig. 8**

in vivo

C3 Isolate AAV mutants with human hepatocyte tropism and immune-evasion capacity.

***Rationale*.**

Aim 2

Aim 2.1

C3.1

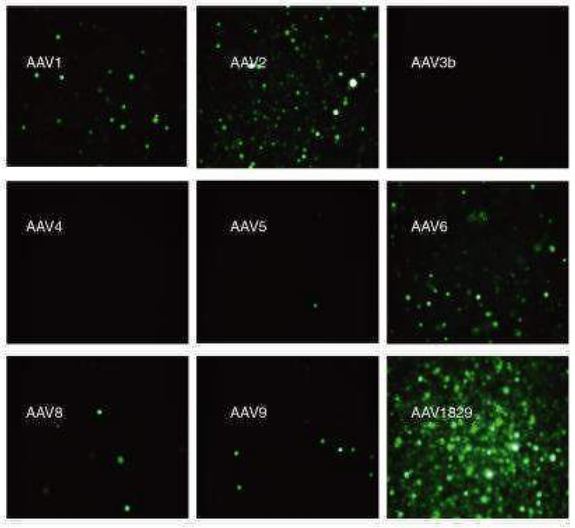

Fig. 9.

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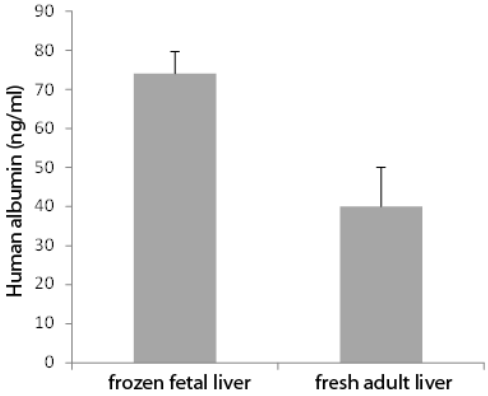

Fig. 10. Engraftment of human liver in FRG mice.

AAV transduction in human liver cells in xenografted mice.

C3.2. Selection of AAV mutants in human liver xenografted mice.

in vivo

T) for mutation (K→R, S/T→A). Mutated AAV capsids will be

DNA shuffling and the generation of a new AAV capsid library.

Aim 3.1

in vivo
Directed evolution in human liver xenografted mice.

rep

in vitro *in vivo*
Isolation of human liver cells.

In vivo characterization of AAV mutants in xenografted mice

C3.1
Structural analysis.

C3.3 Investigation of immune-evasion from humanized AAV mutants.

in vitro,

in vitro *in vivo*

C3.2

In vitro *in vivo*

Detection of AAV capsid ubiquitination in AAV mutant transduced cells.

with 10 µl of protein G PLUS
µl of mouse anti monoclonal antibody clone B1at 4°C for 1 h, followed by the addition of 30 µl of protein G

Antigen presentation in mice:

Anticipated result, potential pitfalls and alternative approaches

in vivo

in vitro

–

Summary:

in vivo

VERTEBRATE ANIMALS

IL2Rγnull (Nod IL2rγnull, NSG) with

Aim 1. Study the effect of AAV empty particles on AAV capsid antigen cross-presentation.

Aim 1.1. The kinetics and dose-response of AAV capsid antigen presentation from AAV2 empty virions *in vivo*.

Aim 1.2. The effect of empty particles on AAV capsid antigen cross presentation from full-particle AAV transduction *in vivo*.

Aim 1.3. AAV capsid antigen presentation in TAP-/- and in Cat S-/- mice.

Aim 2. Investigate AAV capsid antigen presentation following administration of AAV mutants or proteasome inhibitors for enhanced AAV liver transduction.

Aim 2.1. Capsid antigen presentation from AAV mutants with enhanced liver transduction in mice.

in vitro

Aim 2.2. The effect of proteasome inhibitors on AAV capsid antigen presentation *in vivo*.

Table 1

Aim 2.3. The effect of combination of AAV mutants with proteasome inhibitors on antigen presentation *in vivo.*

Aim 3 Isolate AAV mutants with human hepatocyte tropism and immune-evasion capacity.

Aim 3.1. Verify AAV human liver transduction efficiency in xenograft mice.

Aim 3.2 Selection of AAV mutants in human liver xenografted mice.

Aim 3.1

Aim 3.3. Investigation of immune-evasion from humanized AAV mutants

in vitro

in vivo

Aim 1 **Am 2** **Aim 3**

in vivo

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