EIP
European Immunogenicity Platform

13th OPEN SCIENTIFIC EIP SYMPOSIUM

ON

IMMUNOGENICITY OF BIOPHARMACEUTICALS

Immunogenicity Risk Assessments for Cell and Gene Therapies

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Outline

- Novel Biologics- Scope
- Risk Factors and Assessments

- Bioanalytical/ Clinical Strategy

Background

Multiple viral vector-based gene therapy drugs and cellular therapies have now received marketing approval.

Diverse viral vectors and next generation cellular therapies (allogeneic, autologous and TCR-Ts) are in various stages of clinical trials for the treatment of genetic / acquired diseases and cancers

Immunogenicity Risks

GTs; Viral vectors and their transgene products

Cellular Therapies: response to distinct CAR domains and associated residuals can limit efficacy

Mitigation Strategies:

De-risk based on sequence and CQA minimization during Process

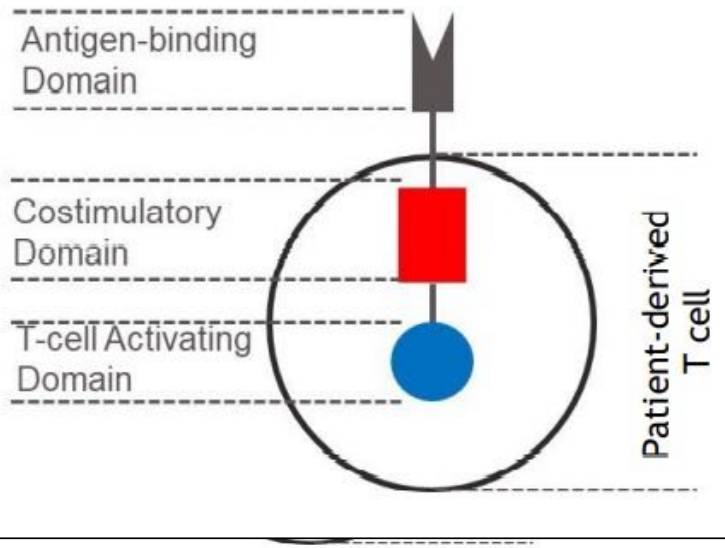
Management

Bioanalytical strategies

Clinical Immune Monitoring

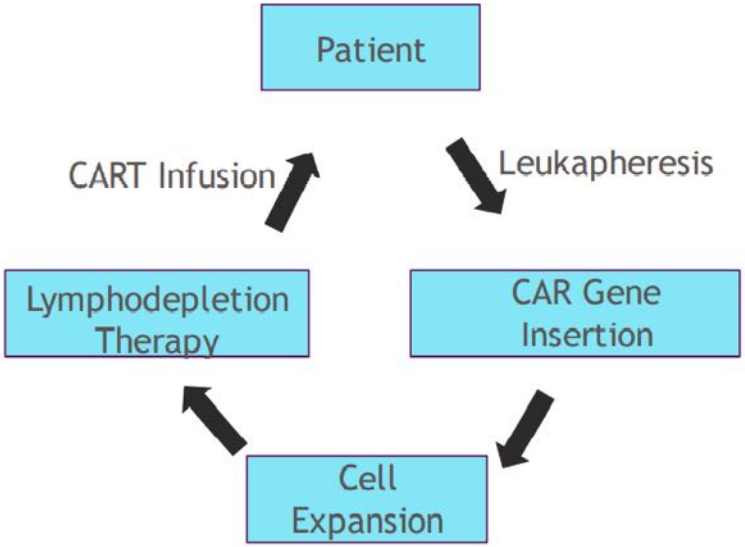
A proactive risk assessment during early development can help drive a robust bioanalytical strategy for clinic

Key Features of CART Therapeutics



- CAR T is an example of an adoptive cell therapy (ACT)
- CAR is a multi-domain protein: antigen-binding, T cell activating, costimulatory
- CAR T activity is dependent on target expression but is HLA-independent
- CAR T therapies targeting liquid tumors have better clinical results compared CART therapies targeting solid tumors

Key Features of CART Treatment Cycle

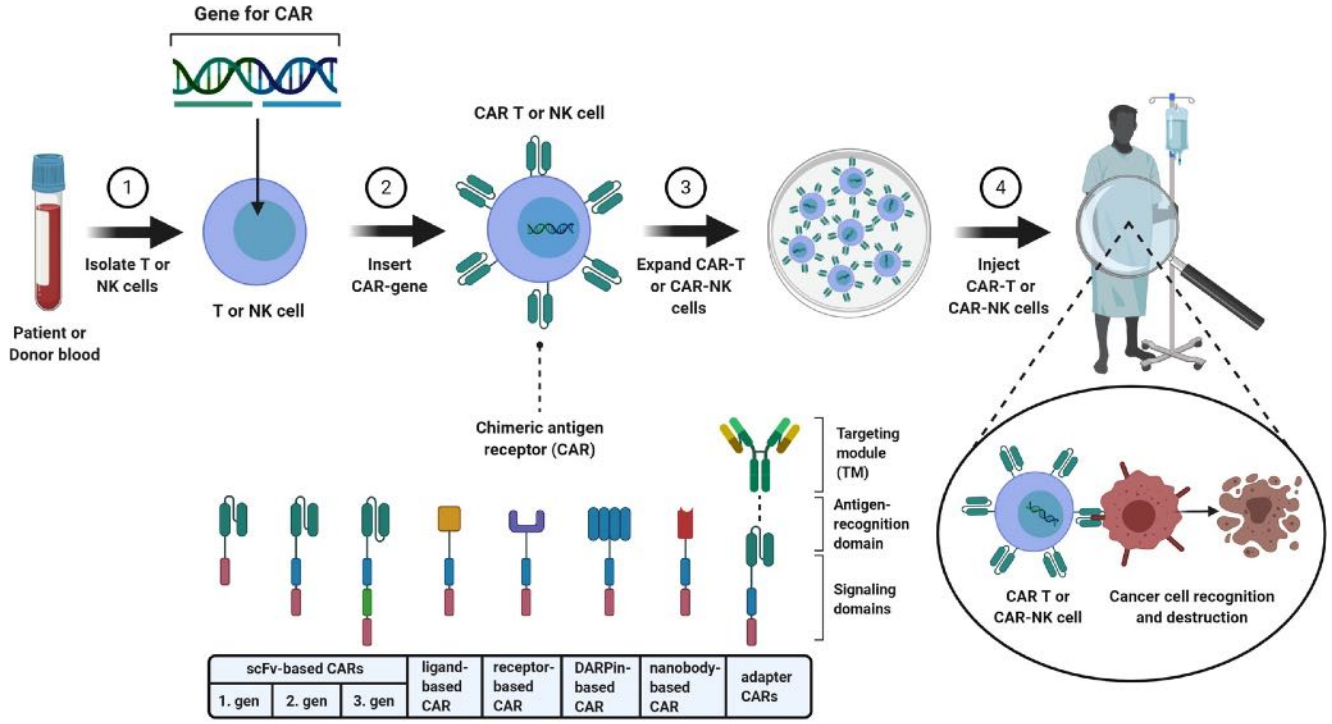


- **Yescarta™ (Axicabtagene Ciloleucel): CD19**
- **KYMRIAH® (tisagenlecleucel): CD19**

Breyanzi® (lisocabtagene maraleucel) : CD19

ABECMA® | (idecabtagene vicleucel) : BCMA

Next Generation CAR-T Modalities



Cells:

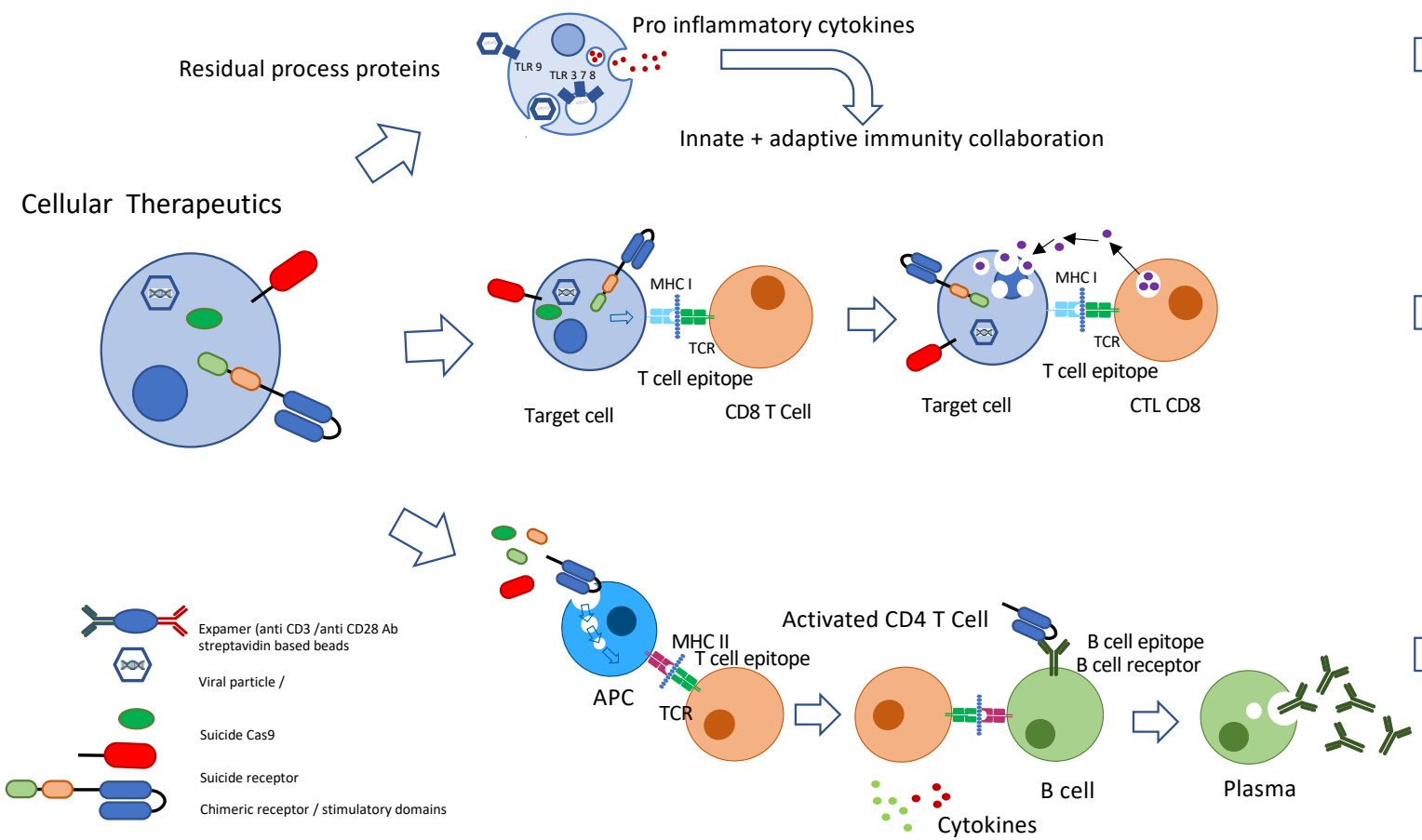
- Autologous CAR- cell Tx
  - T cells / NK cells
- Allogenic CAR "off the shelf"
  - Donor T cells / NK cells
  - IPSC

Receptor:

- ScFv to tumor antigen
- alternative scaffold
- TCR (HLA antigen binding) CAR-T
- Antigen / ligand (CAAR-T)

Adapted from Albinger et al 2021

Immunogenicity Risk of CAR-T Therapeutics



Innate immunity

Activation of innate immune system resulting
In cytokine release and cross activation of adaptive immune system

Adaptive Cellular Immunity

MHC class I mediated CD8 immune response to
Intracellular* antigens resulting in cytotoxic
T cells capable of destruction of antigen expressing cells

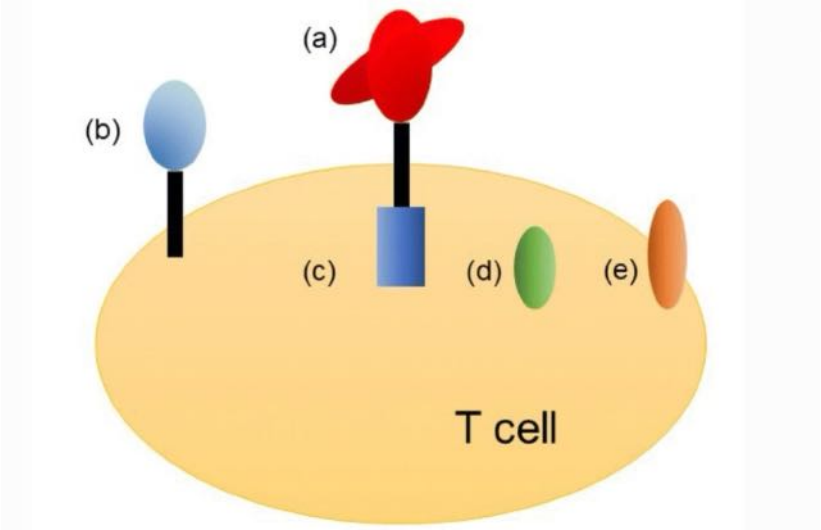
Adaptive Humoral Immunity

MHC class II mediated CD4 immune response to extracellular* antigens resulting in an antibody response

- ADA mediated compliment activation
- NK/Macrophage/Mast Cell/ FcγR mediated destruction

* Cross presentation and cross activation between adaptive and humoral immune response have been described

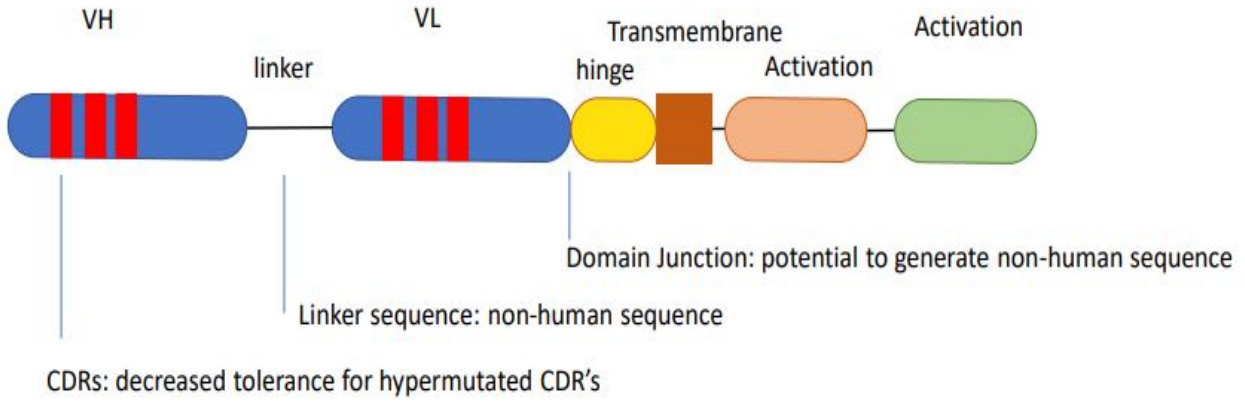
Risk Factors for CART; Structure/Sequence



(a) CAR antigen-binding moiety, frequently designed as scFv
(b) safety or suicide domain
(c) intracellular signaling stimulatory domain
(d) Residual viral
(e) Non-human proteins associated with gene

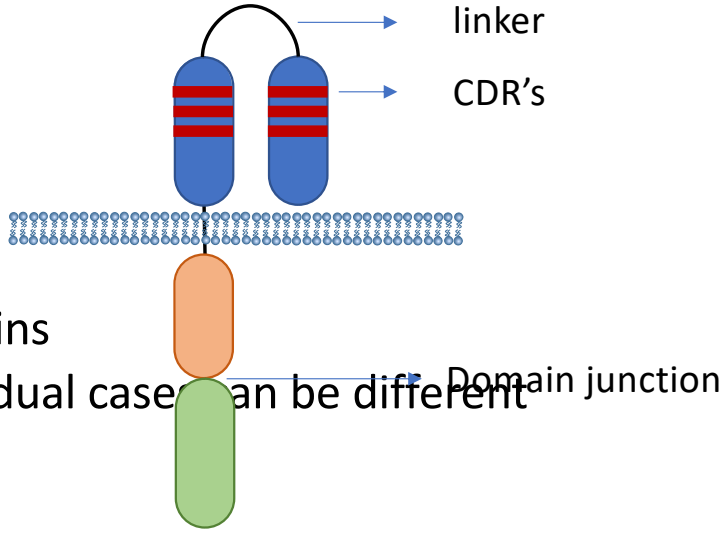
Immunogenicity Risk

- Immunogenicity risk for CAR-T constructs:
  - MHC class I / II due to intracellular expression
  - CDR's and domain junctions



CAR-T Immunogenicity Risks; Process Development

- Residual production related proteins:
  - Viral (AAV Lenti) proteins
  - Expansion mAbs / streptavidin
  - CRISPR / Talen proteins
  - Preexisting reactivity for many typo these residual proteins
  - After expansion → no more detectable protein → individual case can be different
  - WHO standards for residual protein



- Allogenic CAR-T
  - GVH (HVG → risk due to MHC mismatch risk / TCR

CAR-T Immunogenicity Risks; Patient & Disease Related

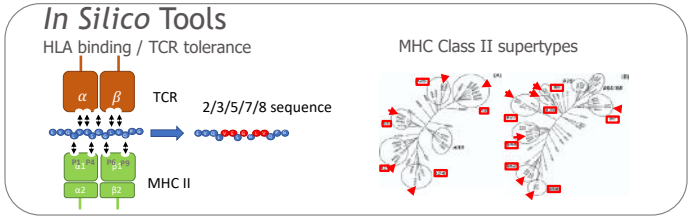
- Disease:
  - Oncology (B cell targets / lymphodepletion) approved CAR-T → Low risk
  - Oncology solid tumor no lymphodepletion → medium risk
  - Immunology / autoimmune disease → medium / high risk

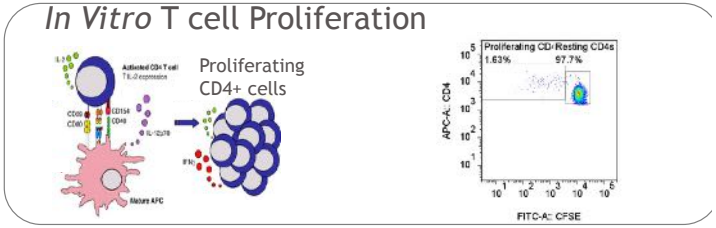
- Patient:
  - Status of immune system
  - Preexisting immunogenicity → can be background /
    - Reactivity to residual process related proteins common in humans (Cas9/AAV)
  - Previously treated with (different) CAR-T (serial dosing)
    - Risk of cross reactivity of immunity to shared elements, increased risk to boost immunity and impact on expansion and persistence

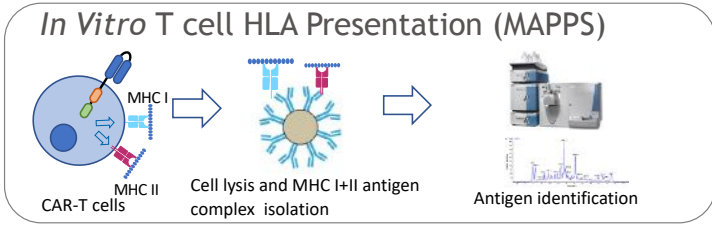
Immunogenicity Risk Assessment Assays and Tools

Immunogenicity tools and assays developed for biologics can be modified to cellular therapeutics

- ***In Silico* tools**
  - MHC Class I and II binding
  - Novel tools predicting antigen processing and presentation and tolerance
- ***In vitro* assays**
- T cell proliferation assay
  - Extracellular vs whole construct (challenge to recombinantly express whole receptor)
  - Overlapping peptides of CDRs/linkers/domain junctions
- MAPPS assay
  - MHC I and II presented peptides processed and presented peptides
  - Can be used to design peptides for clinical ELIspot /CTL assay
  - Can be used for algorithm development
- Innate activation assay
  - Residual process related proteins
  - Whole blood / PBMC / engineered TLR cell line





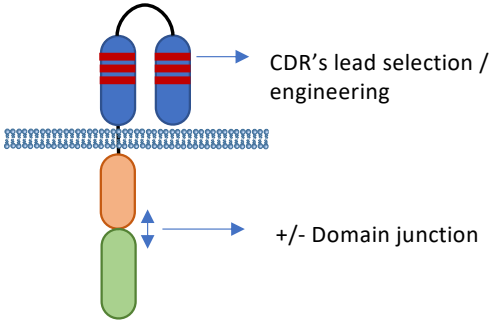


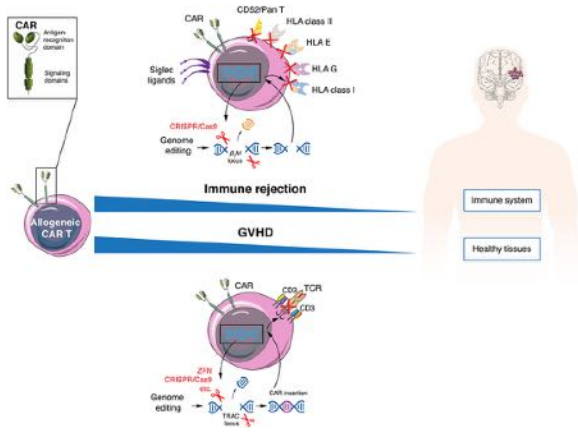
Immunogenicity Mitigation Strategies

- CAR-T Receptor Construct
  - Select ScFv / domain junctions / linkers with decreased IMG risk
    - Fully human ScFv
  - Protein engineering based de-immunization
    - optimization of receptor construct / Wild typing CDRs / moving of junctions

- Allogenic CAR-T (GVH)
  - TCR, HLA I/II, CD52 deletion using CRISPR or TALEN
  - Expression of Siglec ligands

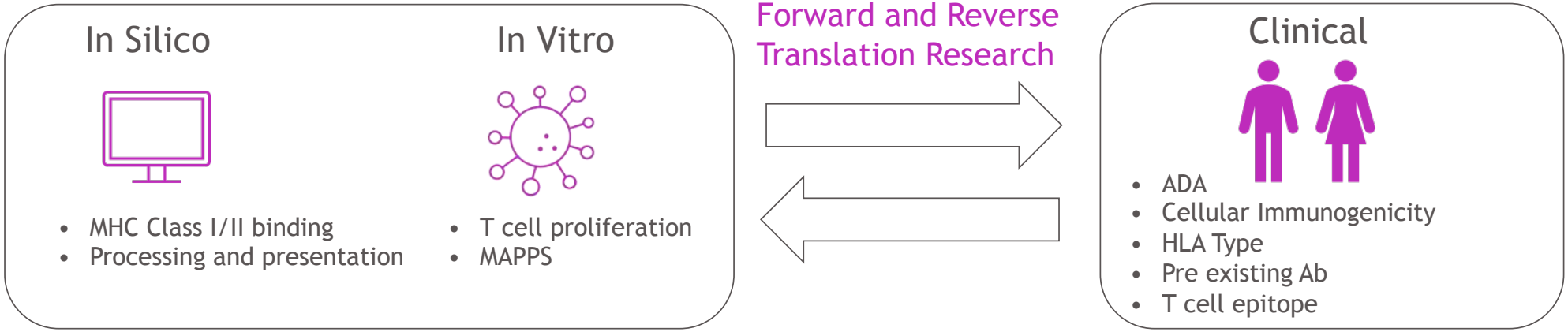
- Process Related Impurities
  - Minimize, monitoring per patient
  - WHO standards
  - Set product specs based on risk (in vitro assay data)





Reverse Translation
Clinical Trial Related Sampling and Data Mining

- Cellular Tx are a novel modality with limited Immunogenicity data
  - Need understanding mechanisms of clinical immunity to cellular Tx
  - Cellular / humoral / innate responses? → impact?
  - Accuracy of predictive tools



Bioanalytical Strategy

- PK assays
  - Cellular kinetics
  - PCR based gene expressions (matrix; blood, bone marrow, *CSF*?)
- Humoral Response
  - Technical challenge ; ECD domains, cell associated, *Nab assays*?
- Cellular Response
  - Trigger based ; loss of persistence in absence of humoral response, safety event,
- Other readouts for Innate Phase Activation
  - Residuals associated with viral vectors, Cas proteins, novel domains and linkers, contaminants in the medium etc.

Immunogenicity Assay Strategy

Autologous T cells pose low to medium immunogenicity risk

Overall strategy is similar to large molecules and large molecule guidelines are applicable

Cell therapies are capable of inducing both humoral and cellular responses

Clear communication with HA: what will be measured at what stage

Critical Attributes to consider

- Impact on exposure and expansion
- Patient and product related attributes

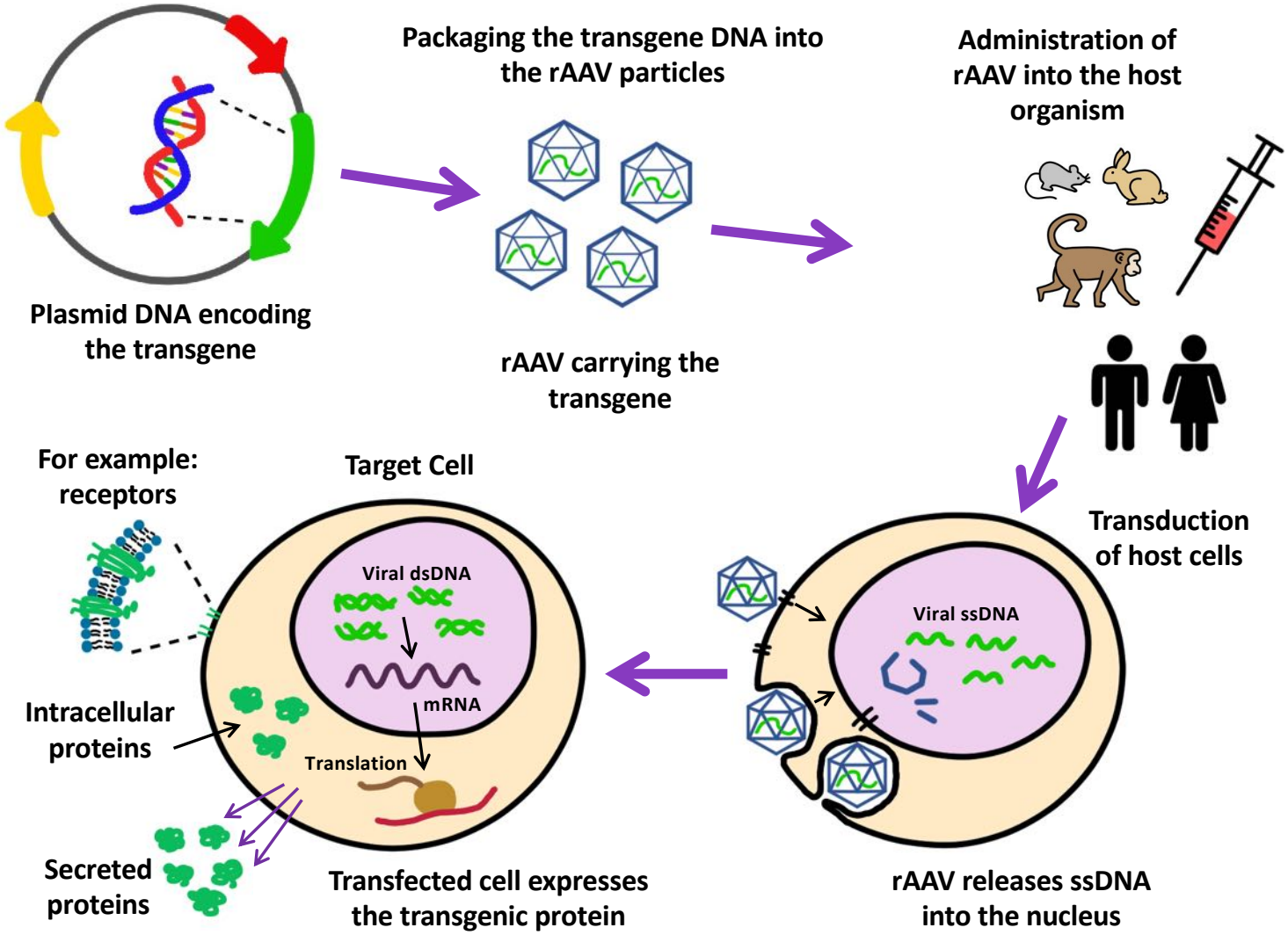
Humoral Immunogenicity

- Tiered phase-based approach for LBA approach
- LBA ADA as the initial assay
- Clear guidance/consensus on requirement of an ADA assay (LBA)
- May need a cell-based FACS assay to detect antibodies to CAR-T expressing cell as opposed to ECD in LBA
  - Eg- Kymriah
- Nab assay requirement is still unclear (Mostly implemented in Phase III)
  - Competitive LBA vs Cell Based Assay

Cellular Immunogenicity

- ELISpot and FluoroSpot widely used
- Clinicians are more interested in Cellular Immunogenicity
- Alternate assays may need to be considered

rAAV: Mode of Action and its implication in triggering immunogenicity



Product

Engineered capsid
Transgene protein: endogenous vs engineered

Enhancer/promoter:

universal vs tissue specific

Process /CQA

(unmethylated) CpG content;

Capsid serotypes;

Empty, Partial and Fully packaged Viral DNA/RNA

Patient

Genetic background;

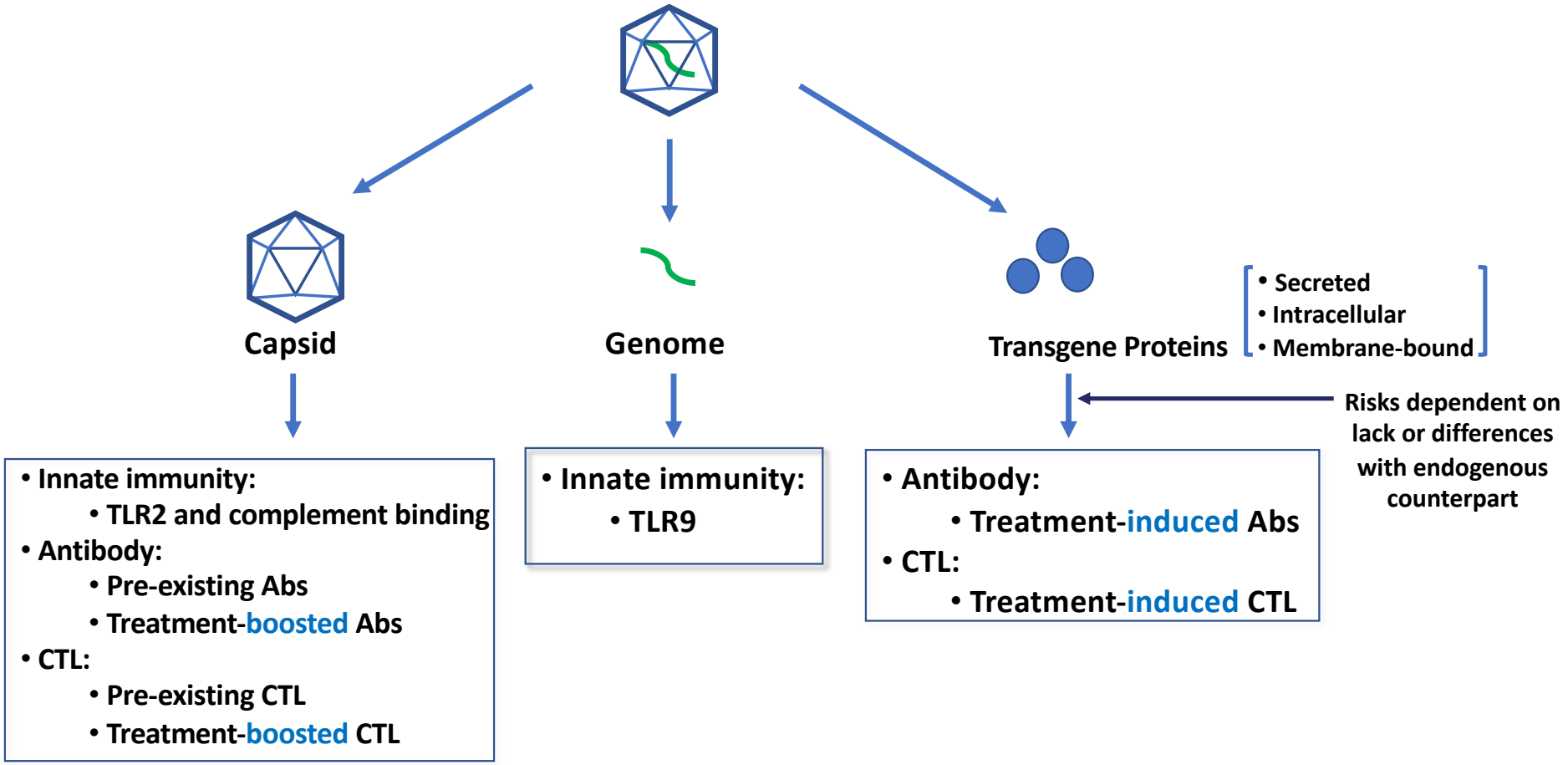
Pre-existing immunity;

HLA types;Underlying disease/immune state; Age

Dose levels; Routes of administration

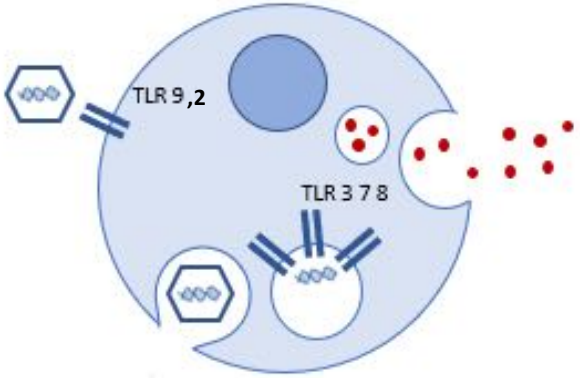
Risk Assessment Factors

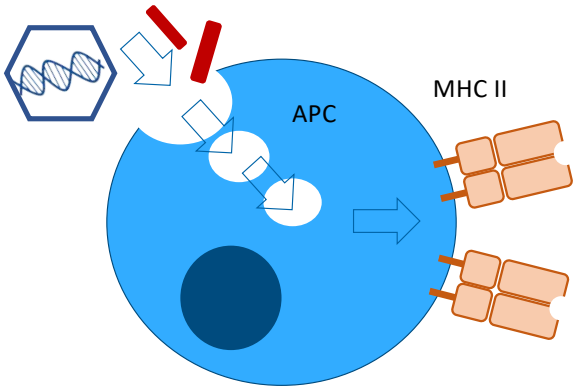
Immunogenicity Considerations for AAV-based Gene Therapies are Far More Complex: Innate and Adaptive Immunity

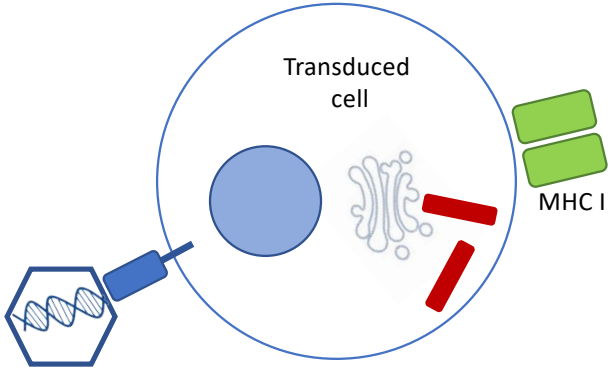


Modified from Shirley et al. Molecular Therapy Vol. 28 (3): 709-722 (2020)

Immunogenicity Risks and Mitigation Approaches







Innate Immunogenicity risk (~hrs)

- TLR mediated activation of the innate immune system
- Innate immune system activation / cytokine release cross talk /danger signal to adaptive and cellular immune system

Mitigation

- monitor TLR activation /cytokines
- CQAs:
  - Process-related impurities
  - Product related impurities
- Immune suppressive medication

Humoral Immunogenicity risk

- Transgene and or viral capsid protein is presented on MHC class II and recognized as non self
- Anti capsid antibodies / anti transgene product antibodies

Mitigation

- Monitor anti transgene and anti capsid Abs in patients (treatment-induced)
- Single dosing
- Optimize transgene and capsid sequence to reduce MHC II binding
- Tolerance induction strategies

Cellular Immunogenicity risk

- Transgene and or viral capsid protein is presented on MHC class I and recognized as non self
- Cellular immunogenicity resulting in CTL reaction to transfected cells /tissue

Mitigation

- Monitor CTL activation in patients
- Single dosing
- Optimize sequence to reduce MHC I binding
- Tolerance induction strategies

Current Learnings

Product related : High unmethylated CpG content led to increased CTL response and reduced efficacy (Wright, JF, 2020 Mol Ther 28:701)

Engineered capsids: reduced surface-exposed tyrosine residues (Li et al., 2013; Martino et al., 2013; Zhong et al., 2008).

Dose dependent complement activation and associated SAEs (Wright, JF, 2020 Mol Ther 28:701)

Host related:

Anti AAV Abs expected against most of the capsids and have led to loss of response

Responsible for immune toxicities, not necessarily responsible for clinical findings

Cellular responses observed but not necessarily connected with humoral response

Gap in understanding of cellular immune response against rAAV

Immunogenicity Assays for AAV and Transgene

- Humoral Responses: ELISA
  - Antibody to Capsid and Transgene in Serum and Ocular matrix (Vitreous and Aqueous humor) : Total antibodies , isotyping and Neutralizing Antibodies

- Cellular Responses: ELISPOT, ICS
  - AAV capsid and transgene specific T cell responses
  - Epitope mapping using specific peptide pools
  - Intracellular cytokine levels and phenotype assessments (CD4+ vs CD8+; activation markers like Ki67, HLA-DR, Bcl2
- Preclinical Studies
  - Histological changes due to delivery of viral vectors and transgenes
  - Infiltrating T cells and activated APCs

What we do not know…

- What are the molecular and cellular mechanisms dictating these immune responses? Is the immunotoxicity in humans a *de novo* immune response, or a recall response, or both?
- What is the best clinical protocol, such as prophylactic regimen, treatment window, patient selection, to prevent product-related immunotoxicity with desired persistence of transgene expression?
- What is the relationship between critical quality attributes (CQAs) and optimal vector dose for each capsid serotype to avoid immunity while providing therapeutic transgene expression?
- Can cellular immunogenicity testing of PBMCs predict CD8+ T cell responses against and rejection of the rAAV therapies? Are the assays adequate to measure cellular immune responses?

Modified from Martino A.T. et al., 2019

Key Challenges

Understand the Relationship between Immunogenicity and Clinical Efficacy, Persistence and Safety to Enable:

- Prediction of novel rAAV design:
  - vector selection, optimal PK/PD, etc.
- Prediction of clinical immunotoxicity:
  - dosing, administration routes, disease indications, selection of patients, etc.
- Optimal prophylactic treatment to promote immune tolerance so:
  - More patients can be treated;
  - More doses and frequencies can be given when needed

Risk Based Clinical Immunogenicity Strategy

Share as part of IND/INTERACT /Pre-IND Meetings

Brief background

- Vector; engineered capsid, serotype, tropism, promoter
- Transgene: target , type of mutation (null, deletion, point)

Risk assessment

- Sequence-based risk; transgene, homology to endogenous
- Mechanism of action-based risk; immune modulatory/target cells
- Population-based risk; pre-existing serotype reactivity; pediatric vs adults; healthy vs diseased state
- Quality attribute-based risk; any residuals in final packaged drug substance

Immunogenicity results from clinic (if available); previous experience

Immunogenicity Strategy

- Frequent monitoring vs. infrequent monitoring vs. storage;
- Bioanalytical assays
  - Tab vs Nab assays
  - What studies will have NAb assay, how long to defer
  - Cellular Immunogenicity assays; decision tree for when to perform;

Conclusions

Gene therapies

- Identification of Risk Factors for Immune Response to Novel AAV requires an end-to-end evaluation of risks
- The systemic immune response to viral vectors and transgenes is an interplay of both innate and adaptive phase response
- Risks differ with target tissues and hence the immunogenicity strategy for clinic will differ
- AAV component specific immune responses and transgene specific immune responses vary with target

Cellular Therapies

- The Cellular therapies would require a similar risk assessment related to the components of the CART as well as cells being targeted
- Optimization of CART domains during engineering can mitigate the risk
- Design of specific primers and probes would lead to a relevant detection of CART domains and immune response

A comprehensive understanding of risks through the course of development can be summarized as part of IND or other pre-IND interactions with health authorities to gain input on clinical monitoring and bioanalytical strategy

Acknowledgements

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- Akbar Nayeem
- Jochem Gokemeijer
- Timothy Mack
- Sekhar Surapaneni

IQ Consortium Novel Modality Working Group

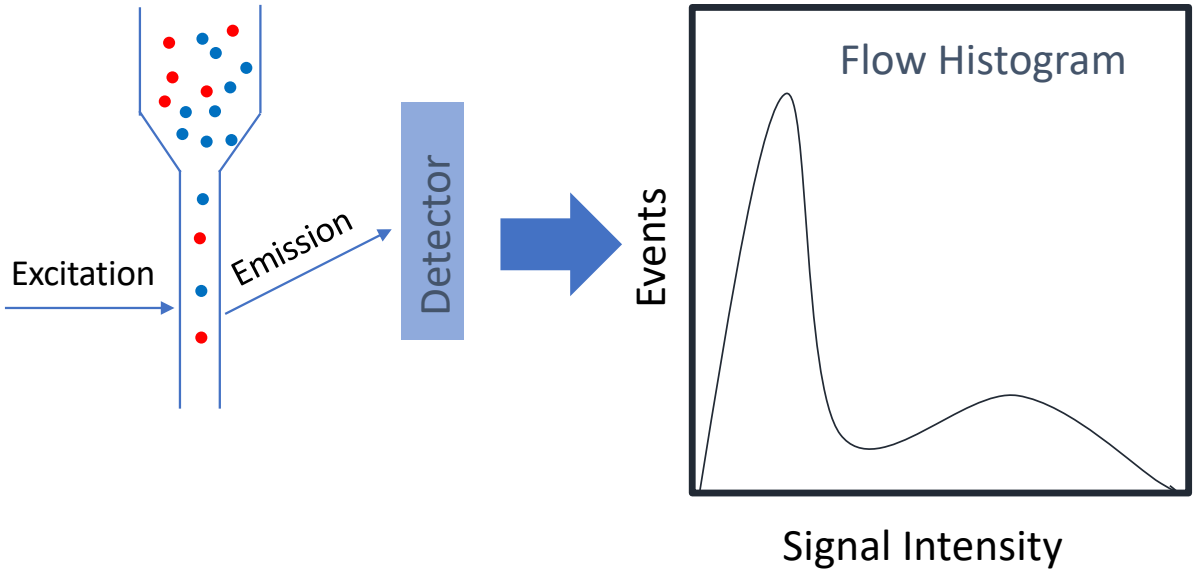
AAPS Therapeutic Product Immunogenicity

AAPS Gene and Cell Therapy Working Group

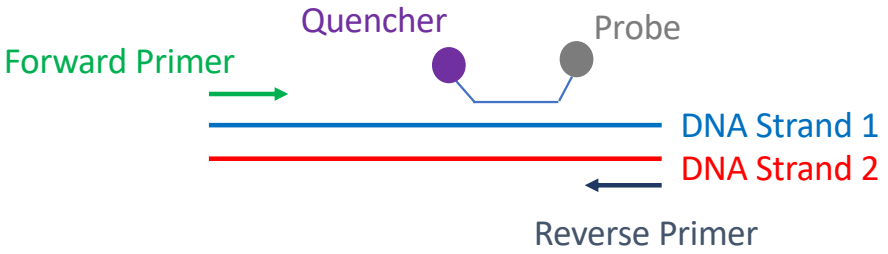
BackUps

Methods for Measuring Cellular Kinetics

Quantify Cells Directly (Flow Cytometry)



Quantify CAR T Transgene (Polymerase Chain Reaction)



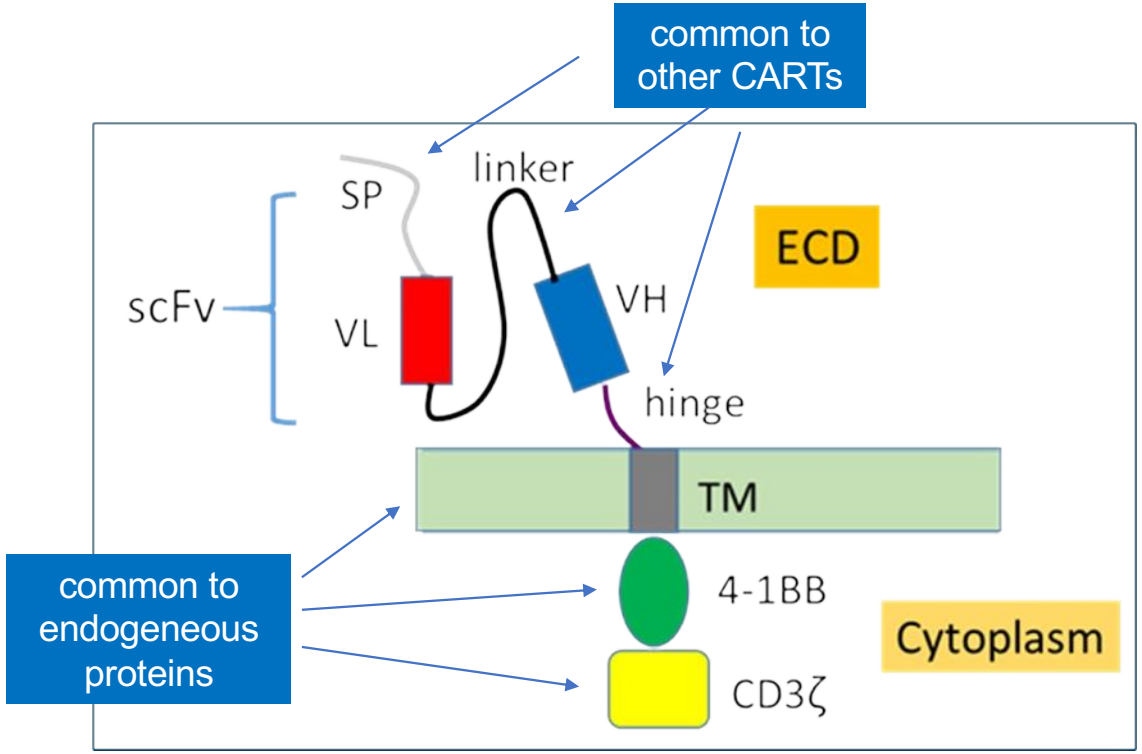
Key Reagents (Flow):

- Antibody Against Cell Surface Antigen

Key Reagents (PCR):

- Primers, probes and target sequence (plasmid or cell line)

Primer design for PK assays



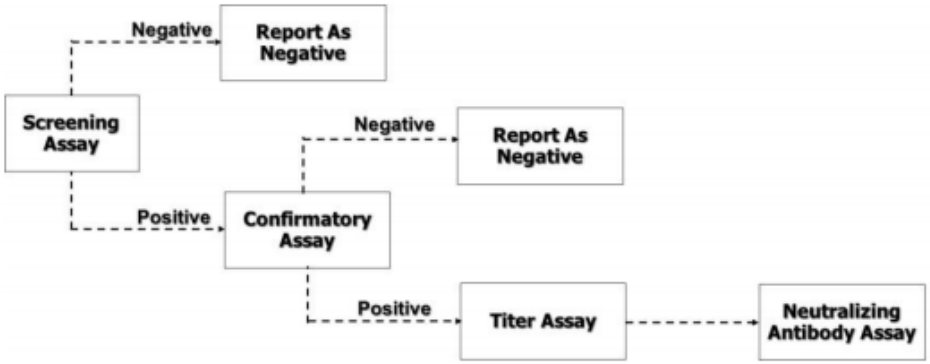
VL and VH are the 2 regions in scFv that most likely have the unique sequences for primer and probe constructs

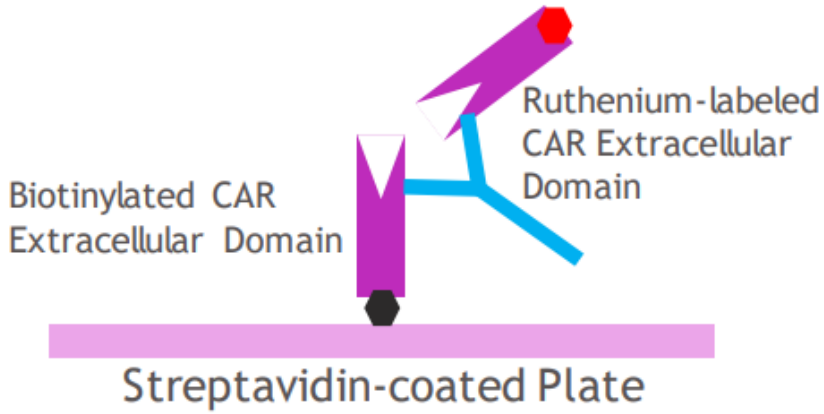
strategy

Search non-target CAR-T sequences and human genome database for sequences unique only to VH and/or VL regions in target CAR-T

Approaches to Evaluating Immunogenicity of CAR Ts : Humoral Immunogenicity

- Overall strategy is similar to large molecules
- Immunogenicity sampling times; lymphodepletion will impact immunogenicity
- Consider potential immunogenic components of CAR T: non-human sequences, residual components from manufacturing process etc.
- Neutralization Antibody Assay Format: cell based vs competitive ligand binding assays
- Established regulatory guidelines for large molecules are applicable
- Assays Utilizing Purified Reagents vs Cells that Express Target





Approaches to Evaluating Immunogenicity of CAR Ts : Cellular Immunogenicity

- Not performed with large molecule protein therapeutics
- Immunogenicity sampling times need to be considered in context of lymphodepletion
- No established regulatory guidelines for large molecules are applicable
- Assay Formats
  - Chromium Release Assay: target cells loaded with ^{51}CR that is released upon lysis mediated by effector cell
  - ELISPOT: PBMCs isolated from patients stimulated with peptides corresponding to the CAR

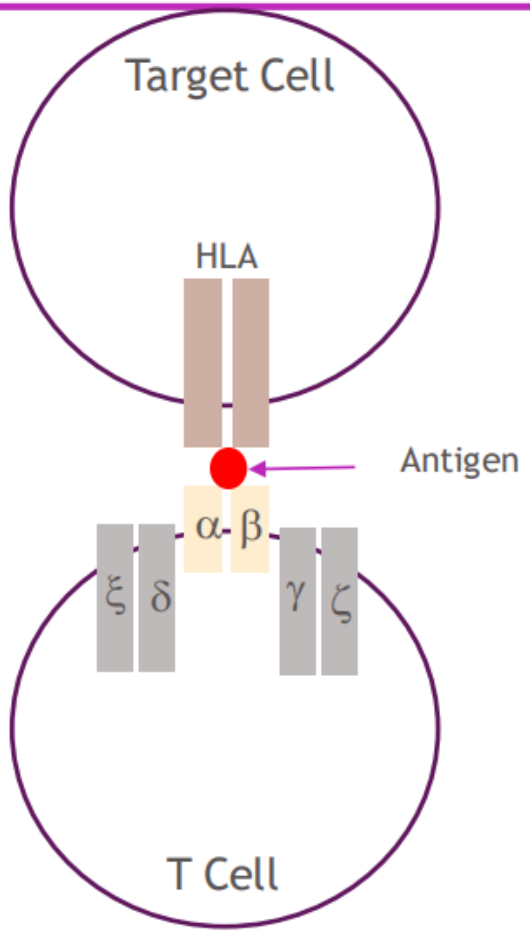


Table 1 Main CAR-T therapeutic modality immunogenicity risk factors and proposed associated mitigation strategies

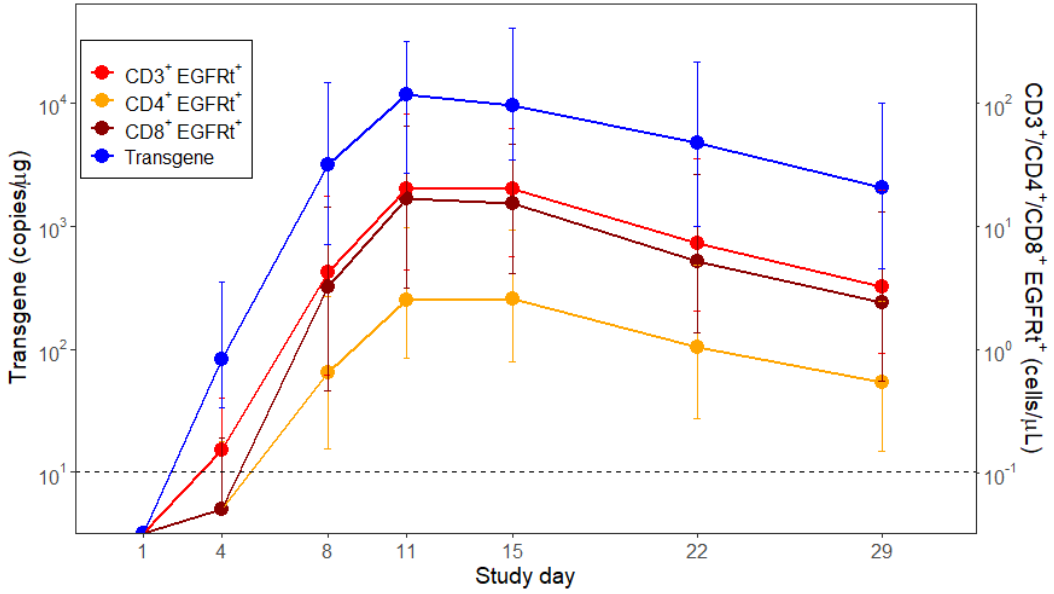
From: Immunogenicity of Chimeric Antigen Receptor T-Cell Therapeutics

Risk factor	Probability of immune response induction	Examples of response specificity	Potential response type	Proposed monitoring strategy	Proposed mitigation strategy
Non-human or partially human nature of the CAR-T construct components including CAR, suicide domain or other domains of the construct	High	Anti-extracellular CAR domain, anti-suicide domain response	Antibody and cellular	Pre- and post-dose monitoring of antibody and cellular response	Reduction or complete elimination of non-human sequence in the extracellular domain of CAR; use of a suicide domain of human origin
Presence of residual viral proteins, Presence of other residual non-human origin proteins, e.g. TALEN Gene editing-associated deletions and mis-expressions	Medium, depends on the level of residual material present	Anti-viral protein response, anti-TALEN protein response	Antibody and cellular	Risk-based approach based on the level of residual proteins present in final product	Control for non-human protein presence

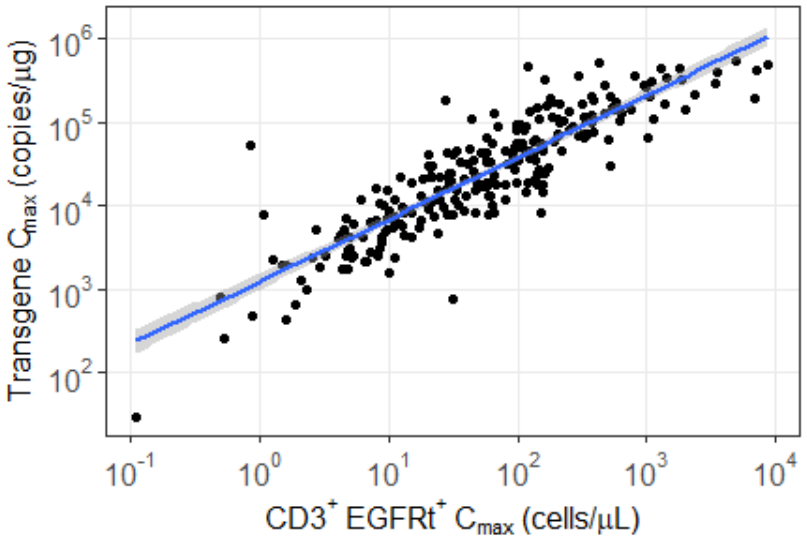
CAR chimeric antigen receptor, *TALEN* transcription activator-like effector nuclease

Case Study Number 1: *in vivo* cellular expansion as assessed by qPCR and flow cytom

Concentration-time Profile

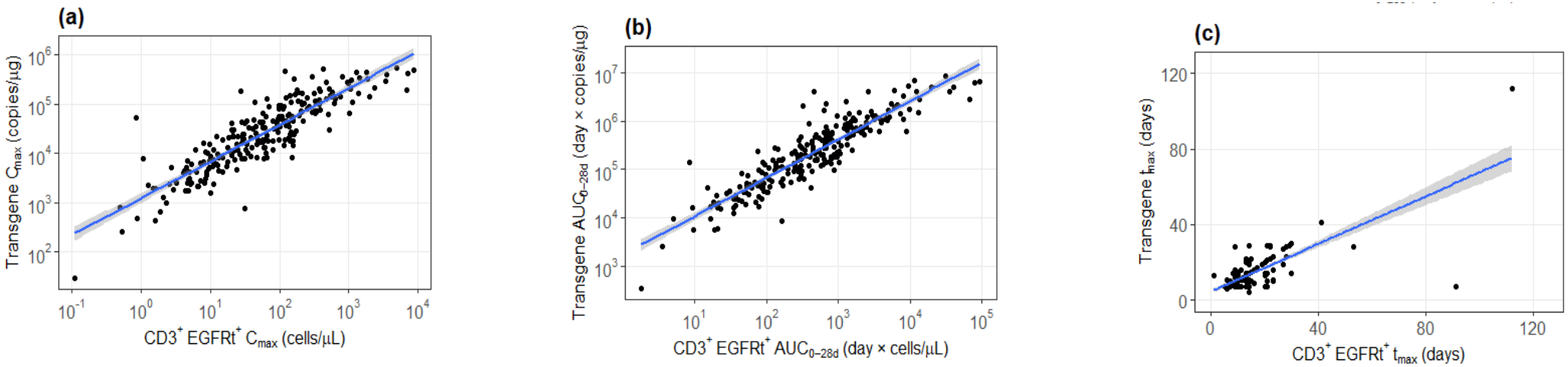


Cmax



Ogasawara, Lymp, Mack, et al. *Under prep*

Case Study Number 1: *in vivo* cellular expansion as assessed by qPCR and flow cytometry



Conclusion: High correlation between qPCR (transgene) and flow cytometry (CD3+ EGFRt+) PK parameters were observed, and flow cytometry PK was generally consistent with qPCR PK.

Ogasawara, Lymp, Mack, et al. *Under prep*