Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

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Overview of GTIB Research Programs

Andrew Byrnes, Ph.D.

Chief, Gene Transfer and Immunogenicity Branch

March 10, 2022

Overview of the Gene Transfer and Immunogenicity Branch

Six laboratories focused on:

- Cell and Gene Therapy
- Immunology
- Virology

Relevance to FDA's mission

- Improving safety and efficacy of cell and gene therapy products
- Characterizing complex products
- Mitigating immune responses to products
- Better preclinical models
- Other FDA and HHS priorities
  - Pandemic influenza
  - COVID-19

GTIB's regulatory review responsibilities

Review of manufacturing and testing for investigational products

Especially gene therapy vectors, T cell therapies, hematopoietic stem cell therapies, and genome editing

License application review, including chairing review committees

Many first-in-class products

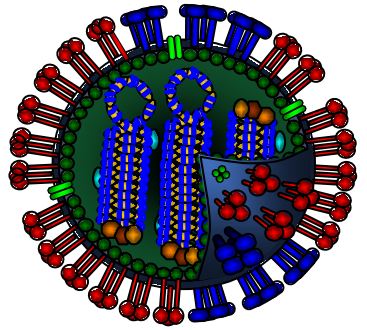
Review of post-licensure manufacturing changes (BLA supplements)

GMP inspections of manufacturing facilities

Policy, guidance, meetings, outreach and training

Immunity and Protection against Viral Infection Induced by Recombinant Vectors

Suzanne Epstein, PhD



Relevance of this work to our regulatory mission

- **Universal influenza vaccine approach: control of seasonal and pandemic influenza is a Center- and Agency-wide public health priority**
- **Immune responses to recombinant vectors used in this work have major impact on safety and efficacy of gene therapy**

  We need to understand these responses – and understand how to measure them – in both preclinical animal models and clinical trials

- **In addition, OTAT regulates a variety of immunologically-based products to control viral infections, including influenza**

Relevant vectors studied in this program currently or previously:

Plasmid, adenovirus, AAV, and poxvirus vectors

Assays:

Mouse, ferret, and human antibody and T cell assays

Immunization of mice with recombinant adenovirus expressing influenza A/NP+M2 or B/NP

Findings include:

- **Antibody and T cell responses persist for over a year after a single intranasal immunization, and so does broad protection against influenza**
  Lo, et al., Vaccine, 39: 4628-4640, 2021
- **Despite pre-existing immunity to the vector, a second dose a year later is not blocked, elicits an immune response to a new antigen**
  Lo, et al., Vaccine, 39: 4628-4640, 2021
- **Transmission to naïve contacts is reduced for at least a year after immunization**
- **Safety: Mucosal immunization does not impair lung function**
  Dhakal, et al., Journal of Virology, 95:e02359-20, 2021

Broad cross-protection for control of influenza

Future plans:

- **Analyze in more detail possible adverse consequences of vaccine-induced T cell responses in the lungs**
  Excessive cytokine secretion, cell killing by cytotoxic T lymphocytes

Public health implications:

Broadly-protective influenza vaccines could be used off-the-shelf

Early in an outbreak, before matched vaccines are available

Potential to reduce illness, death, viral titers, transmission of infection

Understanding Mechanisms for Immunogenicity and Inflammatory Toxicities Associated with Gene Therapy Products

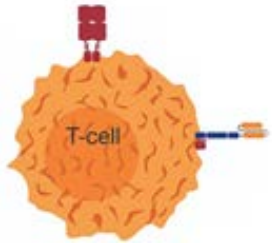
Nirjal Bhattarai, Ph.D. (PI)

Alan Baer, Ph.D. (Staff Fellow)

Lab research overview & significance

- The Bhattarai Lab aims to improve manufacturing and decrease immunogenicity of cell and gene therapies

A. Cell-based gene therapy products (e.g., CAR-T cells)

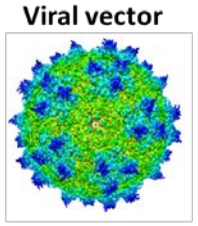


Manufacturing Challenges: Developing manufacturing strategies that improve product quality

Safety concerns:

- Understanding mechanisms contributing to inflammatory toxicities (e.g., Cytokine Release Syndrome, CRS) during CAR-T cell therapy
- Developing strategies to reduce inflammatory toxicities

B. Immunogenicity of viral vectors (e.g., AAV)



Innate immune response: Developing *in vitro* systems to study innate immune response induced by viral vectors

T cell response: Developing novel strategies to reduce T cell response against viral vectors

Significance: This work addresses important challenges with cell and gene therapy products, aiming to improve safety and efficacy of these products

Major findings and future directions

CAR-T cells

Manufacturing:

- Identified a novel role of Src-kinases in CAR-T cell activation
- Identified a method to improve CAR-T cell product attributes during manufacturing

Baer A et al., PLoS One, 2017
Lamture G et al., Journal of Immunotherapy, 2021

Safety concerns:

- Identified a novel candidate inflammatory factor that contributes to CAR-T cell mediated inflammatory toxicity *in vitro*

Immunogenicity of viral vectors

- Reduced T cell responses against AAV vectors by incorporating a viral immunomodulatory peptide

Colon-Moran W, Baer A et al., Gene Therapy, 2021

Future Directions

CAR-T cell project

1. Understand how a novel inflammatory factor contributes to toxicity during CAR-T cell therapy
2. Develop strategies to improve CAR-T cell safety by inhibiting expression of inflammatory factor, and test safety and efficacy of these strategies

Viral vector immunogenicity project

1. Study immunogenicity of AAV vector expressing immunomodulatory peptide *in vivo* (e.g., mice)
2. Develop strategies to reduce vector-induced activation of innate immune responses

Safety-Enhanced Lentiviral Vectors for Gene Therapy

Jakob Reiser, Ph.D. (PI)

Takele Argaw, D.V.M. (Staff Scientist)

Safety issues with lentiviral vectors used in patients

- Potential to form replication-competent lentivirus
- Potential for insertional gene activation/inactivation
- Potential for off-target transduction

The goal of the Reiser lab is to develop safer lentiviral vectors by:

- Directing vector integration to genomic “safe harbor sites”
- Narrowing the vector’s cell tropism

Directing vector integration to genomic “safe harbor sites”

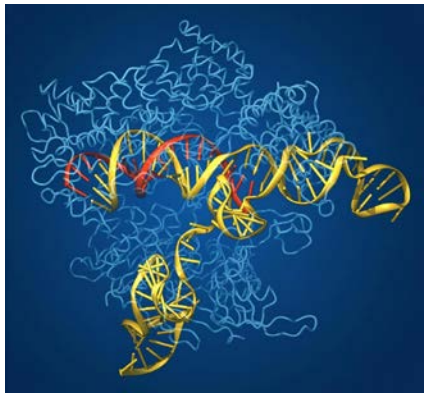
- Design strategies for site-specific genomic insertion of vector/transgene sequences without introducing DNA breaks using engineered recombinases
- Test engineered recombinases bearing specific DNA binding domains targeting safe harbor sites
- Improve the specificity and efficacy of engineered recombinases by regional hypermutation and directed evolution using replication-competent Rhabdovirus vectors
- Test integrase-defective lentiviral vectors or virus-like nanoparticles for transient delivery/expression of engineered recombinase proteins or RNAs attached to HIV-1 Gag domains

Narrowing the vector’s cell tropism

- Design targetable envelopes for pseudotyping lentiviral vectors and virus-like nanoparticles
- Improve the specificity and efficacy of targetable envelopes by directed evolution using replication-competent Rhabdovirus vectors
- Test targetable lentiviral vectors bearing improved cell-specific envelopes in vitro and in vivo
- Test targetable virus-like nanoparticles bearing improved envelopes for transient and cell-specific delivery of proteins and RNAs

Development and Evaluation of Cell Engineering Technologies

Zhaohui Ye, Ph.D.



Differentiation of human induced pluripotent stem cells (iPSCs)

Ongoing Research

- Optimize hematopoietic differentiation conditions
- Develop characterization methods for iPSC-generated cell types

CBER Mission Relevance

- Support development of manufacturing platforms using iPSCs
- Improve quality assessment of stem cell-derived products

Evaluation of genetic engineering technologies

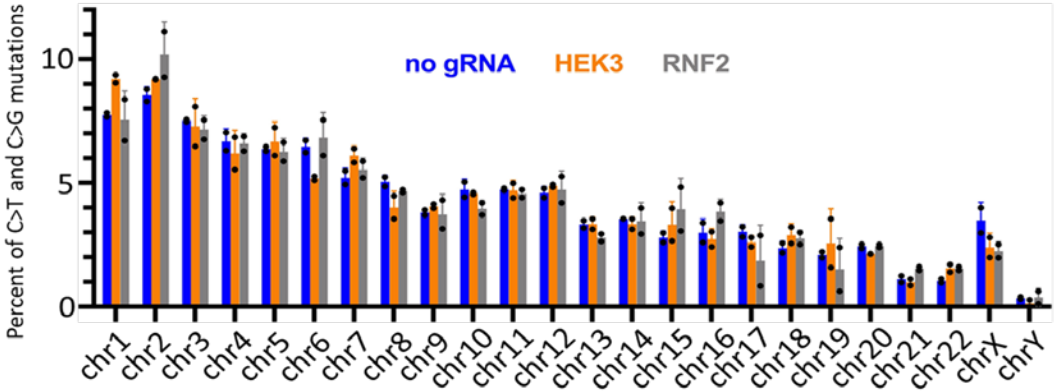
Ongoing Research

- Evaluate specificity of emerging CRISPR-based genome editing tools

CBER Mission Relevance

- Develop technology to improve product manufacturing
- Improve safety evaluation of gene therapies incorporating genome editing

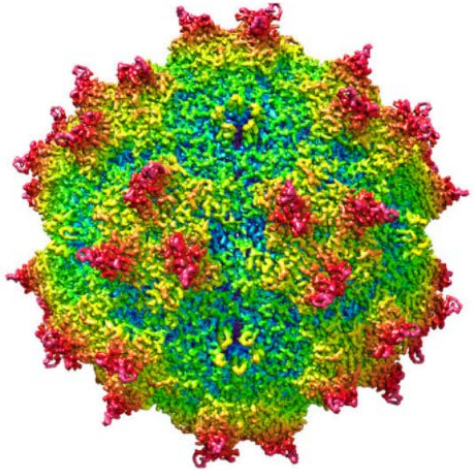
Random distribution of mutations induced by cytosine base editor



McGrath, Shin, et al., *Nat. Commun.* 2019

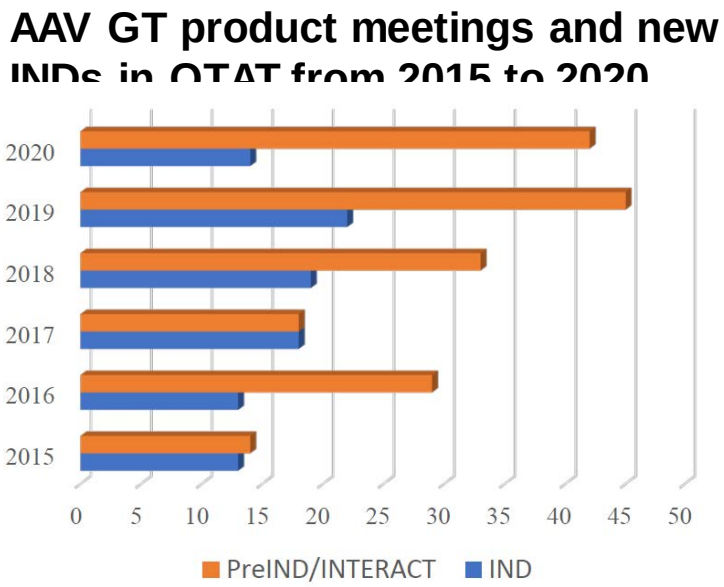
Immunogenicity of AAV Vectors Used in Gene Therapy

Ronit Mazor, Ph.D.



Adeno-associated virus vectors in gene therapy

- AAV vectors are highly utilized in gene therapy
- Two FDA-licensed AAV products
- More than 170 active INDs across multiple indications
- AAV is a major part of the regulatory portfolio in our office



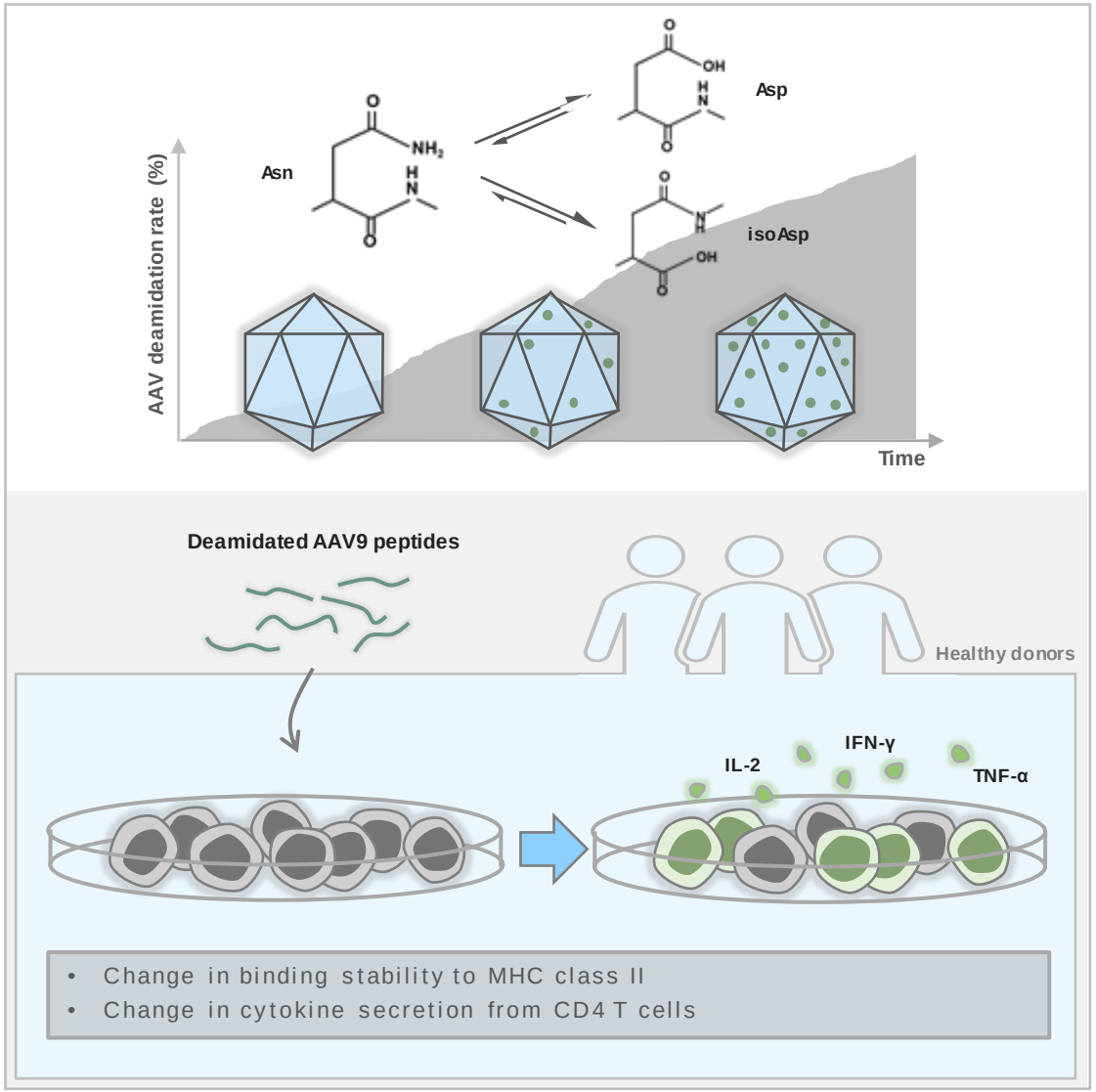
Goals

The Mazor lab develops platform technologies to investigate, monitor and mitigate adaptive immunogenicity of AAV vectors

Ongoing projects:

- **Identification of T cell epitopes in AAV vectors**
- **Design of novel controls for immune monitoring assays**
- **Rational design of AAV vectors with lower immunogenicity**
- **Study the impact of capsid protein changes on AAV immunogenicity**

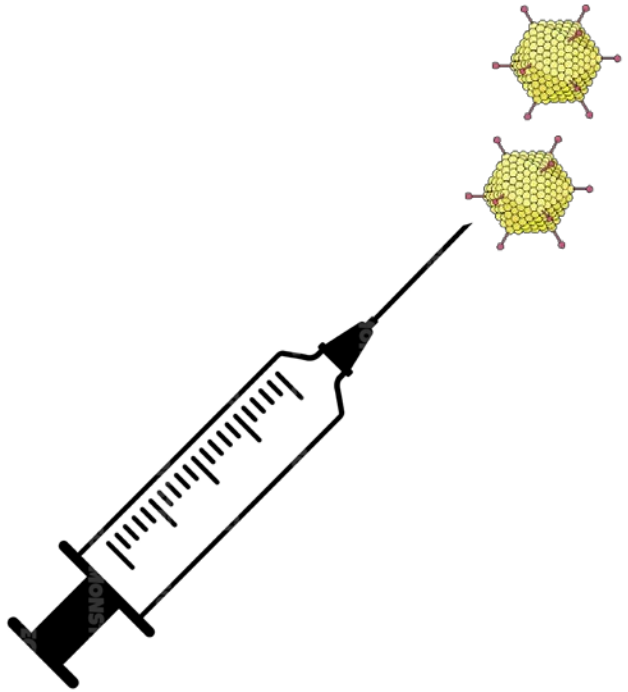
Differential T cell immune responses to a deamidated AAV vector



Bing et al. Mol. Ther. Methods Clin. Dev. 2022

Adenovirus Vector Biodistribution and Toxicity

Andrew Byrnes, Ph.D.



Adenovirus: a popular vector in clinical trials

Adenoviruses can be engineered to create non-replicating or conditionally-replicating vectors

There are many active clinical trials with adenovirus vectors

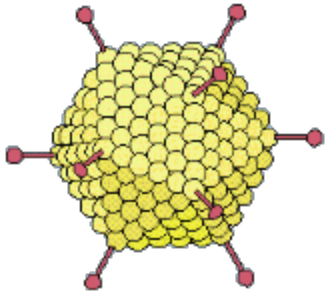
- > 90 Ad-based gene therapies and oncolytic adenoviruses
  - Most are cancer therapies

We study systemic IV gene therapy

- How to prevent immediate clearance of vector by the liver?
- How do animal models differ from humans?

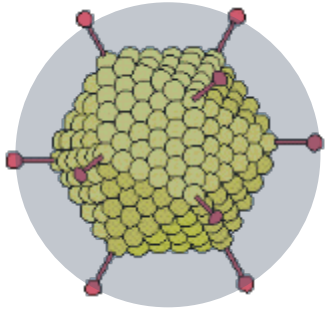
Plasma proteins bind to adenovirus vectors and change vector biodistribution

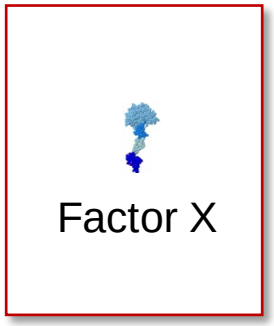
In vitro



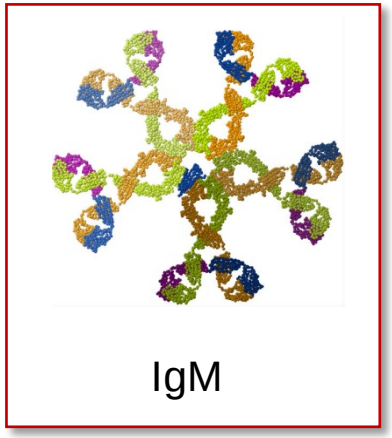
Plasma proteins

In vivo

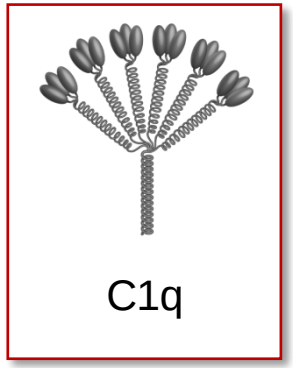


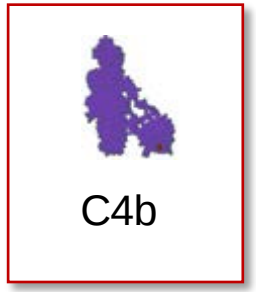


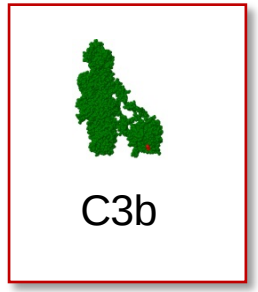
Coagulation factors



Antibodies







Complement proteins

Ongoing work and future directions

Our focus is on host proteins that interact with Ad vectors

- How do these proteins influence vector biodistribution and toxicity?
- How do these interactions differ between mice and humans?

Expanding our studies to many different Ad serotypes

- Different serotypes have quite different properties as gene therapy vectors

Goals and mission relevance:

- Better vectors that can be targeted to specific tissues or tumors
- Understanding the benefits and limitations of preclinical animal models

Questions?

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Gene Transfer and Immunogenicity Branch

Epstein lab

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Bhattarai lab

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