Strategies for Clinical Assessment of Immunogenicity for Multidomain Therapeutics and Gene Therapy

Breakthroughs that change patients' lives

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Global Product Development

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Disclaimer

- The contents of this presentation reflect the personal opinion of the author and may not represent the official perspectives of the affiliated organization.

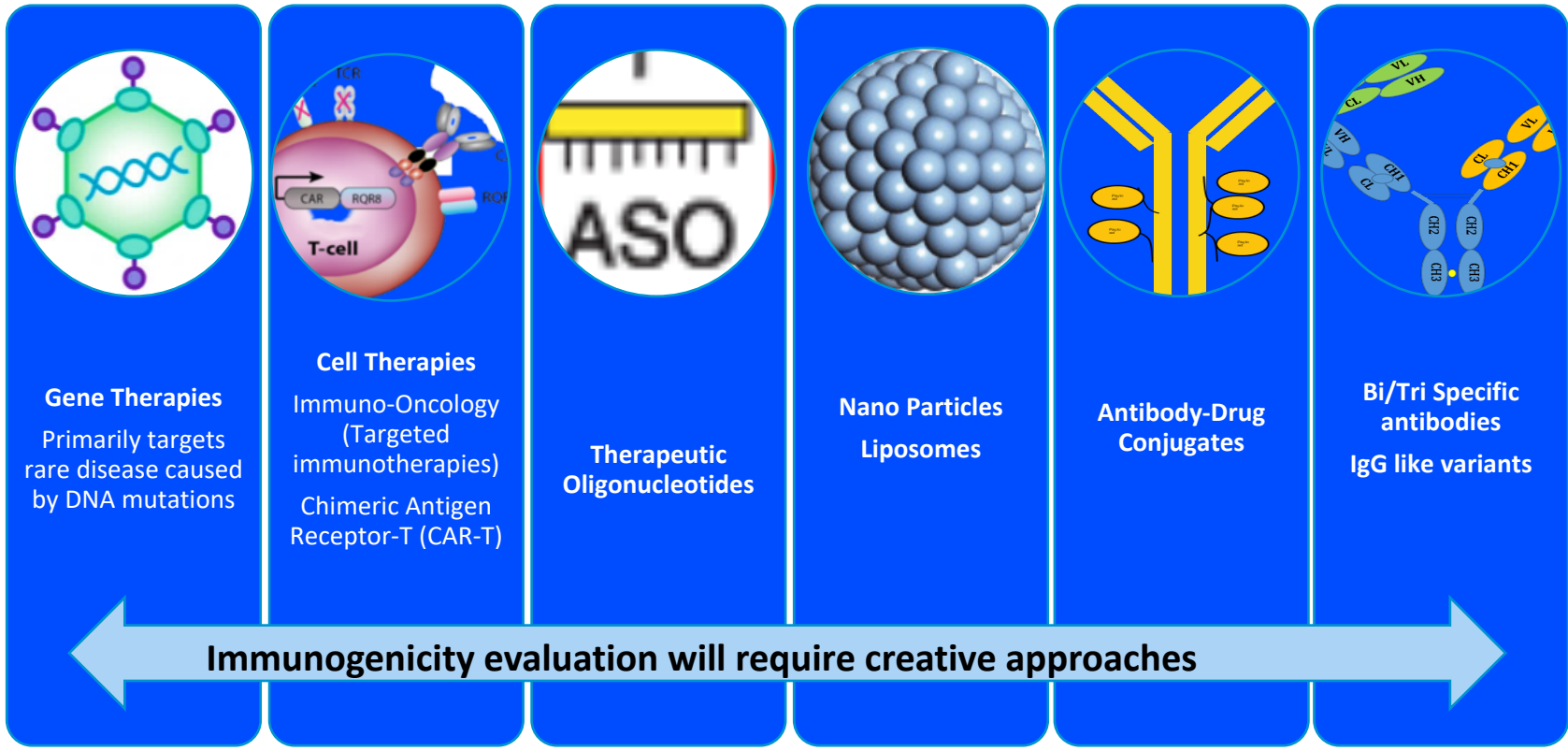
Agenda

- Emerging modalities
- Immunogenicity Assessment Roadmap
- Case Studies
- Take Home Message

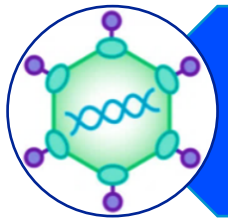
Beyond Antibodies…

- Rare diseases - SMA, DMD, Sickle Cell Anemia, Hemophilia etc.
- Neurological diseases
- Inherited retinal diseases
- Cardiovascular and Metabolic

Innovation of Science and Technology

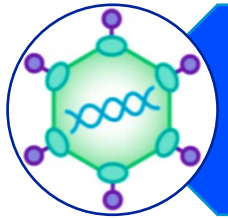


New Modalities New Challenges



Scope of Work

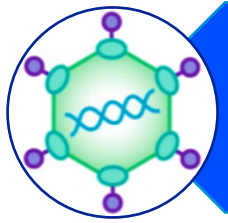
Lead Time



Critical Reagents

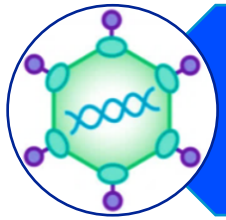
Reference Material

Positive Control



Technologies

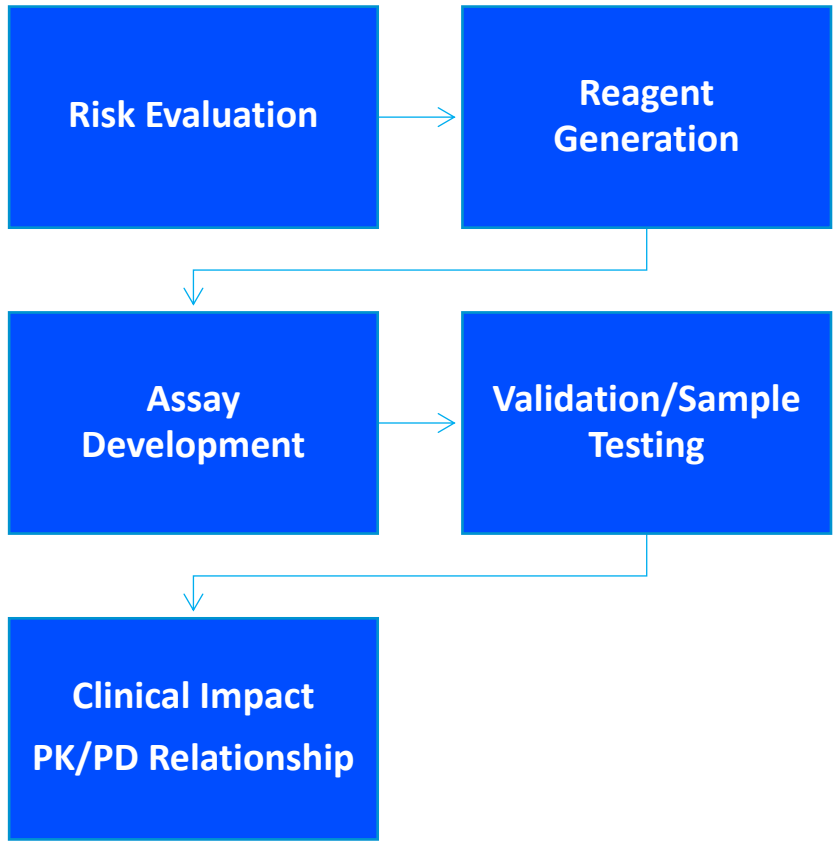
Beyond Traditional Assays



Assay Expectations

Regulatory Component Evolving

Immunogenicity Assessment Roadmap



Risk Evaluation And Reagents

- Patient related factors
- Product related factors
- **Resources**
- **Time commitment**
- **Drug**

Ownership of Risk evaluation report?

Significant Lead Time

Step 2 Assay Development

- Platform selection
- **Multi-Tiered vs Unique assays**
- Matrix selectivity
- Cut point calculation

Method development/Qualification report

Step 3 Assay Validation

- IM guidance/EMA guidance
- **Scientific judgement for Unique assays**
- Generate acceptance criteria for production

Working SOP/Method validation plan/report

Step 4 Sample Testing

- Track controls
- In study cut points if warranted

Final SOP/sample analysis report

Pfizer | Breakthroughs that change patients' lives

Unique Points to Consider for Risk Evaluation

Antibody Drug Conjugates

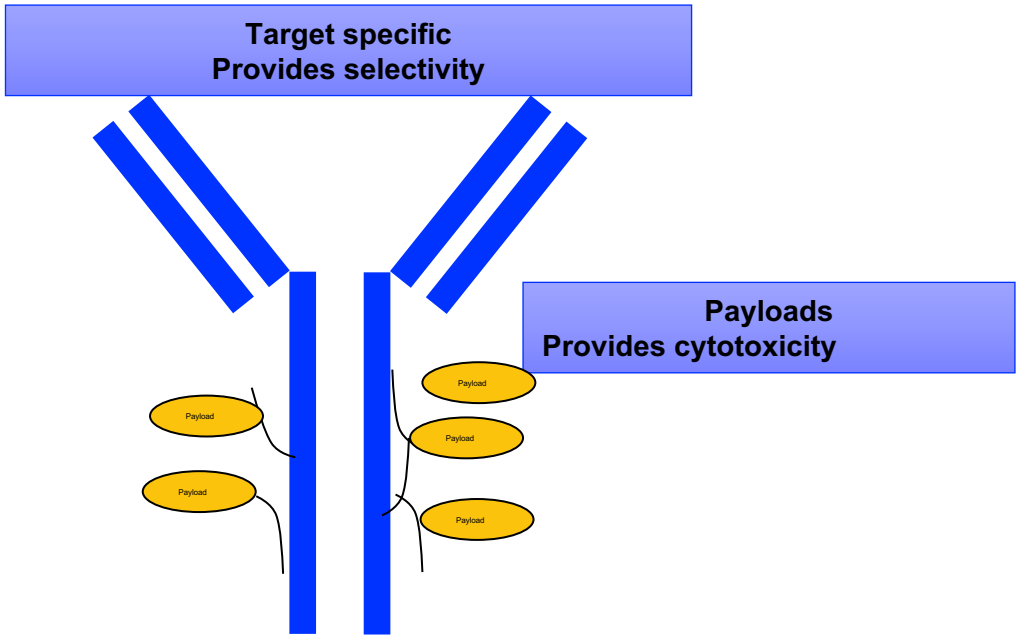
- Dose and frequency of administration
- Patient level of immunocompetence
- Antigen presentation of cell surface
- Degree of homology with endogenous counterpart

Gene Therapy

- Size, type and molecular structure of virus
- Physical properties like aggregation
- Route of administration
- Genetic status of patients; each patient will react differently
- Physical factors e.g. storage conditions
- Pre-existing antibodies

Six ADCs have market approval

Antibody Drug Conjugates



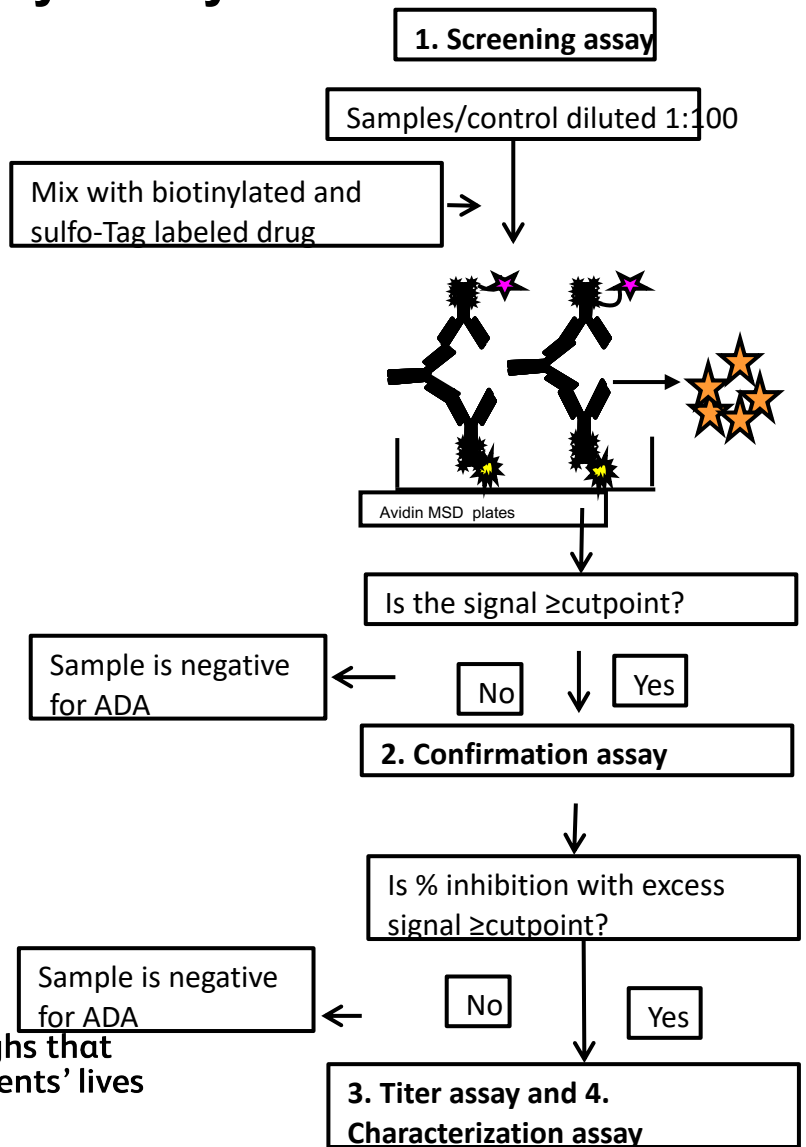
Immunogenicity Assay list for ADC

- Anti-drug Ab (ADA)
- ADA specificity to Antibody
- ADA specificity to Payload
- Neutralizing Ab to Antibody
- Neutralizing Ab to Payload

Drug	Maker	Condition	Trade name
Gemtuzumab ozogamicin	Pfizer	relapsed acute myelogenous leukemia (AML)	Mylotarg
Brentuximab vedotin	Millennium/Takeda	relapsed HL and relapsed sALCL	Adcetris
Trastuzumab emtansine	Genentech, Roche	HER2-positive metastatic breast cancer (mBC) following treatment with trastuzumab and a taxane	Kadcyla
Inotuzumab ozogamicin	**Pfizer**	**relapsed or refractory CD22-positive B-cell precursor acute lymphoblastic leukemia**	**Besponsa**
Moxetumomab pasudotox	AstraZeneca	adult patients with relapsed or refractory hairy cell leukaemia (HCL) who have received at least two prior systemic therapies	Lumoxiti
Polatuzumab vedotin-piiq[18]	Genentech, Roche	relapsed or refractory (R/R) diffuse large B-cell lymphoma (DLBCL)	Polivy

Besponsa® Immunogenicity Evaluation

Binding Antibody Assay



Neutralizing Antibody Assay

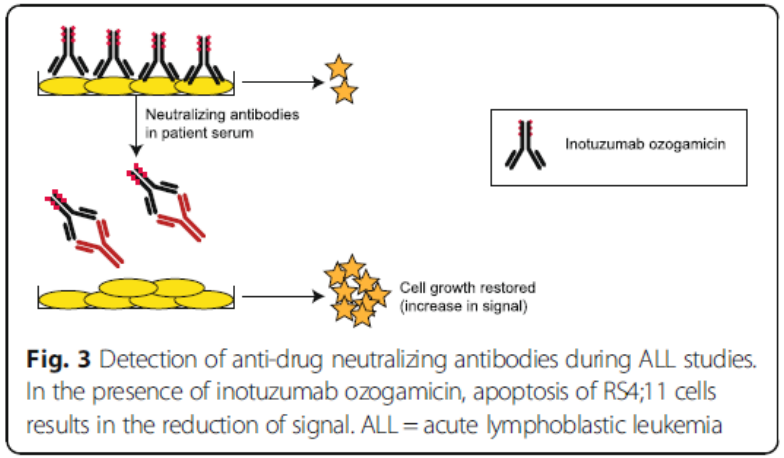
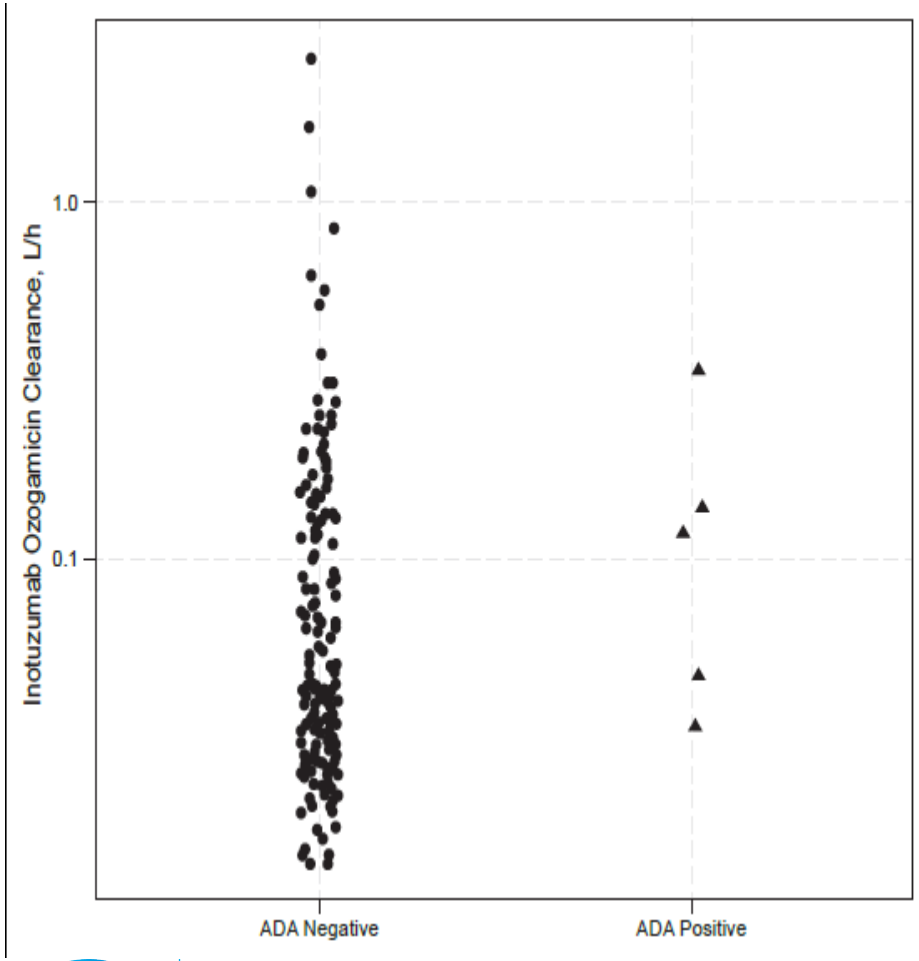


Fig. 3 Detection of anti-drug neutralizing antibodies during ALL studies. In the presence of inotuzumab ozogamicin, apoptosis of RS4;11 cells results in the reduction of signal. ALL = acute lymphoblastic leukemia

Phase 3 pivotal study

- Anti-drug antibodies were detected in 7 of 159 (4%) ALL patients
- Neutralizing antibodies were not detected in any of the 7 patients

Overall, no impact of ADA on PK parameters



- Anti-drug antibodies were detected in (4%) ALL patients
- Neutralizing antibodies were not detected in any of the ADA positive patients

Besponsa® Global approval-Feb 2017 US; June 2017 in Eu

Jani *et al. AAPS Open*
DOI 10.1186/s41120-018-0021-5

AAPS Open

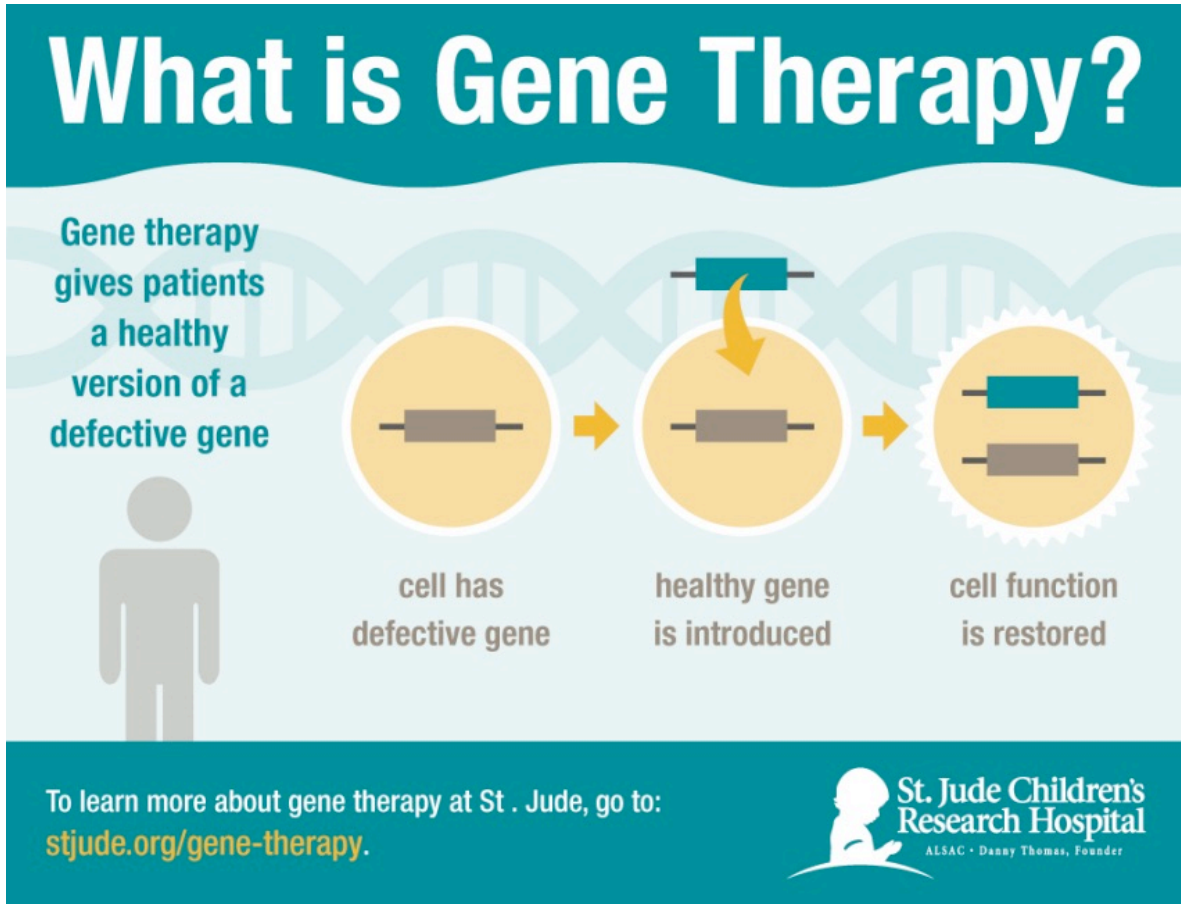
RESEARCH Open Access

Assessment of clinical immunogenicity of inotuzumab ozogamicin in patients with non-Hodgkin lymphoma and acute lymphoblastic leukemia

Darshana Jani[1*], John Nowak[2], Ying Chen[3], Joseph Boni[4] and Boris Gorovits[2]

Gene Therapy - Virus Based

- Defined as the insertion, removal or manipulation of one or multiple genes inside a cell to treat a specific disease
- More frequently, simply means “gene transfer’ and can be accomplished by transient expression or permanent insertion of gene
- Disease caused by absence of functional gene product normally produced by a single gene
- Genes delivered usually upregulated by strong promoter leading to protein expression



https://www.stjude.org/research/initiatives/gene-therapy.html

Approved Therapies

Product	Condition	Delivery
Gendicine	Head and neck squamous cell carcinoma	Adenovirus vector carrying the p53 tumour-suppressor gene
Oncorine	Late stage refractory nasopharyngeal cancer.	Oncolytic Adenovirus (H101)
Glybera	Lipoprotein lipase deficiency (LPLD), a rare inherited disorder which can cause severe pancreatitis	AAV1 viral vector with an intact copy of the human lipoprotein lipase (LPL) gene for delivery to muscle cells
Imlygic	Melanoma	HSV-1, two genes are removed and one gene is added. gene coding for granulocyte colony-stimulating factor (GM-CSF) is inserted to promote an immune response
Zalmoxis	Lymphoma or Leukemia patients receiving haematopoietic stem cell transplant	T cells modified with a Retroviras vector encoding for a human nerve growth factor receptor and the herpes simplex I virus thymidine kinase
Strimvelis	ADA-SCID (Severe Combined Immunodeficiency due to Adenosine Deaminase deficiency).	Retrovirus containing the human adenosine deaminase gene and then reinfused into the patient.
Luxturna	Retinal dystrophy	AAV2-based treatment with RPE65 gene
Kymriah	B-cell acute lymphoblastic leukemia	T-cells engineered to target CD19 receptors on the tumor B cells.
Yescarta	B-cell acute lymphoblastic leukemia	
Zolgensma	Spinal muscular atrophy	AAV9 viral vector delivers the SMN1 transgene to cell nuclei where the transgene begins encoding SMN protein

Courtesy of: Genetherapynet.com
Gene Therapy Review
Katherine High, N Engl J Med 2019; 381:455-464
DOI: 10.1056/NEJMra1706910

Understanding Challenges is the Key for Proper Support

- Risk assessment based on vector/therapeutic area – high risk for humoral immunity
- Immunogenicity evaluation requires
  - multiple assays, reagents and lead time
  - use of novel assays such as ELISPOT and other technologies
- Scientific and technical judgement is critical while regulatory environment is evolving.

Regulatory Guidance on Immunogenicity Assessment of Gene Therapy Products

EMA: Guideline on follow-up of patients administered with gene therapy medicinal products, 2010:

If it is clinically relevant, antibody and cell mediated immunity testing shall be a part of the clinical trial and the observation period should be sufficient to detect a signal. If the antibody is a non neutralising antibody, not targeting epitopes linked to the activity of the protein, and therefore without any impact on the efficacy of the GT medicinal product, then screening tests are not needed

FDA: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products, Draft Guidance, 2013:

If immunogenicity is a concern, then each subject's immune response to the product should be evaluated. This evaluation may include monitoring for evidence of both cellular and humoral immune responses."

Three Components play Role in Immunogenicity for Virus based Gene Therapy

Vector
Viral

Nucleic
Acid

Therapeutic
Protein
encoded by
nucleic acid

Immune
Response

Humoral

Cellular

Safety
Efficacy

Example: Immunogenicity Assays Using AAV Vector

Assay	Commonly Used Assay Platform	Assay Characteristics
Anti-Capsid (e.g. AAV00) antibodies	Immunoassay (MSD) Bridging format	Tiered approach of screen, confirm, titer; End Point Titer
Anti-Transgene antibodies	Immunoassay to measure antibodies against transgene –protein using surrogate recombinant protein	Tiered approach of screen, confirm, titer
Anti-Capsid (AAV00) NAB	Cell based assay	Inhibition of transduction
Anti-Capsid or anti-transgene T cell response measured by cytokine production	ELISPOT	Relative index
Binding Antibodies: IgG and IgM isotyping	Immunoassay Multiplex	Isotype positive/negative Semi Quantitative approach
Anti-Capsid or anti-Transgene immune cell population ratio before and after treatment	Flow Cytometry	Relative index

Commonly available assays to evaluate cytokine production for immunogenicity assessment

- Enzyme-Linked Immunosorbent Assay (ELISA)
- Luminex & Cytometric Bead Array (CBA)
- Enzyme-Linked Immunospot Assay (ELISPOT)
- Fluorescence-Activated Cell Sorter (FACS)
  - Intracytoplasmatic Cytokine Staining (ICS)
  - Tetramer staining
  - Pentamer staining
  - Surface marker staining

Desirable features of assays for immunogenicity assessment in regulated environments

- Performs identical with fresh and previously frozen samples
- Works with genetically diverse populations
- Uses the least amount of cells and clinical sample material
- Scalable to accommodate large-volume testing with hundreds of samples in later stage clinical trials
- Can be standardized; to enable, for example, inter-study comparisons to make data more robust for multicenter or large international clinical trials

Take Home Message
New Modalities: New Challenges: Break the Rules….

New Modalities are an exciting area of drug discovery/development

Immunogenicity assessment will continue to evolve **Novel techniques emerge.** **Novel algorithms may be needed**	**Assay approaches will need thinking** **Approach can be simple or complex depending upon the needs of the project**	**Design, execution and reporting of data from these assays have not been standardized.**

A combination of Gold standard methods and new creative approaches generate confidence in assay performance and data.

Acknowledgement

- Many Pfizer scientists
- AAPS/APA/PBSS/EBF Scientists