Best Practices for Bioanalytical and Immunogenicity Assessment aspects of CAR-T Cellular Therapies Development: An Industry Perspective

Moderators (Live Q&A): Shirley Chang PhD (J&J) and Lucy Xu PhD (Regeneron)

Identify risks associated with the current and next gen cell therapies and develop a risk-based bioanalytical strategy to support preclinical and clinical studies (15 mins)

Jochem Gokemeijer PhD (BMS)

Recommendations on novel bioanalytical and immunogenicity assay format, best practices and standardized approaches for assay development and validation (15 mins)

Nanda Balasubramanian PhD (BMS)

Review of available clinical data and clinical relevance of the bioanalytical strategy and assays used current (15 mins)

Weifeng Xu PhD (Merck)

Live Q&A section (12 mins)

Affiliation Communities
CGRN
Oncology (ONC)

Acknowledgement

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Novel Modalities (Cell/Gene/Viral Therapy)Working Group Of Translational ADME Leadership Group (TALG)

Co-Chairs: Vibha Jawa (BMS) and Nagendra Chemuturi (Takeda)

- **Mission / Objectives**:

  Identify risks associated with the next generation therapies using cell, viral and gene-based platforms. Develop a risk-based strategy that can drive the bioanalytical strategy for preclinical and clinical studies;

  Work on recommendations on novel bioanalytical and immunogenicity assays, best practices and standardization approaches for development and validation of these assays

  Develop a roadmap for PK/PD analysis and dose translations from non-clinical models to patients

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Acknowledgement (not in any particular order)

Name	Institution
Kate Herr	Johnson & Johnson
Vibha Jawa	Bristol Myers Squibb
Timothy Mack	
Cindy Xia	
Michael Swanson	Johnson & Johnson
Sophie Tourdot	Pfizer
Maya Vinzing	Bayer
Nanda Balasubramanian	Bristol Myers Squibb
Jochem Gokemeijer	Bristol Myers Squibb

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Joanna Grudzinska	Bayer
Edit Tarcsa	Abbvie
Hardik Mody	Johnson & Johnson
Tong-Yuan Yang	Johnson & Johnson
Robert Dodge	Novartis
Swati Gupta	Abbvie
Weifeng Xu	Merck

Jochem Gokemeijer, Ph.D.,

Director, Discovery Biotherapeutics

Bristol Myers Squibb

Jochem has been at Bristol-Myers Squibb for 17 years in different roles of responsibility focused on biotherapeutic drug development.
For the last 10 years he has been focused on building a group for pre clinical immunogenicity risk assessment and mitigation. He received his training at the University of Groningen and the Dana Farber Cancer Institute

Weifeng Xu, Ph.D.,

Director, Regulated Bioanalytics,

Merck and Co.

Weifeng has been in the field of immunogenicity for more than 10 years. He is an active member in AAPS neutralization antibody (NAb) work group as well as EBF (European Bioanalysis Forum) NAb team; he is also co-leading the NAb assay drug tolerance subteam at AAPS. After join Merck at the end of 2018, Weifeng is now leading Cell Assay group within PCD Regulated Bioanalytics to develop immunogenicity assays for both biologics, vaccines, and cell therapy.

Nanda Balasubramanian, Ph.D.,

Senior Principal Scientist, Clinical Pharmacology Pharmacometrics and Bioanalysis

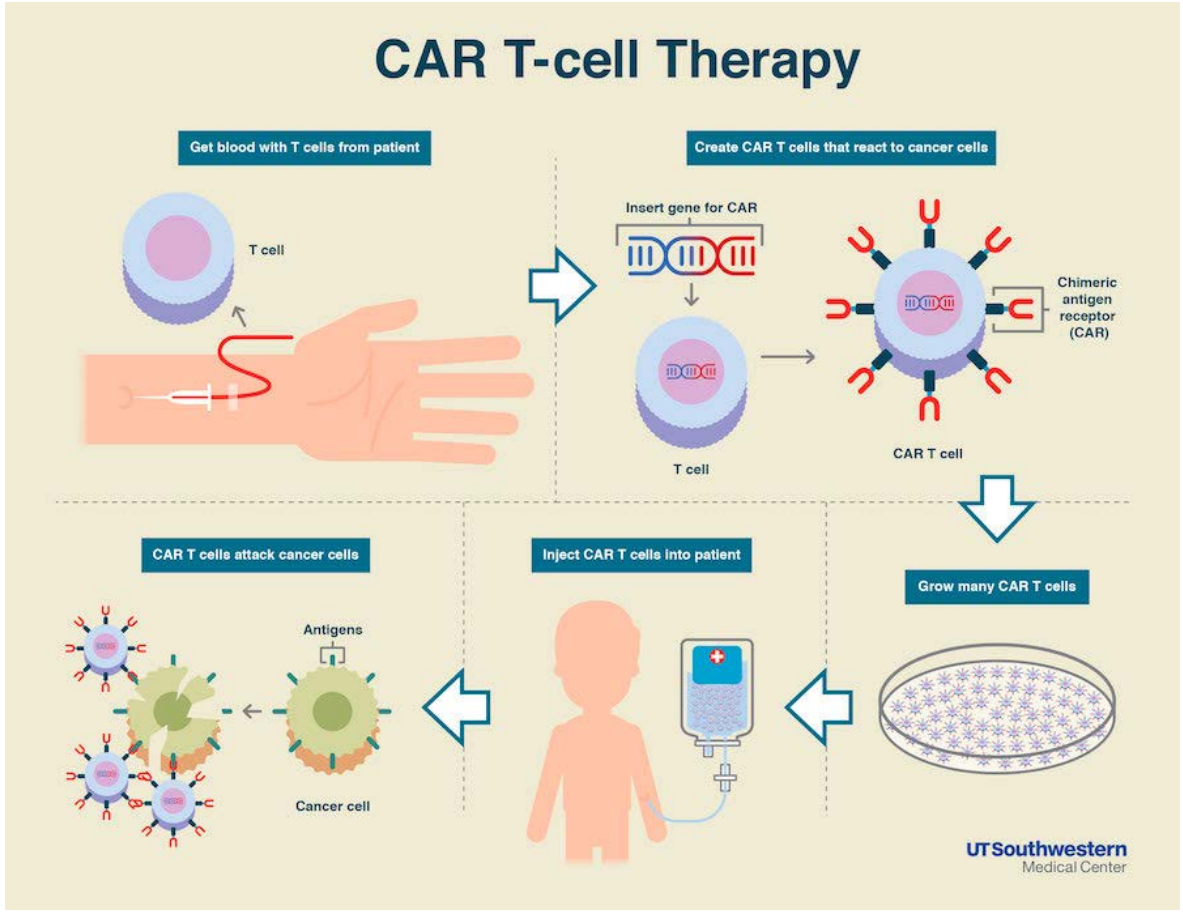
Bristol Myers Squibb

Nanda has 8+ years of immunoassay and bioanalytical experience. In his current role at BMS he supports bioanalysis for CAR-T, gene therapy and large molecules. Prior to BMS he has held the bioanalytical lead scientist role with increasing responsibilities at Alexion, Pfizer and Astra Zeneca, supporting Oligonucleotide therapeutics, gene therapy and large molecules.

Outline

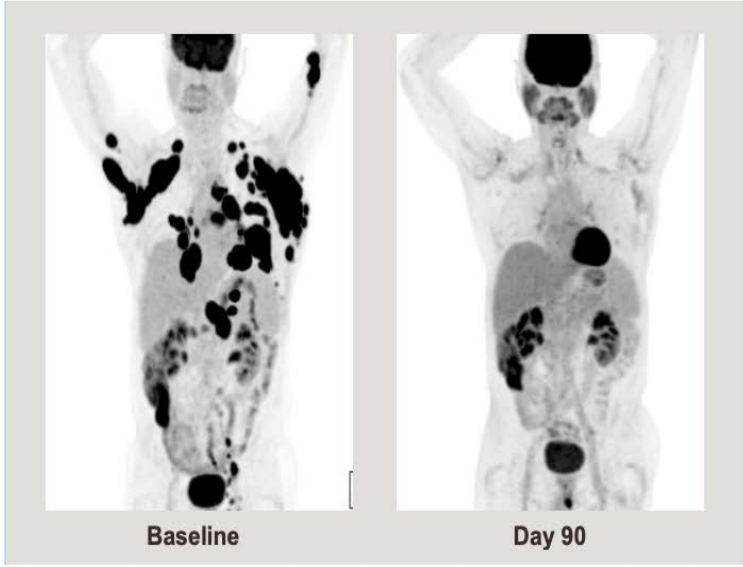
- Immunogenicity Risk Assessment
- Bioanalytical Strategy and Challenges
- Clinical Relevance

Chimeric Antigen Receptor (CAR)-T cell Therapy



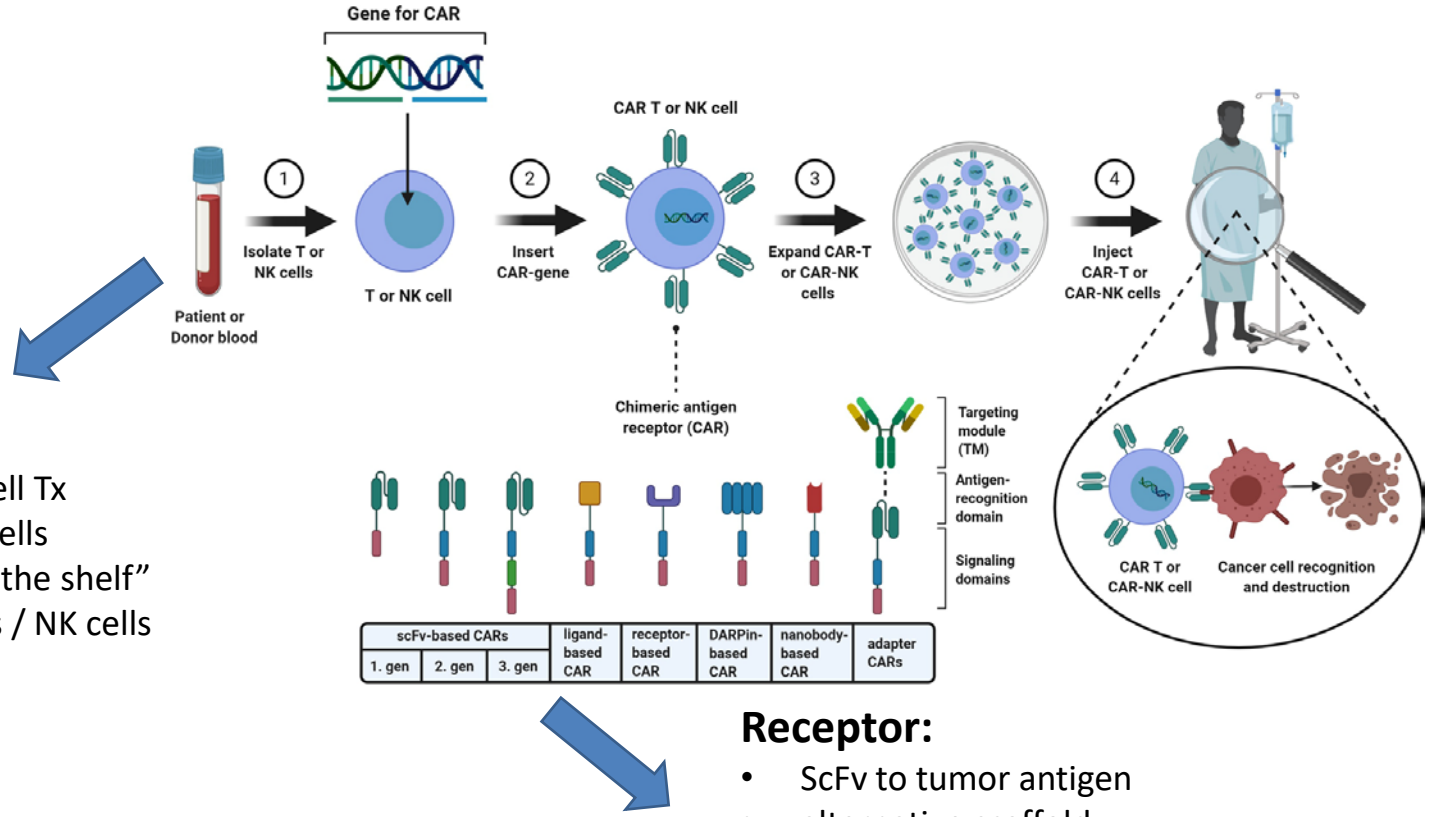
Clinical Efficacy

62 yo man with Diffuse Large B cell Lymphoma (DLBCL)
Extensive prior therapies (R-CHOP/R-GDP/R-ICE/R-Revlimid)



Adapted from Kite Therapeutics

CAR-T Modalities



Cells:

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

Receptor:

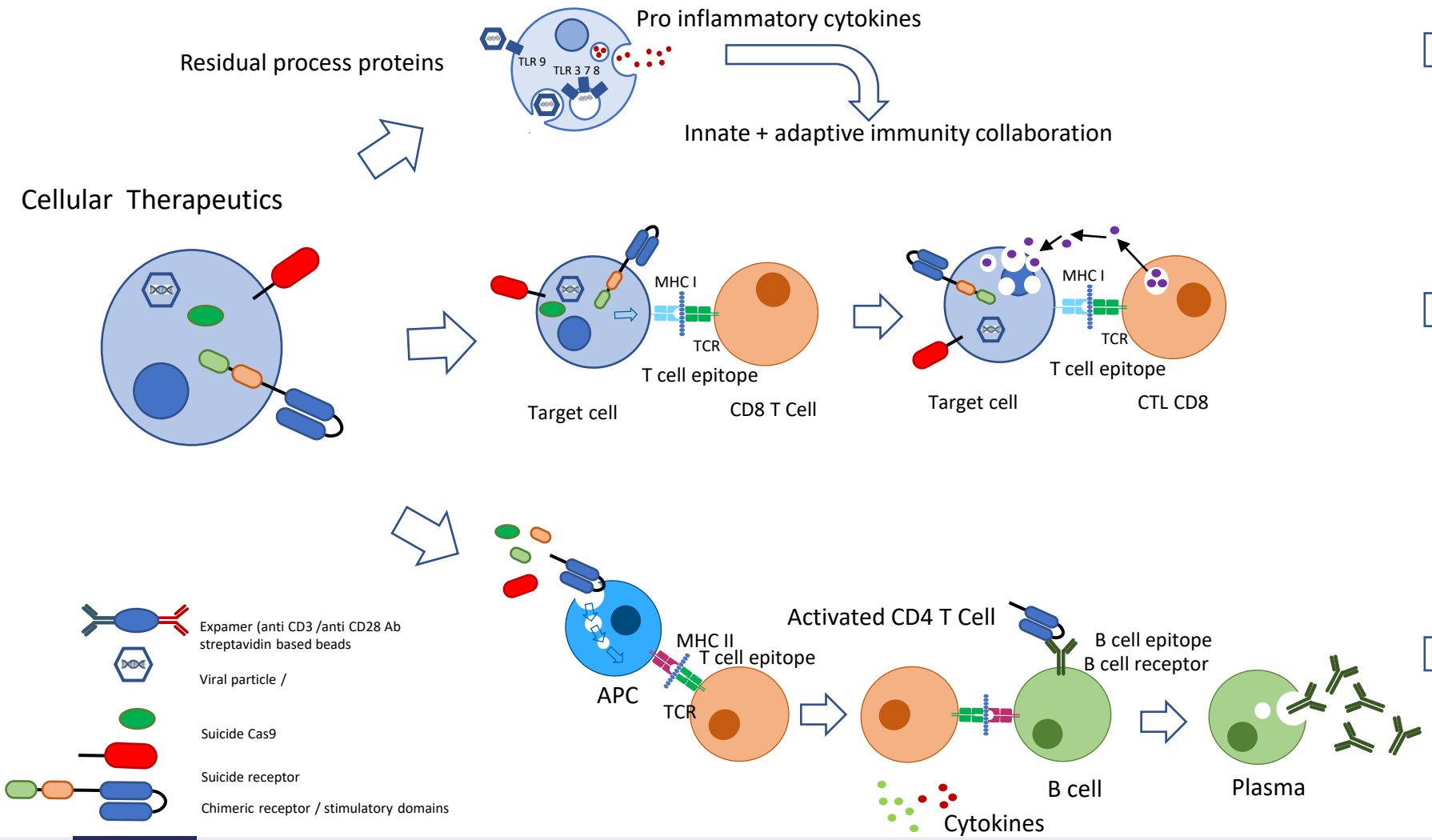
- ScFv to tumor antigen
- alternative scaffold
- TCR (HLA antigen binding) CAR-T
- Antigen / ligand (CAAR-T)

Adapted from Albinger et al 2021

Immunogenicity Risk Assessment

Jochem Gokemeijer

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

Adapted from Gokemeijer et al 2023

Potential Immunogenicity Impact

Patient safety

- Injection site reaction / anaphylaxis

Efficacy

- Destruction of CAR-T → decreased expansion / persistence
- Effects much more could be more pronounced in redosing
- ADA mediated Neutralizing of CAR function

CAAR-T (chimeric autoantibody receptor)

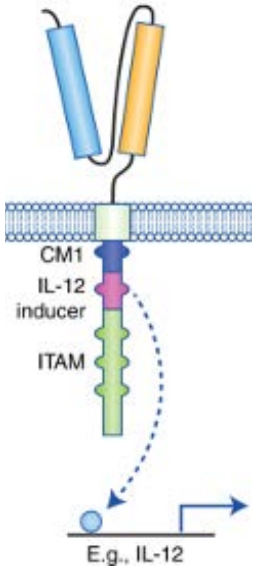
- Potential of ADA to cross react with endogenous antigen protein

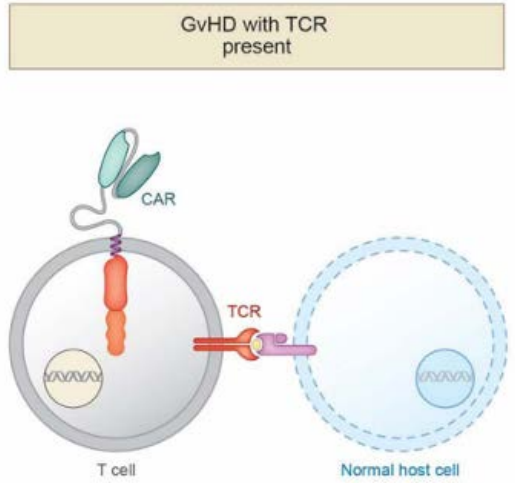
Allogenic CAR T immunogenicity

- GVH response to CAR-T
- Increased immunogenicity risk due to redosing / shorter persistence

Armored CAR's

- Secreted cytokines enables the potential of immunity to cross react with endogenous counterpart (Armored CAR)





Turtle et al. J Clin Inv 2016 / Brudno et al. Nat Med 2020

Adapted from Allogen Therapeutics

Observed Immunogenicity/Impact in Patients

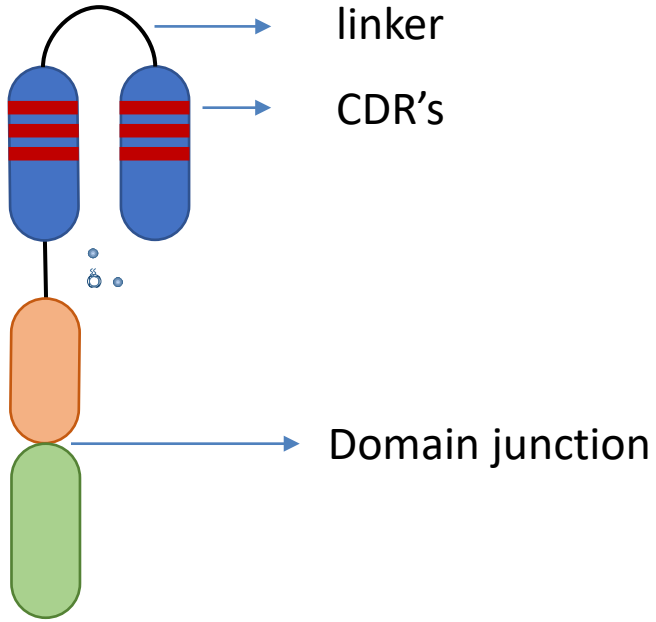
CAR-T Tx	Target	ScFv	IMG (ADA)	IMG (Cell)	Impact
Kymriah	CD-19	murine	86%-91% preexisting ADA 5% treatment induced	No T cell responses observed	No impact on response or expansion
Abecma	BCMA	human	3% preexisting ADA 47% treatment induced	NA	No impact safety, expansion or effectiveness
Breyanzi	CD-19	murine	11% preexisting ADA 11% treatment induced/boosted	NA	impact not conclusive due to small number of patients
Tecartus	CD-19	murine	2% ADA positive (16% screening positive / confirmation negative)	NA	No evidence of impact on expansion , persistence , safety or effectiveness
Yescarta	CD-19	murine	0% ADA positive (13% pretreatment positive / 2% post treatment, all negative in confirmation)	NA	No evidence of impact on expansion , persistence , safety or effectiveness
Carvykti	BCMA	murine	19.6% ADA positive		No evidence of impact on expansion , persistence , safety or effectiveness

Note: some clinical studies for in development programs have shown cellular immunity impacting expansion and efficacy

CAR-T Immunogenicity Risks

Receptor construct:

- Non-human sequence can be presented on MHC class I/II and be recognized
- Mouse vs fully human ScFv
- CDR's can be recognized as non-self
- TCR variability
- linkers
- domain junctions



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 cases can be different
- WHO standards for residual protein

Allogenic CAR-T

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

Patient & Disease Related Immunogenicity Risk Factors

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

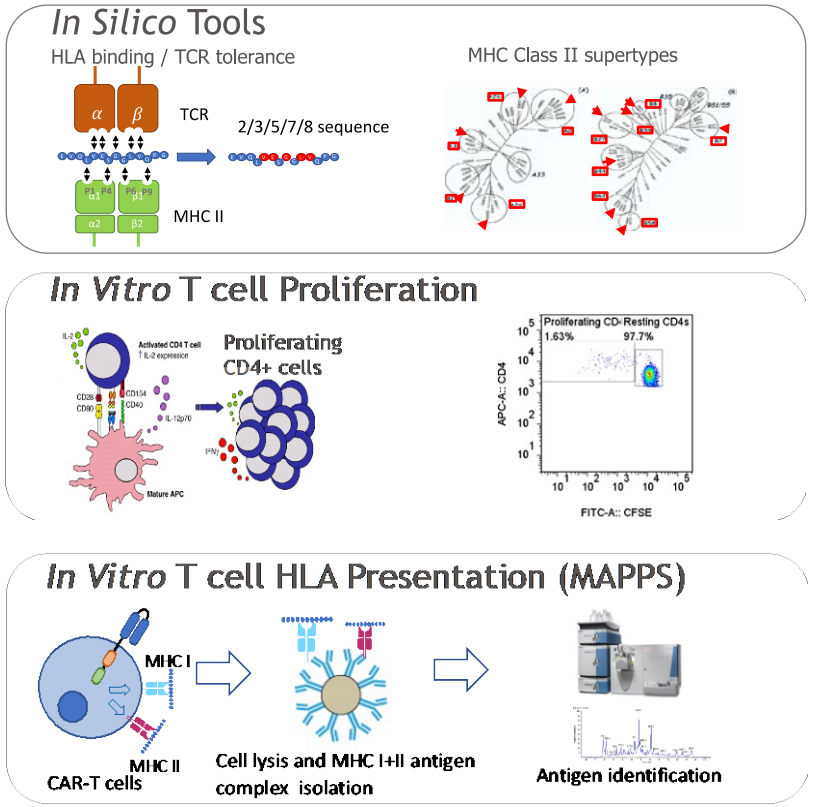
- Extracellular vs whole construct (challenge to recombinantly express whole receptor)
- Overlapping peptides of CDRs/linkers/domain junctions

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

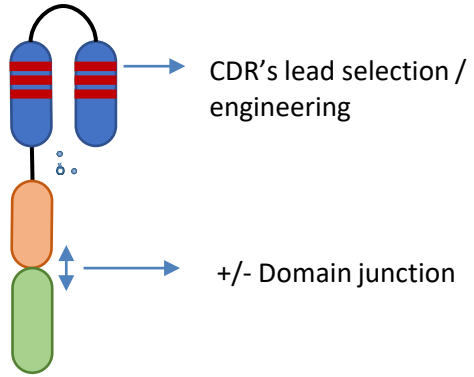
- Residual process related proteins
- Whole blood / PBMC / engineered TLR cell line



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

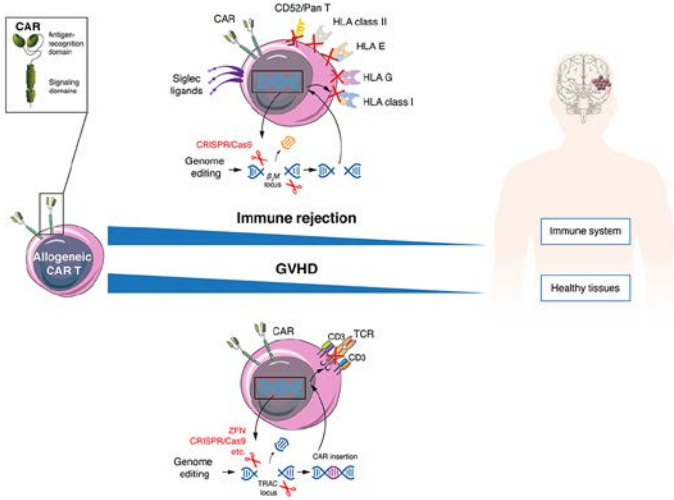


Allogenic CAR-T (GVH)

- 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)

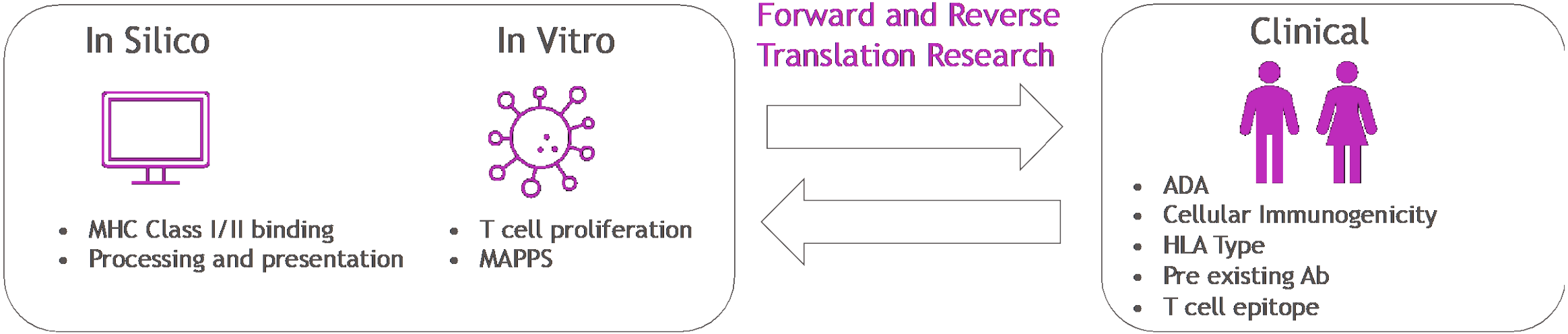


Bedoya et al 2021

Immunogenicity Translational Data

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 and Challenges

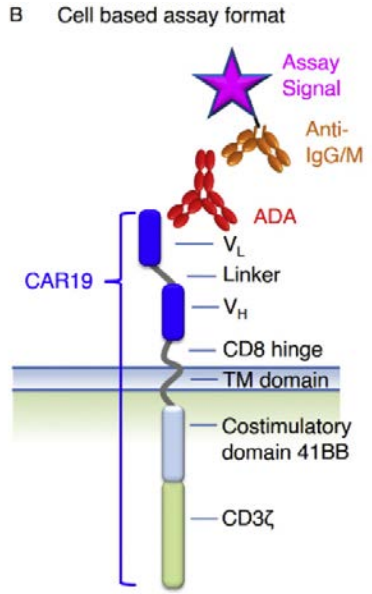
Nanda Balasubramanian

Immunogenicity Assay Strategy

- Autologous T cells pose low to medium immunogenicity risk but can induce both innate and adaptive immune responses
- Cell therapies are capable of inducing both humoral and cellular responses
- Overall strategy is similar to large molecules and large molecule guidelines are applicable
- Critical attributes to consider
  - Patient, process and product related attributes
  - Impact on exposure and expansion
- Humoral Immunogenicity
  - Tiered phase-based approach for LBA assays
  - LBA based 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
  - Alternate assays may need to be considered

Humoral Immunogenicity

- anti-CAR antibody assay usually implemented at FIH start
- Tiered approach (for LBA)
  - Screening → Confirmatory → Titer → Nab assay
- Assays could utilize purified reagents or cells that express target
- When soluble ECD domain is available a bridging LBA may be adequate
- Drug interference /matrix interference???
- A cell-based assay format is appropriate when
  - Insoluble ECD domain
  - Risk of masking immunogenic epitopes during labeling
  - Need to monitor immunogenicity to entire CAR
- Cell-based assay formats
  - Plate based assay where cell line expressing CAR or cell membrane bound ECD domain is immobilized
  - Flow cytometry-based assay using recombinant cell line expressing CAR



Potthoff B et al- (2020)

ADA assay formats comparison and challenges

Advantages of LBA based ADA assay

- Easy implementation when appropriate reagents are available
- Standard sample collection and processing

Challenges of LBA based ADA assay

- Availability of soluble CAR reagents (ECD domain, ScFv, hinge region and entire CAR)
- Masking of domains during labeling
- Matrix and drug interference

ADA Domains and features	Bridging ELISA with Soluble CAR	Bridging ELISA with source antibody (Yescarta)	Cell based assay (Kymriah)
VAR	🟢	🟢	🟢
ScFv	🟢	🔴	🟢
Linker, Hinge	🟢	🔴	🟢
Membrane Protein	🔴	🔴	🟢
Insoluble ECD	🔴	🔴	🟢
Label-Free	🔴	🔴	🟢

Potthoff B et al- (2020)

ADA assay formats comparison and challenges cont.

Advantages of Cell Based ADA assay

- Transduced cell lines –higher transduction rate – consistent CAR expression long-term
- No labelling required for CAR proteins
- Solubility is not a concern
- Enables CAR presentation in the native state with any post translational modification

Challenges of Cell Based ADA assay

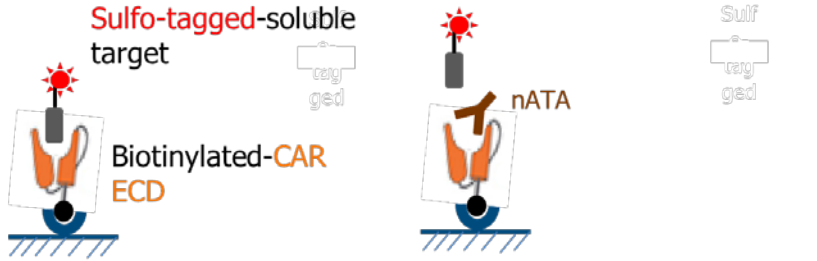
- Availability of patient-specific T cells
- Adaptation of cut point
- Loss of CAR expression and population shift is a concern
- High background potentially non-CAR specific responses
- Wild type T cells may be needed as controls
- Complex sample collection
- Require specialized labs (Not all CROs can do FACS)

ADA Domains and features	Bridging ELISA with Soluble CAR	Bridging ELISA with source antibody (Yescarta)	Cell based assay (Kymriah)
VAR	🟢	🟢	🟢
ScFv	🟢	🔴	🟢
Linker, Hinge	🟢	🔴	🟢
Membrane Protein	🔴	🔴	🟢
Insoluble ECD	🔴	🔴	🟢
Label-Free	🔴	🔴	🟢

Potthoff B et al- (2020)

Neutralizing ADA/ATA Assay: Formats

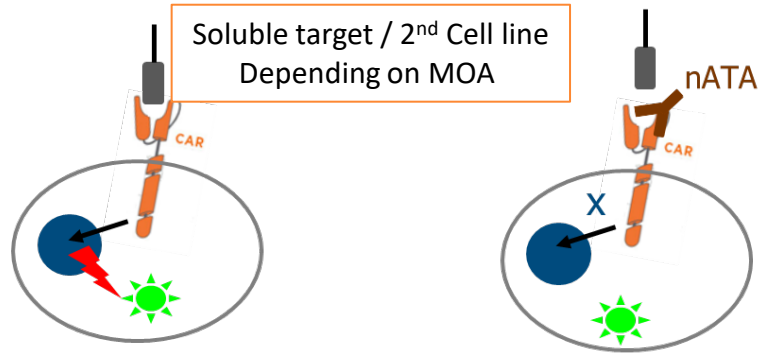
Solid-phase nATA assay



Target-bound signal

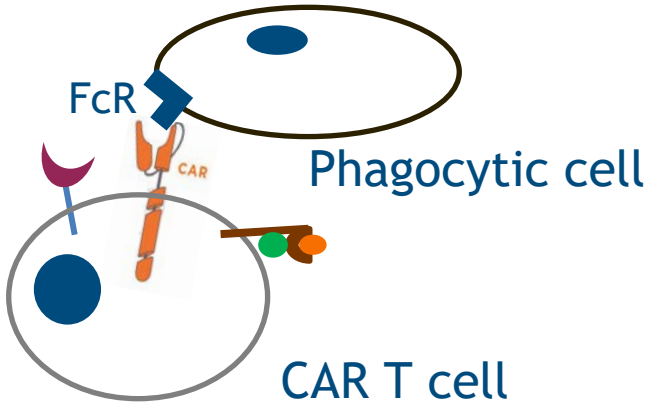
nATA binding to CAR reduces target-bound signal

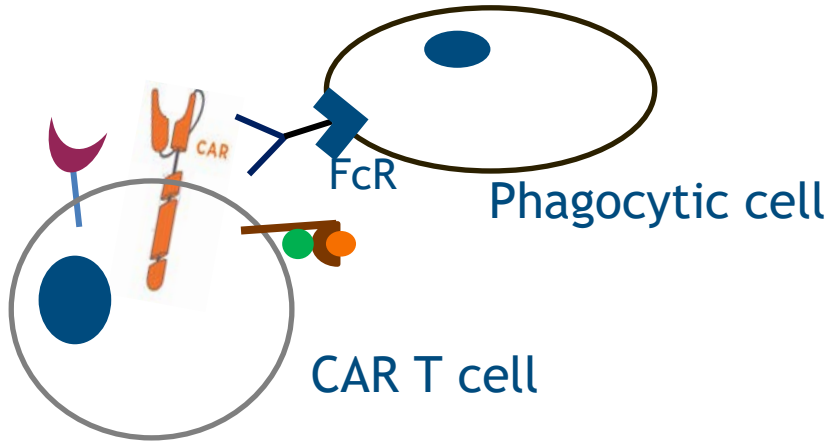
Cell-Based nATA assay



Cell-line expressing CAR receptor
Target-induced reporter read-out

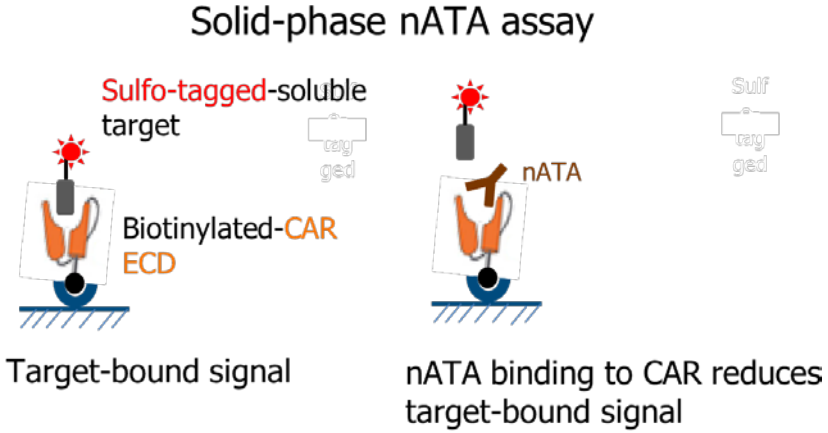
nATA prevents target-CAR interaction, reduction in signal

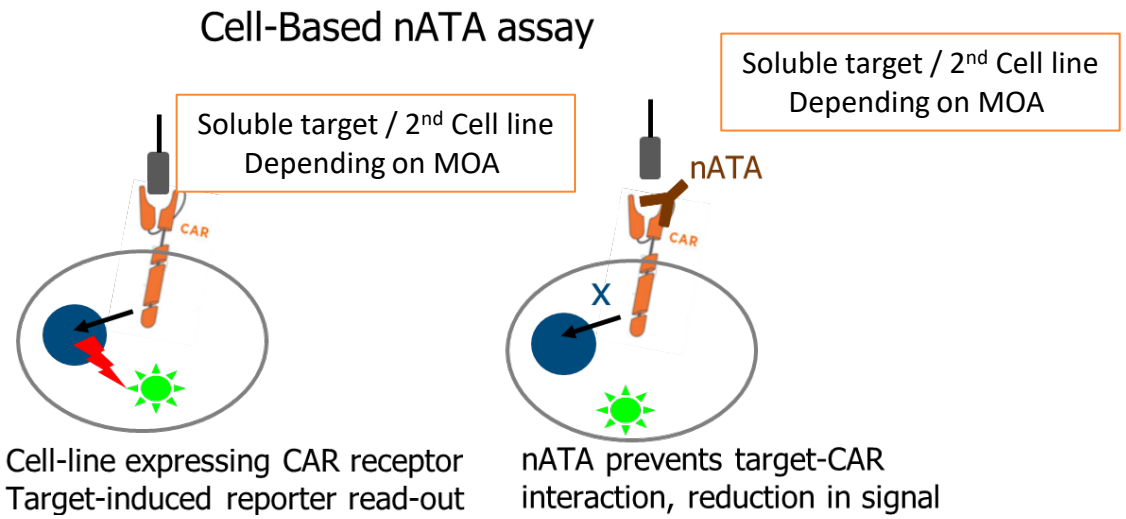




Potency/ Cell killing assays can be repurposed for Nab assays

Neutralizing ADA/ATA Assay: Challenges

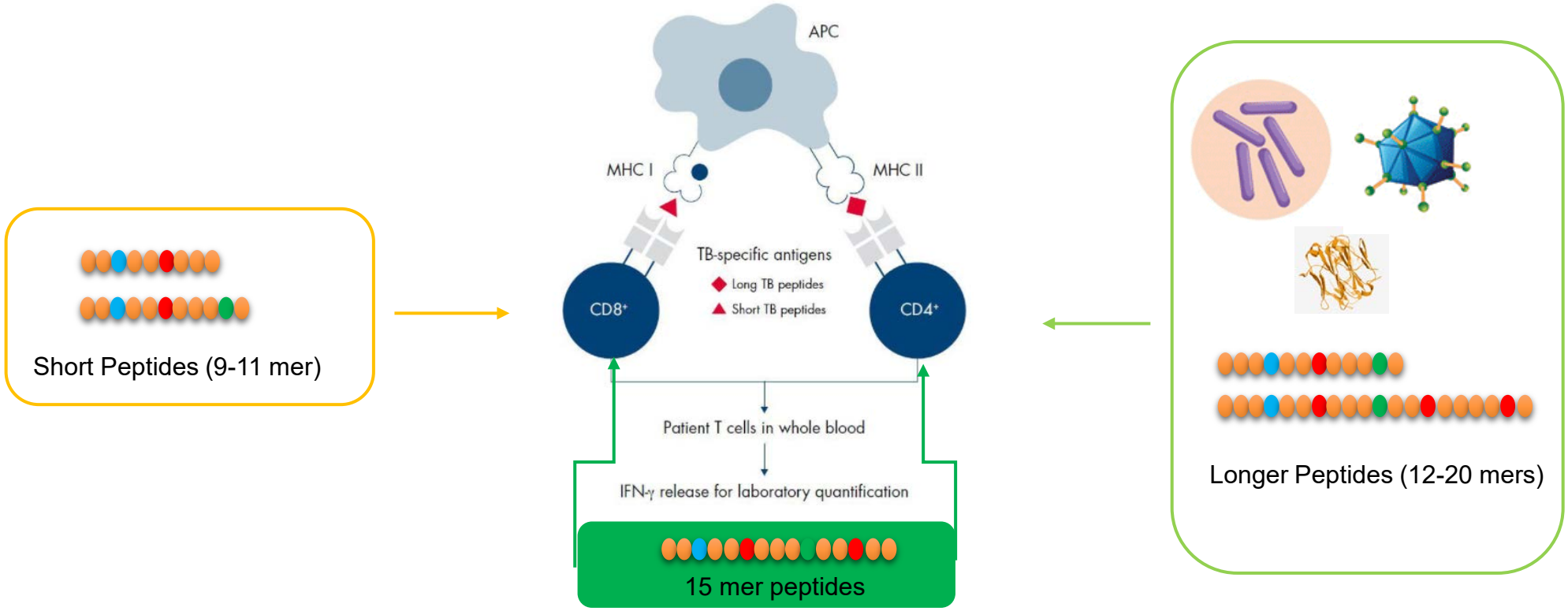




- Availability of soluble CAR reagents (ECD domain, scFV, hinge region and entire CAR)
- Masking of domains during labeling

- Availability of patient-specific T cells
- Time needed for generation and expansion of CAR expressing cell lines
- Loss of CAR expression and population shift is a concern
- High background potentially non-CAR specific responses
- Wild type T cells may be needed as controls

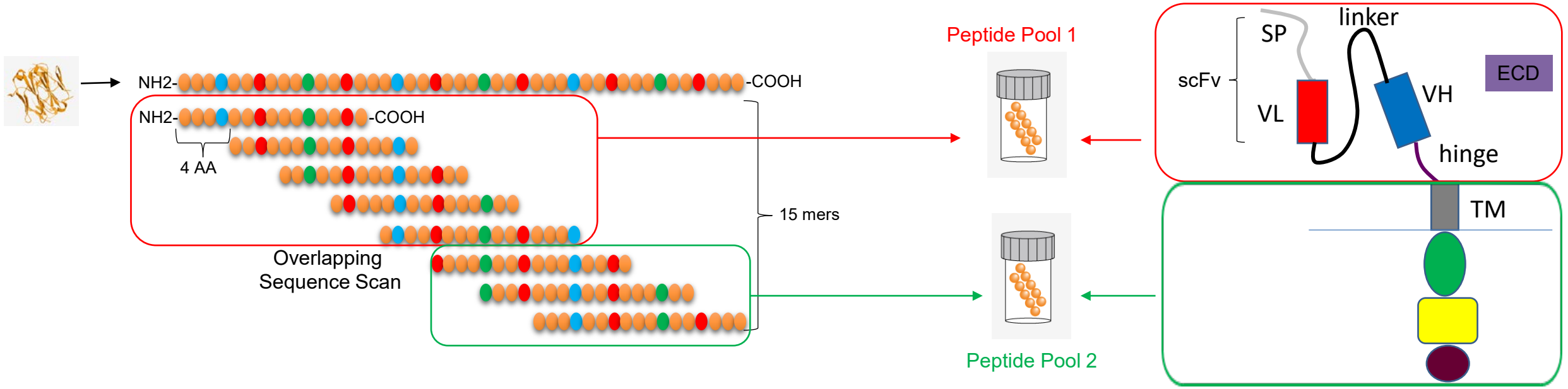
Cellular Immunogenicity



Enables activation of MHC Class I and Class II

ELISPOT and FluoroSpot assays are widely used

Peptide Design for Cellular Immunogenicity



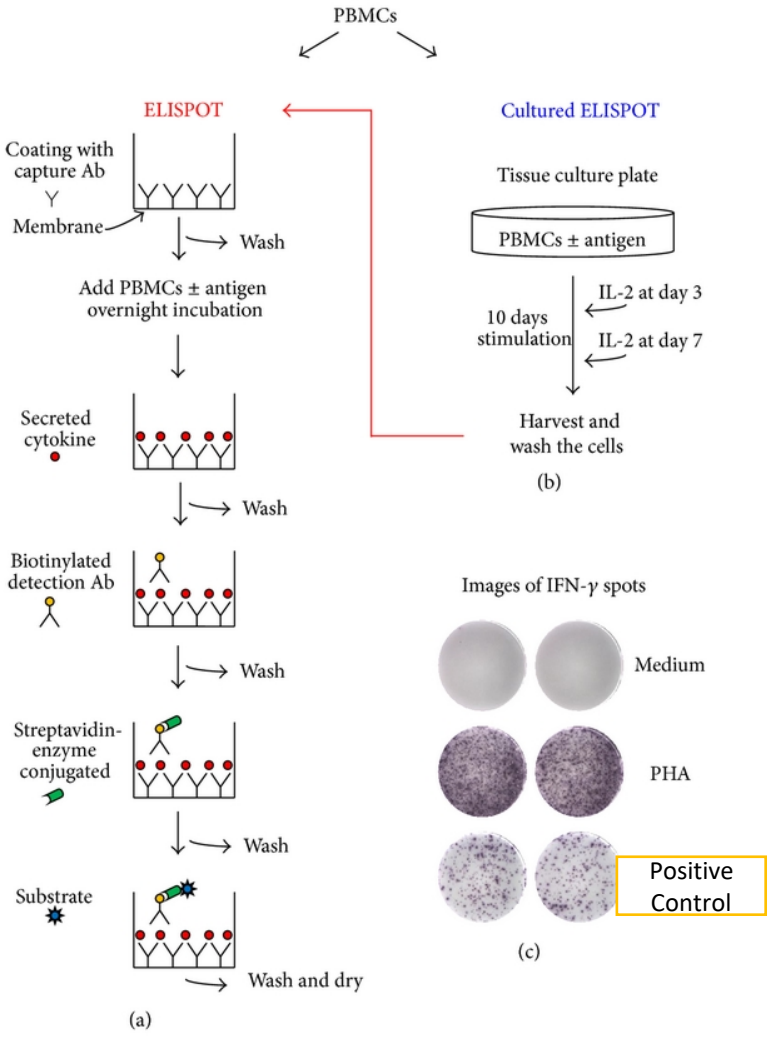
Peptide Design Strategy

- 15 mer peptides with 11 mer overlap
  - covering the entirety of the transgene
  - Immuno-dominant regions
- Capped Peptide Synthesis to ensure correct sequence

Peptide Pooling Strategy

- Pooling strategy to enable domain mapping

ELISpot Assay



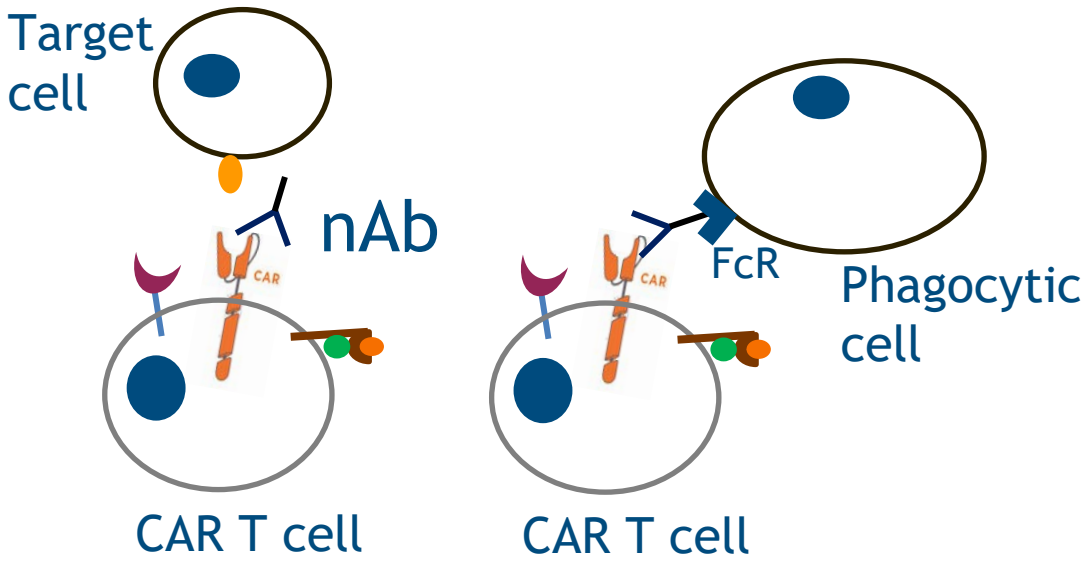
Attributes and Challenges

- PBMCs isolated from patients and stimulated with peptides corresponding to the CAR
- PBMC isolation is critical and requires high blood volume
- Sampling time should be determined in context of lymphodepletion
- No established guidelines for validations parameters
- Non availability of patient PBMCs
- Sample transport logistics could be an issue
- Assay implementation and cost
- Need for Orthologous methods
  - Chromium Release Assay
  - Luminex Assay

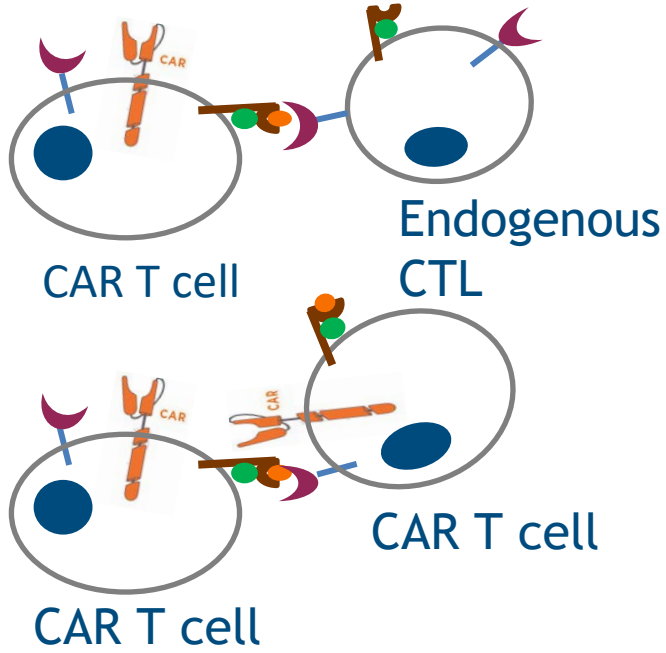
Adapted From Calarota SA and Baldanti F J, Journal of Immunology Research, 2013

Potential Inhibitory Mechanisms of Humoral and Cellular Immunogenicity of CARTs

Humoral Immunogenicity

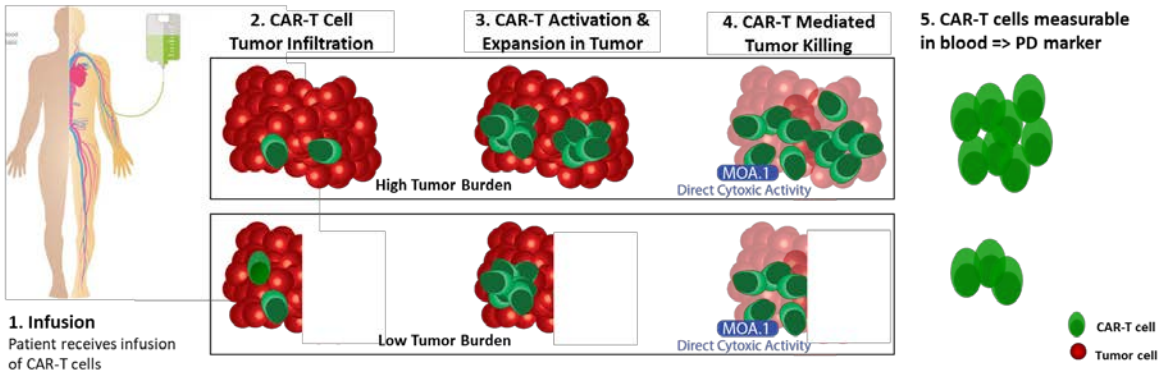


Cellular Immunogenicity



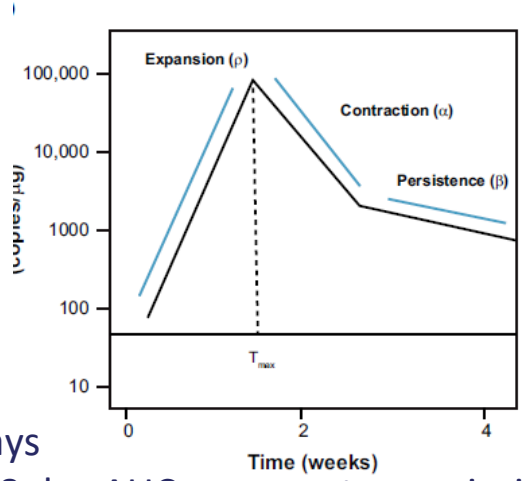
What is the impact on exposure, expansion and persistence of CAR-T cells?

General Features of CART Cellular Kinetic Profiles



Property	Small/Large Molecule	CART
Ability to Proliferate	No	Yes
Reason for α and β phases	Distribution and Elimination	Contraction and Persistence
Terminal $T_{1/2}$ Scale	Hours, days, weeks	Years
Product Variability	Minimal	Variability exists
Dose-Exposure Relationship	Yes, may be nonlinear	No strong relationship between dose and exposure

Cells expand in patients upon target engagement and can persist for years

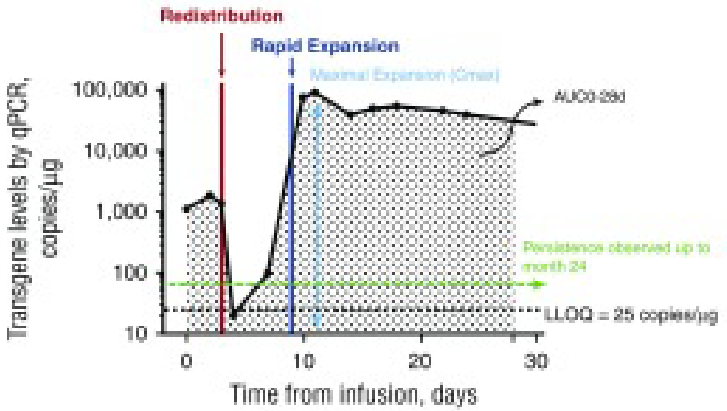


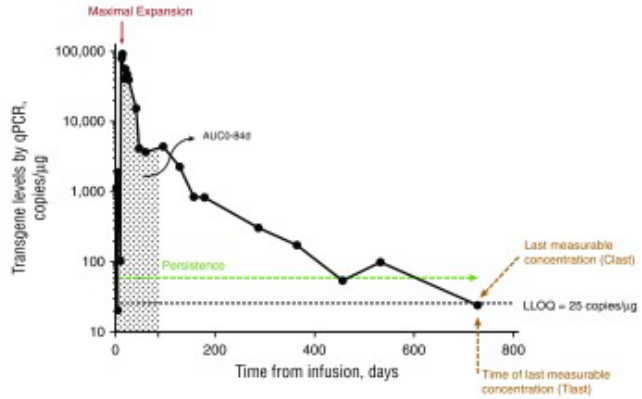
T_{max}: 10-14 days

Exposure: 0-28 day AUC represents a majority total exposure

Persistence: may be upwards of 1 year or greater

Matrix: Blood and Bone Marrow



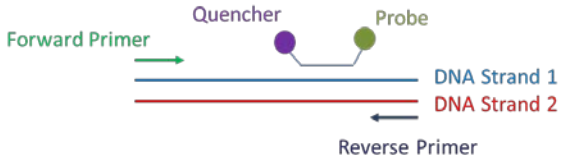


Setting up the right sampling plan is critical

https://uihc.org/health-topics/car-t-cell-therapy Adapted From June C. 2019, Pharmacometrics Syst Phamracol. *Blood Adv* (2020) 4 (19): 4898–4911.

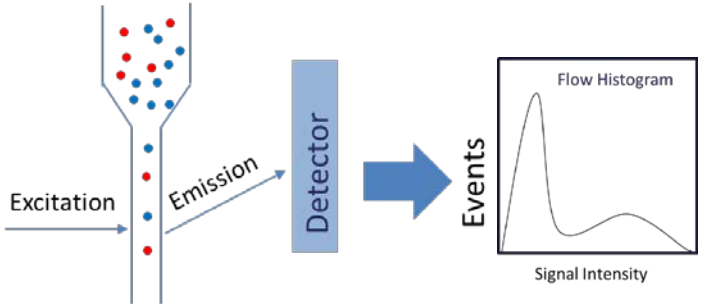
Methods for Measuring Cellular Kinetics (CK)

Quantify CAR T Transgene Indirectly (Polymerase Chain Reaction)



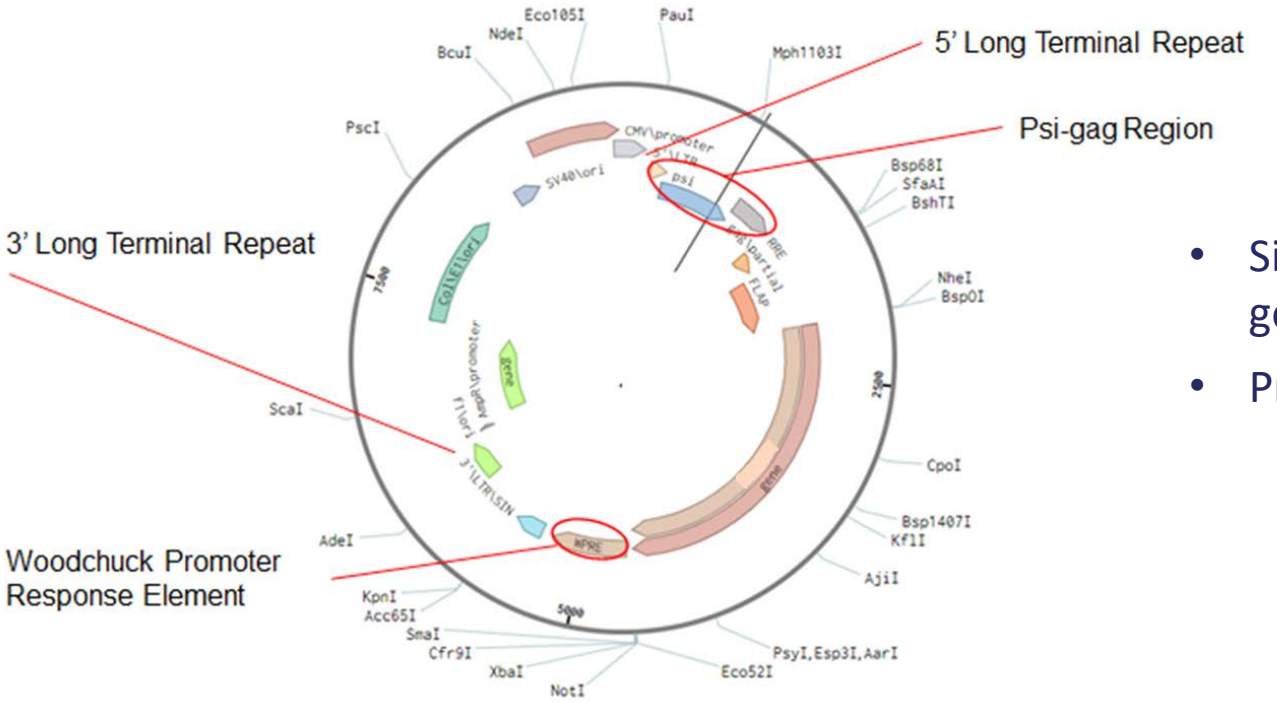
- Key Reagents: Primers, probes and target sequence (plasmid or cell line)
- Assay details
  - Analyte: Genomic DNA
  - Normalization to a reference gene required
  - Sensitivity 50 copies/ug
  - No concerns of ADA interference
- Sample details
  - Samples are stable and frozen after collection
  - DNA extraction is well established

Quantify Cells Directly (Flow Cytometry)



- Key reagents: Ab to Cell surface antigen and control cell lines
- Assay details
  - Analyte: Cell Surface antigen
  - Normalization is not needed
  - Variable sensitivity
  - Useful to understand distribution of CAR expression
- Sample Details
  - Suspension of PBMCs should be analyzed fresh
  - Implementation requires a trained analyst to ensure proper gating and analysis
  - Potential for ADA interference

Primer and Probe Design: CK Assays



- Single copy gene as the reference gene (RG) to normalize the genomic DNA (gDNA) input
- Primer/Probe designing
  - Target conserved DNA sequences between species to enable cross species comparison
  - Similar amplification efficiency (target vs reference gene (≤5%))
  - For re-dosing with different product, may be necessary to differentiate between CAR-T products
  - Avg copy number from cell product is known, but cells with different copy numbers may expand differently in vivo

Courtesy: Akbar Nayeem

Summary

- CAR-T modality is complex and presents a variety of bioanalytical challenges
- CK methods
  - Flow Cytometry- Direct Method
  - PCR- Indirect Method
- Risk based, tiered implementation of immunogenicity assays to evaluate impact on exposure and expansion
- A validated humoral immunogenicity assay is needed
- Cell based assays provide distinct advantages in comparison with LBA assays in certain scenarios
- Consistent interaction and collaboration with discovery and CMC teams will be important to ensure reagent availability and assay implementation

Clinical Relevance

Weifeng Xu

Anti-CAR Abs and Impact on Efficacy of Approved CAR-T

Kymriah – Cell based assay

- 91.4% pre-existing and 5% treatment-induced anti-mCAR19 Abs

Yescarta – Bridging assay

- Pre-existing anti-mCAR19 Abs at baseline in 3% patients
- No additional treatment emergent ADA detected

Tecartus – cell based and Bridging assay

- No pre-existing anti-CAR Abs!

Breyanzi –Bridging assay

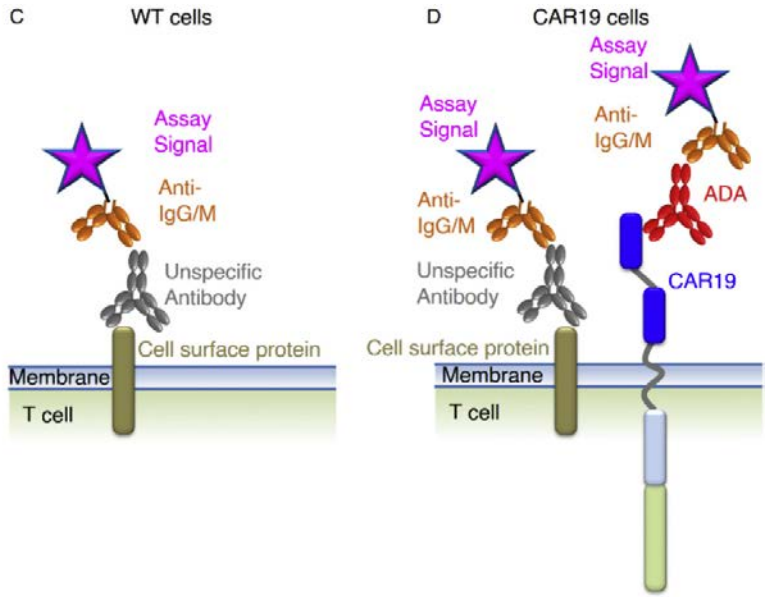
- 11% pre-existing and 11% treatment-induced anti-CAR Abs

Abecma (anti-BCMA)– Bridging assay

- 3% pre-existing and 47% treatment-induced anti-CAR Abs

Carvykti (anti-BCMA) – Bridging assay

- 19.6% ADA positive e



- Stark differences in level of pre-existing antibodies despite similar scFv (FMC63) in Kymriah and Yescarta maybe attributable to cell-based assay format
- Anti-CAR Abs have no impact on clinical efficacy for all 6 approved CAR-Ts; due to B-cell targeting?

Blood Adv. 2020 Feb 11; 4(3): 560–572
Potthoff et al; J Immunol Methods, 2020

Courtesy of: Siddha Kasar, PhD

Delayed ADA Response for Approved CAR-Ts

Anti-CAR Abs are seen 3-6 months after dosing for approved CAR-Ts:

- If lymphodepletion then B cells need time to be recovered.
  - How complete is the depletion?
  - Is lymphodepletion always done? (not in all solid tumors)
- Also related to target/indications: for approved: 4 targeting CD19 and 2 targeting BCMA, all to deplete B cells.
- ADA might be absorbed to CAR-T at early stage when ADA level is still low and CAR-T is plenty?

Clinical Relevance of NAb against CAR

- None of the approved product mentions NAb in the label.
  - Currently mostly for exploratory.
  - Is NAb really needed if PK is already impacted?
- Was requested by FDA even with low ADA rate and no impact on CK.
- Could be more relevant for NAb testing if repeated or dosing with similar constructs.
- Impact assessment: NAb+/- analysis (Need a NAb assay).
- New trends in biologics NAb testing:
  - PK/PD and ADA could replace NAb testing;
  - No/low ADA rate could have no NAb testing

Anti-CAR Abs and Impact on Efficacy of CAR against Solid Tumors

Early generation of CAR-T in solid tumor:

1. Targeting α-folate receptor with metastatic ovarian cancer:
   - Ab against CAR-T was associated reduced CAR-T activity against tumor cells; may have led to the rapid clearance of CAR-T as well;
2. Targeting tumor-associated glycoprotein (TAG-72):
   - NAb against ScFv was associated with elimination of CAR-T;
3. Targeting carbonic anhydrase IX (CAIX) on RCC:
   - High incidence of HACA (6/7, 85.7%) compared with using mAb alone (30%), suggesting higher immunogenicity when on cell surface than in a soluble form; HACA also inhibited cytotoxic activity of CAR-T;

Newer generation of CAR-T against solid tumor:

1. FRP5-ScFv against HER2-positive sarcomas and glioblastoma:
   - CAR transgene can be detected up to 2 years;
   - Clinical responses were unsatisfactory
   - Cellular and humoral anti-CAR was not intensely investigated.

- Cellular and humoral anti-CAR was not intensely investigated for solid tumors;
- Lymphodepletion might be helpful to reduce CAR-T immunogenicity and helpful for efficacy.

Kershaw et al; Clin. Cancer Res. 12, 6106–6115
Hege et al; J. Immunother. Cancer 5, 22 (2017)
Lamers, et al; Blood. 2011; 117(1):72-82
Ahmed et al; JAMA Oncol. 3, 1094–1101 (2017)

Clinical Observation of Cellular Immune Response to CAR-T

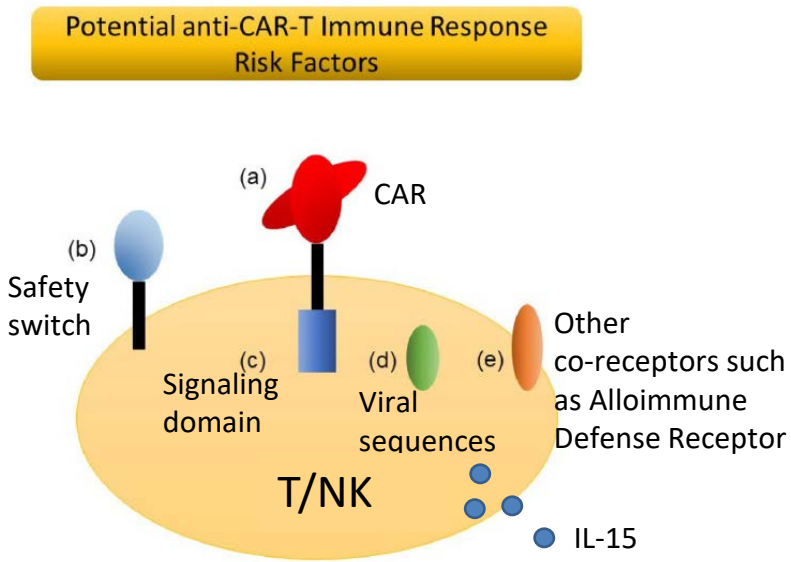
Cases:

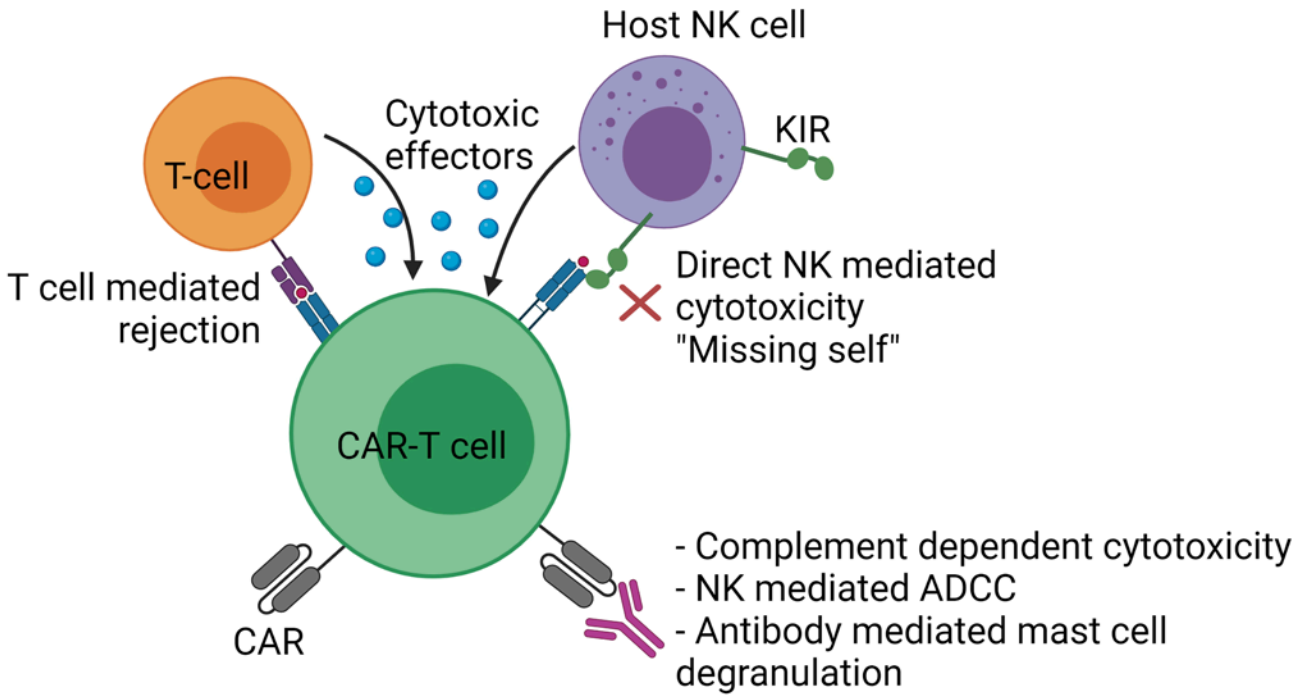
1. Autologous CD19 and CD20 CAR-T:
   - PBMC Chromium 51 releasing assay demonstrated cytotoxic against Neomycin as well as HSV-1 thymidine kinase (HyTK) selection-suicide domain; despite rituximab pretreatment. (Jensen, et al, Biol Blood Marrow Transpl. 2010;16(9):1245–56)
2. Targeting carbonic anhydrase IX (CAIX) on RCC:
   - Anti-CAIX-CAR-T cytotoxic cellular reactivity was detected in post-infusion samples, against mostly CAR protein, but also retroviral epitope, only after several cycle of PBMC stimulation and expansion. (Lamers, et al; Blood. 2011; 117(1):72-82)

- Only one approved CAR-T products has label information for cellular response with no impact.
- FDA has requested cellular assay (may depend on the complexity of engineering)
- Lymphodepletion may reduce immunogenicity for both humoral and cellular immune response. However, lymphodepletion with repeat dosing may pose life-threating risk of myeloablation to subjects. (FDA new CAR-T guidance)
- Clinical relevance of cellular response data? May not be the main driving factor for decision.
- Sample collection and testing is challenging, need good logistic for sample collection, shipment and isolation/storage.
- Transient transfection of vectors could reduce immunogenicity

Key factors influencing the complexity of bioanalytical plan for immunogenicity assessment

Product Design + Mechanism of Immune Rejection + Type of Product Auto vs allo





Adapted from BioDrugs (2019) 33:275–284

Courtesy of: Siddha Kasar, PhD

>20 assays needed for exhaustive immunogenicity assessment

Component	Humoral Immunogenicity			Cellular Immunogenicity	
	Screen/Confirm	Titer	Nab	T cell mediated	NK cell mediated
HLA (allogeneic product)	v	v		?	
CAR	v	v	?	?	?
Armoring molecule (IL-15, etc)	v	v	?		
Suicide/safety switch (surface or intracellular)	v (surface)	v (surface)	?		
Transduction/gene editing sequences				?	?

Monitoring procedures relevant to specific CGT products or study populations include the following:

- If immunogenicity is a concern (e.g., with viral capsids or allogeneic cellular products), 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. If adequate assays are not yet available, baseline and post-treatment blood and/or plasma, as appropriate, should be cryopreserved for later evaluation, once assays have been developed.

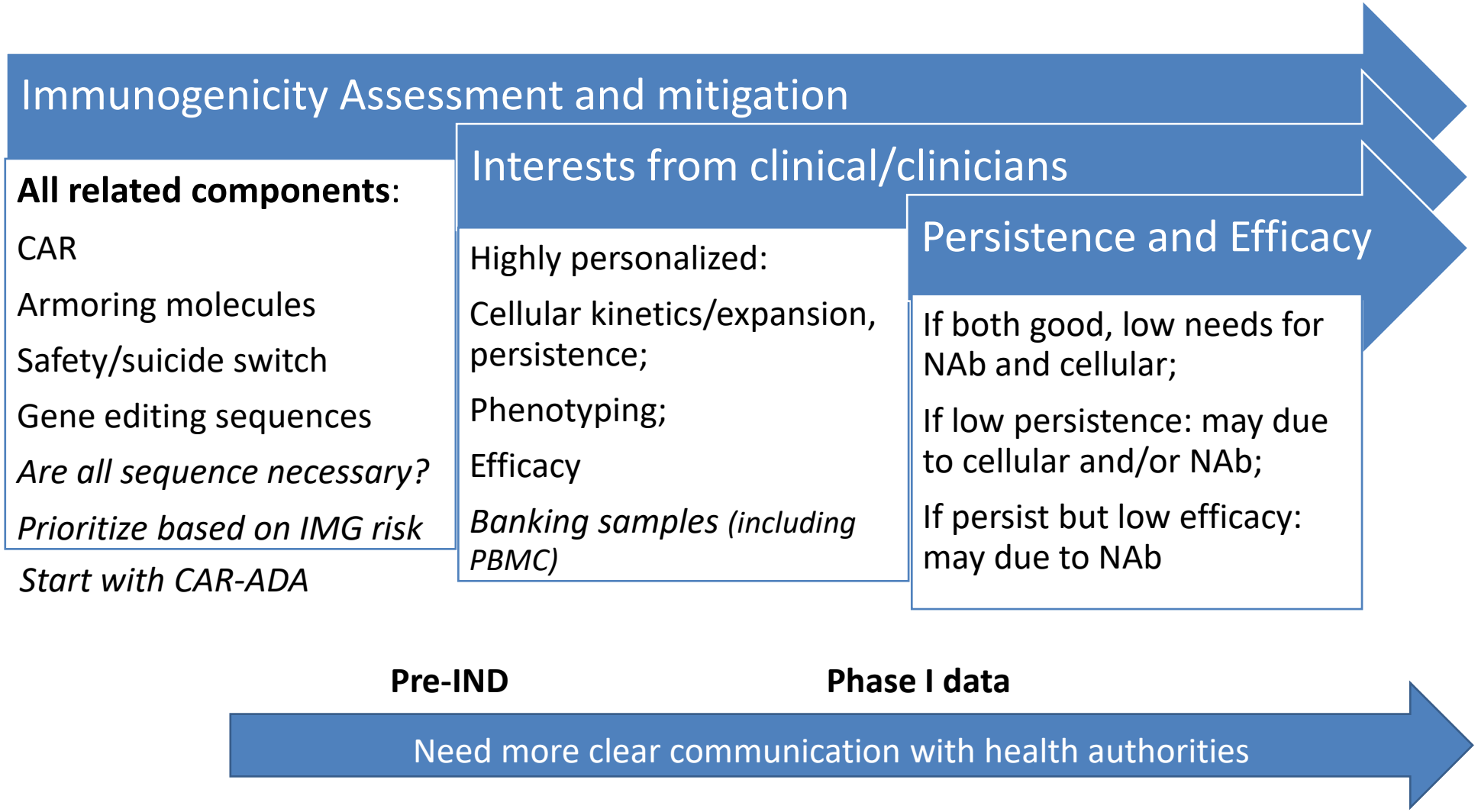
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considerations-design-early-phase-clinical-trials-cellular-and-gene-therapy-products (2015)

Modified from: Siddha Kasar, PhD

New FDA Draft Guidance on the Development of CAR-T Products

- Should include an assessment of any impact that these additional elements will have on CAR T cell specificity, functionality, immunogenicity, or safety.
- Transgene sequences that are unnecessary for the biological function of a product may be immunogenic in vivo or have other unanticipated effects on product persistence or activity. As a general guiding principle, we recommend that unnecessary transgenes should not be included in the vector.
- Previously administered CAR T in the starting material may impact safety, efficacy and immunogenicity.
- Section IV.D describes lots of manufacturing change, for example, vector, growth factor for expansion, etc. (These could also cause change in immunogenicity, hence any change in the manufacturing could be noted and linked for immunogenicity testing.)

CAR T Immunogenicity Strategy



Melenhorst, J.J., Chen, G.M., Wang, M. *et al.* Decade-long leukaemia remissions with persistence of CD4⁺ CAR T cells. *Nature* **602,** 503–509 (2022). https://doi.org/10.1038/s41586-021-04390-6

Conclusions and Discussion

- Cellular therapeutics have been shown to be remarkable efficacious
- Immunogenicity and Bioanalytical strategy in place and being developed
- Unknowns remain regarding many questions and challenges
- Best practices need to be developed within industry

Clinical Pharmacology & Therapeutics

White Paper

An IQ Consortium Perspective on Best Practices for Bioanalytical and Immunogenicity Assessment Aspects of CAR-T and TCR-T Cellular Therapies Development

Jochem Gokemeijer, Nanda Balasubramanian, Ken Ogasawara, Joanna Grudzinska-Goebel, Vijay V. Upreti, Hardik Mody, Siddha Kasar, Venkata R. Vepachedu, Weifeng Xu ... See all authors

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