SOT 59th Annual Meeting & ToxExpo
March 15–19, 2020 • Anaheim, California

Continuing Education Course

Sunday, March 15 | 1:15 PM to 5:00 PM

PM12: Harnessing the T Cell for Cancer Immunotherapy: A Course on T Cell Redirection

Chair(s)

Jessica L. Lynch, Janssen Research & Development
Rafael Ponce, Shape Therapeutics

Primary Endorser

Immunotoxicology Specialty Section

Other Endorser(s)

Biotechnology Specialty Section
Regulatory and Safety Evaluation Specialty Section

Presenters

Jessica L. Lynch, Janssen Research & Development
Rodney Prell, Genentech Inc.
Rafael Ponce, Shape Therapeutics
Thomas J. Long, Juno Therapeutics
Jacintha Shenton, Janssen Research & Development
Alyssa Galaro, US FDA/CBER

SOT Anaheim, California 2020

Course Participant Agreement

Continuing Education Committee

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SOT Anaheim, California 2020

Harnessing the T Cell for Cancer Immunotherapy: A Course on T Cell Redirection

1:20 PM–1:30 PM	**An Introduction to Cancer Immunotherapy and the T Cell Redirection Course** Jessica L. Lynch, Janssen Research & Development, Spring House, PA	4
1:30 PM–2:05 PM	**Generation of Synthetic Tumor Immunity through the Development of T Cell Redirecting Modalities** Rodney Prell, Genentech Inc., South San Francisco, CA	11
2:05 PM–2:40 PM	**Engineered T Cells as Cancer Therapeutics: An Update on Their Design, Manufacture, and Clinical Experience** Rafael Ponce, Shape Therapeutics, Seattle, WA	27
2:40 PM–3:00 PM	**Part 1: Preclinical Safety Assessment of CAR and TCR T Cell Therapies** Thomas J. Long, Juno Therapeutics, Seattle, WA	51
3:00 PM–3:30 PM	**Break**	
3:30 PM–3:45 PM	**Part 2: Preclinical Safety Assessment of CAR and TCR T Cell Therapies** Thomas J. Long, Juno Therapeutics, Seattle, WA	
3:45 PM–4:20 PM	**Getting the Most Out of Your Nonclinical Safety Studies for Antibody-Based CD3 Redirectors to Inform Deselection or Enable First-in-Human Clinical Trials** Jacintha Shenton, Janssen Research & Development, Spring House, PA	60
4:20 PM–4:45 PM	**Regulatory Perspective on the Pre clinical Development of T Cell Immunotherapies** Alyssa Galaro, US FDA/CBER, Silver Spring, MD	77

An Introduction to Cancer Immunotherapy and the T Cell Redirection Course

Jessica L. Lynch PhD, DABT
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Spring House, PA
Email: jlynch18@its.jnj.com

Conflict of Interest Statement

Jessica Lynch is a paid employee of Janssen Research and Development, a pharmaceutical company of Johnson & Johnson.

Abbreviations

- CAR: chimeric antigen receptor
- TCR: T cell receptor
- TLR: Toll-like receptor
- LPS: lipopolysaccharide
- MHC: major histocompatibility complex
- TAA: tumor-associated antigen

T Cell Redirection Course Agenda

- **1:15 pm–1:20 pm:** Opening Remarks
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Brief History of Immunotherapy

1890s

Coley's Toxins
- Noted cases in which patients with cancer went into spontaneous remission after developing erysipelas infection
- Began injecting mixtures of live and inactivated *Streptococcus pyrogenes* and *Serratia marscens* into patient's tumors with mixed results

1957

Theory of Cancer Immunosurveillance
- Thomas and Burnett suggested that lymphocytes acted as sentinels to identify and eliminate somatic cells transformed by spontaneous mutations

During this time the concept of cancer immunotherapy was not widely accepted and even scorned by the American Cancer Society

Late 1990s

Bruce Beutler and colleagues demonstrated that LPS could agonize TLR, which in turn could active the immune system against cancer

2011

US FDA approved first checkpoint inhibitor (anti-CTLA4)

2013

Science named cancer immunotherapy its Breakthrough of the Year

2014

First T cell redirector approved by US FDA (CD19xCD3 BiTE®)

2017

First autologous CAR-T cells approved (Kymriah® and Yescarta®)

Immune System Plays Distinct Roles in Preventing Cancer

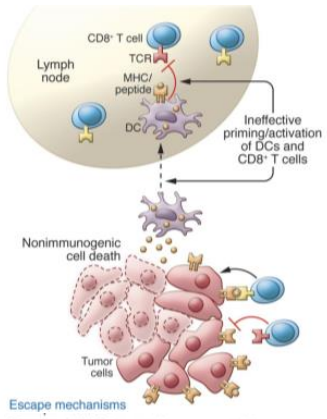
1. Protects host against viral infection suppressing virus-induced tumors
2. Prevents establishment of an inflammatory environment that facilitates tumorigenesis by eliminating pathogens and resolving inflammation
3. **Eliminates tumor cells in tissues**
   - Tumor-associated antigens (TAA) are co-expressed with ligands that activate immune cells and are recognized by the immune receptors on lymphocytes
   - Concept that cancer cells express antigens that differentiate them from their non-transformed counterparts

Schreiber et al., (2011) *Science* 331:1565

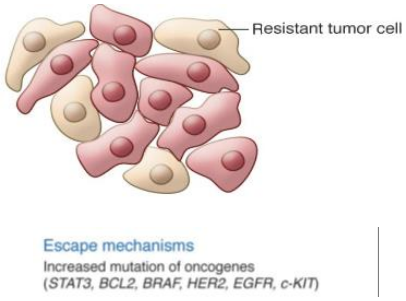
Tumors Circumvent Immune Detection and Elimination

Major mechanisms of tumor escape

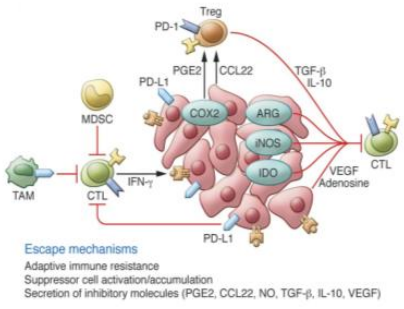
Reduced immune recognition and immune stimulation



Upregulation of resistance against cytotoxic effectors of immunity or increased expression of pro-survival or growth factors



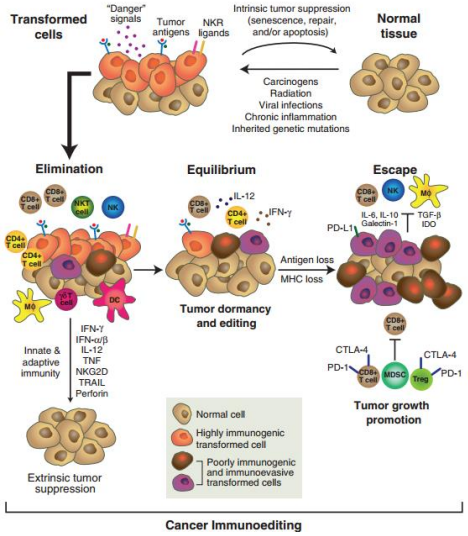
Establishment of an immunosuppressive tumor environment

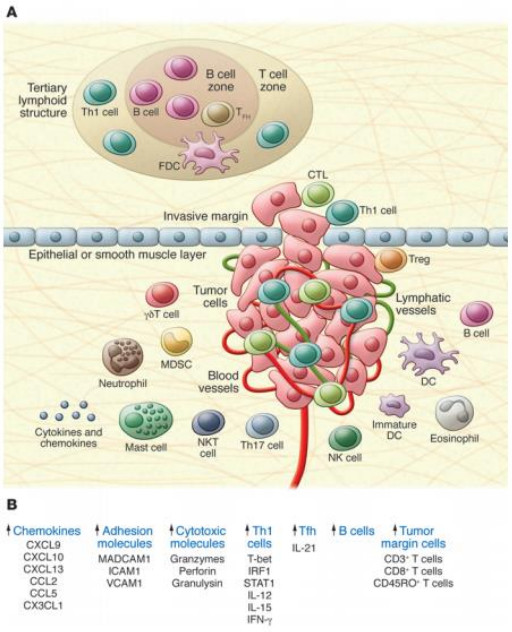


Adapted from: Teng et al., (2015) *J Clin Inv* 125:3338

Cancer Immunotherapy: Key Paradigms

Co-evolution of tumors and immunity ⟷ Tumor immune contexture and survival





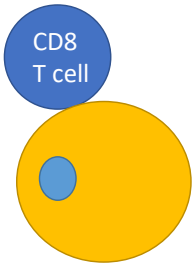
Schreiber et al., (2011) *Science* 331:1565
Teng et al., (2015) *J Clin Inv* 125:3338

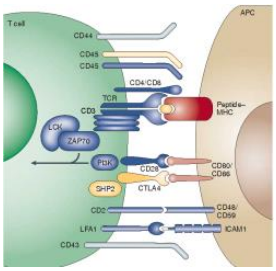
Immunological Synapse

Mechanism of action for lymphocytes to communicate via cell-cell interaction with antigen-presenting cells (APC), antigen-specific targeted cells, and other lymphocytes

1. Circulating CD8+ T cell

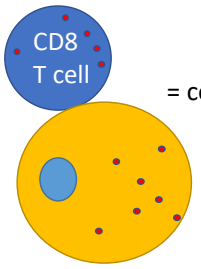
2. Circulating CD8+ T cell finds antigen-presenting cell





3. Interaction of clustered TCRs on the surface of T cells with peptide antigen loaded MHC results in differentiation and activation of CD8Tcells to antigen-specific T cells

4. T cells form cytolytic synapse with target cells and release cytolytic toxins to kill those cells

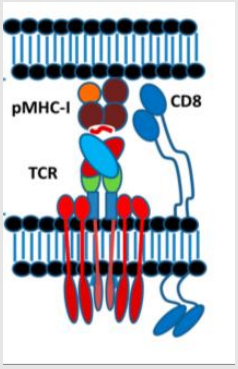


= cell death

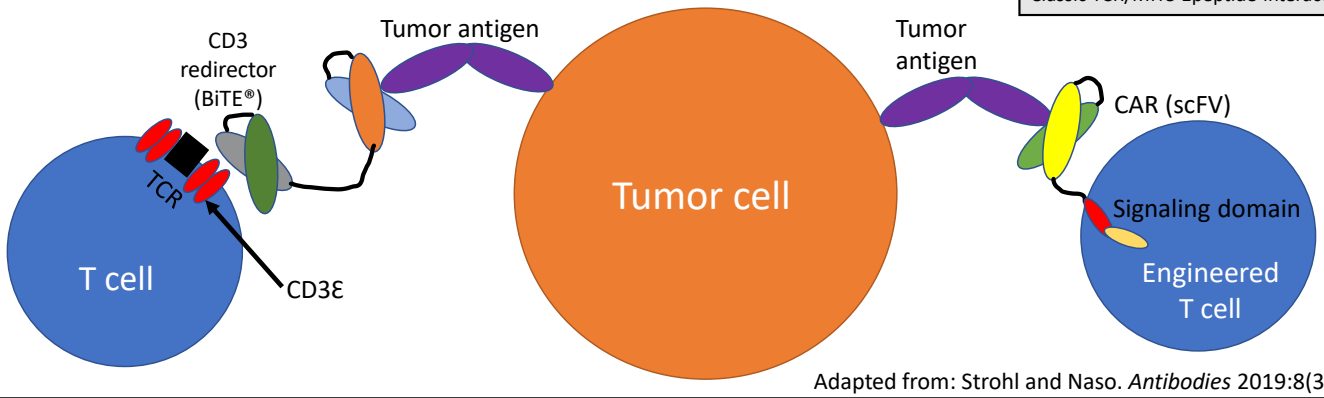
Adapted from: Huppa and Davis. *Nature Reviews Immunology* 2003

T Cell Redirection as a Therapeutic

- CAR-T and CD3-redirection rely on antibodies to replace the function of the TCR, making them independent of the TCR and its MHC-1/peptide recognition
- CAR-T and CD3-redirection are employed to recognize and target tumor-specific antigens outside the realm of MHC-1 displayed neo-antigen peptides



Classic TCR/MHC-1peptide interaction



Adapted from: Strohl and Naso. *Antibodies* 2019:8(3)

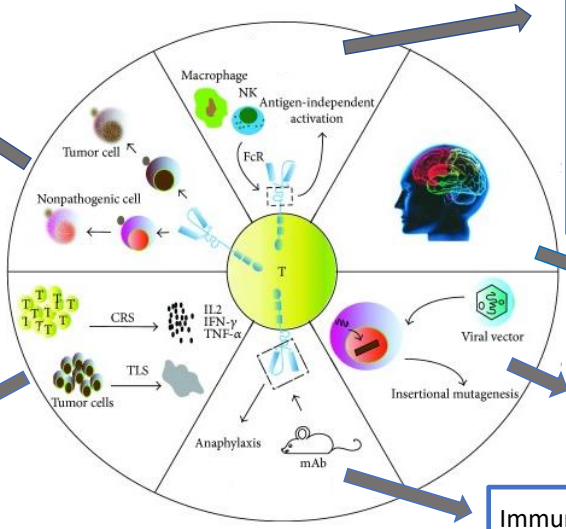
Overarching Safety Concerns with T Cell Redirection

On-target off-tumor toxicity

- Direct attack on normal tissues that have shared expression of the TAA
- Targeting melanoma antigen (gp100 and MART1)
  - Normal distribution in melanocytes in eye, skin, and ears
  - Safety: transient toxicity of melanocytes in skin, eye, and ears

On-target on-tumor toxicity

- Triggered by excessive cytokine release or tumor cell necrosis. Adverse reactions but also required for efficient T cell expansion.
- Cytokine release syndrome
- Tumor lysis syndrome



Off-target off-tumor toxicity

- Direct attack of an antigen other than the intended one or antigen-independent activation
- Targeting melanoma antigen family A3 (MAGE-A3) in melanoma, sarcoma, and carcinoma
  - Normal distribution germ cells and activated in some cancers
  - Safety: cardiac shock and death
  - TCR cross-reacted with unrelated heart muscle protein Titin

Neurotoxicity

Insertional mutagenesis (CAR-T)

Immunogenicity

Adapted from: Sun et al., (2018) *J Immunol Res* 2018:3338

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Generation of Synthetic Tumor Immunity through the Development of T Cell Redirecting Modalities

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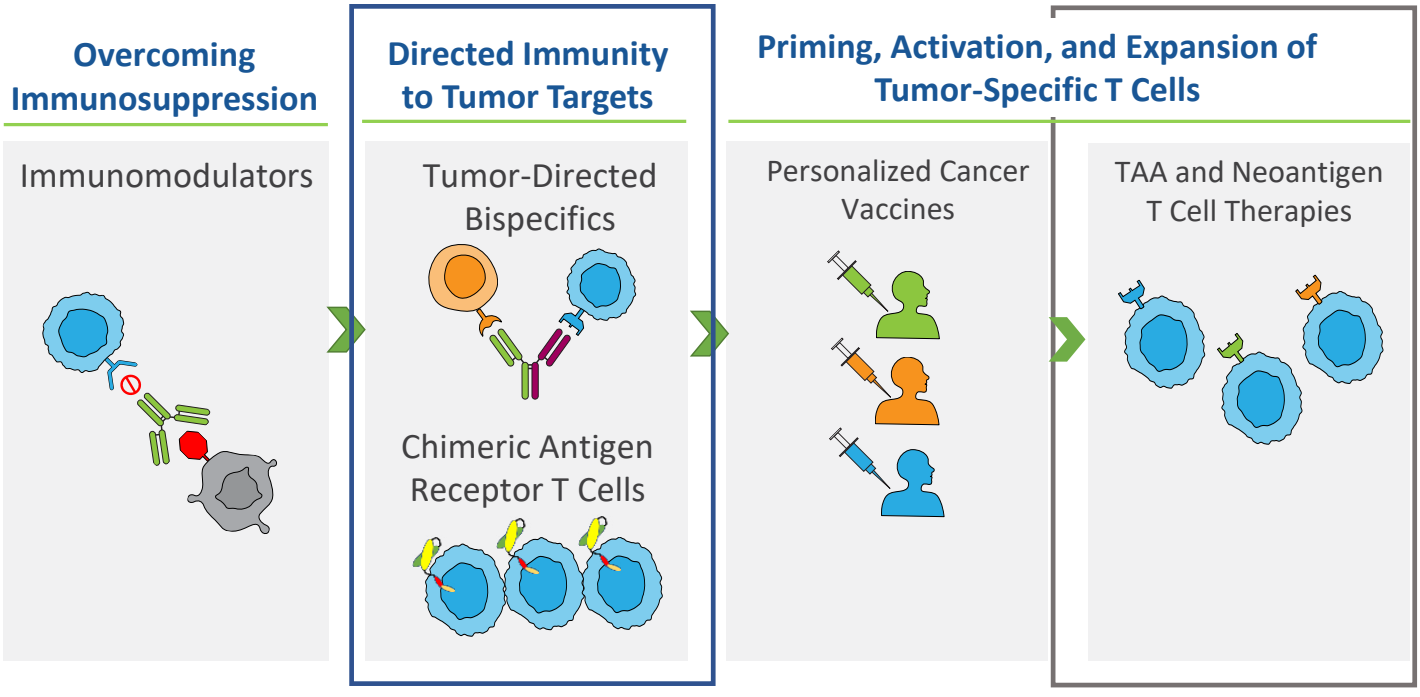
Conflict of Interest Statement

Rodney Prell is a paid employee of Genentech, A Member of the Roche Group.

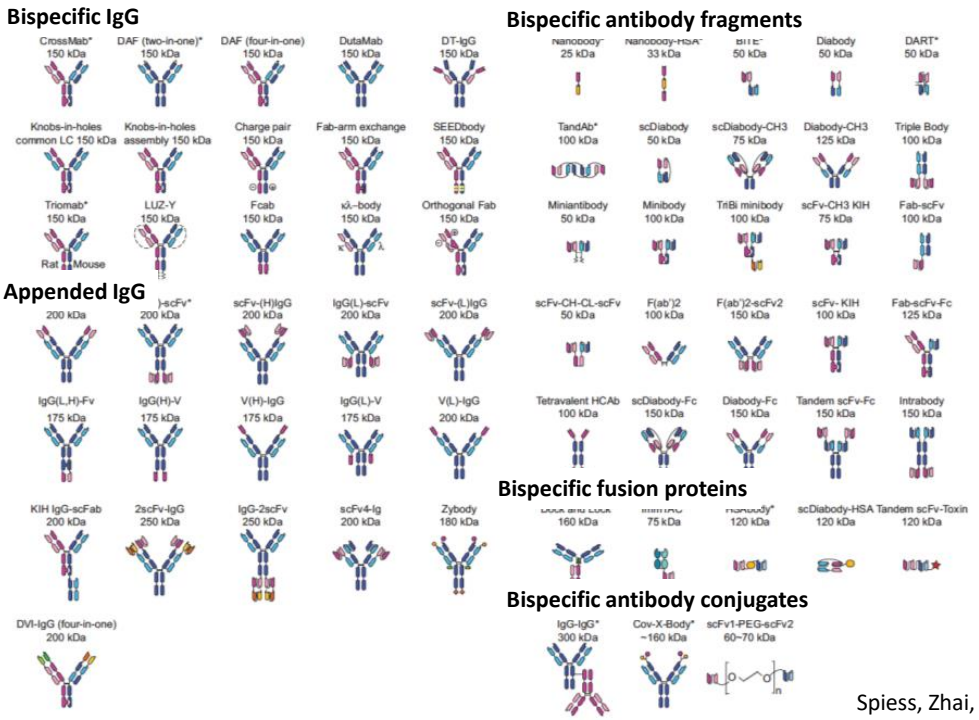
Abbreviations

- CAR: chimeric antigen receptor
- FIH: first-in-human
- ImmTAC: immune mobilizing monoclonal TCR against cancer
- NK: natural killer cells
- TA: tumor antigen
- TAA: tumor-associated antigen
- TCR: T cell receptor
- TDB: T cell-dependent bispecific
- TI: therapeutic index

Ways to Raise a Tumor-Specific T Cell Army: T Cell Redirectors



Multi-targeting Biotherapeutic Formats



>60 different bispecific and multispecific antibody formats

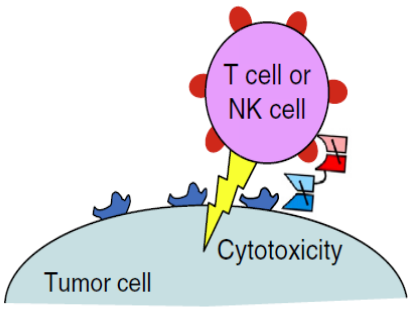
>16 different formats have reached clinical trials

Unique formats create unique challenges due to the difference in size, valency, flexibility, half-life, and biodistribution

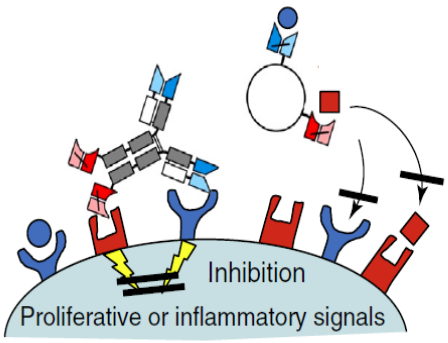
- *PK*
- *Immunogenicity*
- *Effector function*
- *Redirected lysis*

Spiess, Zhai, and Carter (2015) *Molec. Immunol.* 67: 95

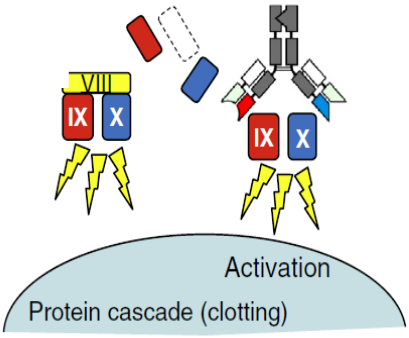
Leveraging Multi-targeting Abs for Specific Mechanisms of Action



Immune cell recruitment and redirected lysis



Receptor/ligand antagonism



Receptor/ligand agonism

Kontermann and Brinkmann, *Drug Disc Today* 2015

T/NK Cell Redirecting Therapeutics in Clinical Development

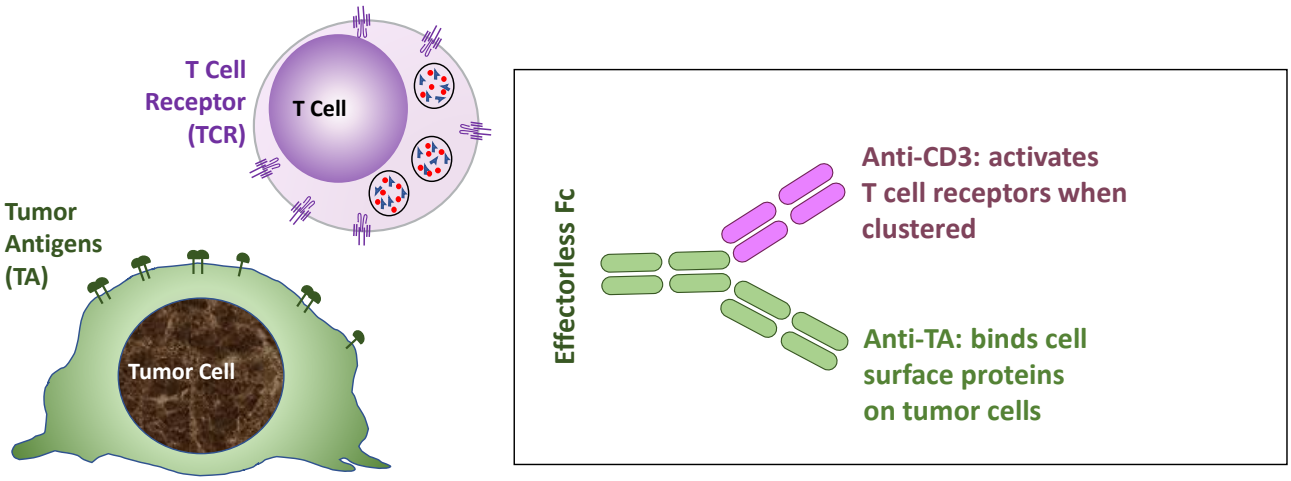
Bispecific T- or NK-Cell Redirecting Antibody Format ***	Clinical Stage			Total
	Phase I/II	Phase III	Approved	
Short half-life bivalent fragments (e.g., BiTE®s, DART®s, ImTACs, other bivalent fragments)	15	0	1	16
Half-life extended bivalent fragments (e.g., DART®-Fc, Extended half-life BiTE®s, TriTAC)	11	0	0	11
Asymmetric bivalent IgG-like (e.g., Trion, BEAT, Xencor H/A platform, Duobodies, other asymmetric platforms)	21	0	1 ****	22
Roche TCB 2:1, Chugai ART-Ig®-scFv and Teneobio 2:1 platforms (two binding sites for target cell, one for CD3)	4	0	0	4
ADAPTIR®and TandAb platforms (tetravalent platforms)	4	0	0	4
Chemically conjugated IgGs (tetravalent; two IgGs)	4	0	0	4
Total	59	0	2	61

**** Withdrawn

- >60 in preclinical development
- ~60 in clinical trials
- Main focus initially in hematological malignancies (CD19, CD20, BCMA, CD123, CD33, etc.)
- Several under development for solid tumors (EpCAM, PSMA, HER2, gpA33, B7H3, CEA, etc.)

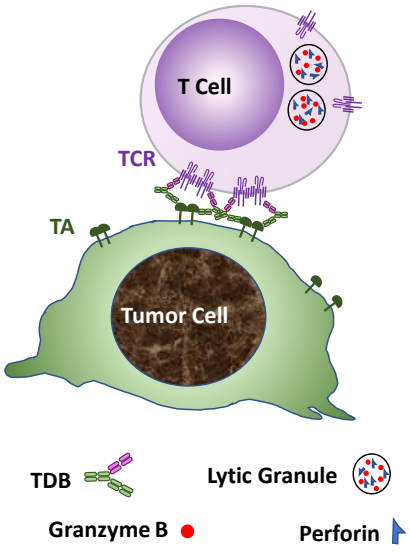
Strohl and Naso, *Antibodies* (2019); Sedykh et al., *Drug Design Development Therapy* 2018

T Cell-Dependent Bispecific Antibodies (TDBs)



Mechanism of Action of T Cell Redirecting Biotherapeutics

Tumors and TA^{high} Tissues



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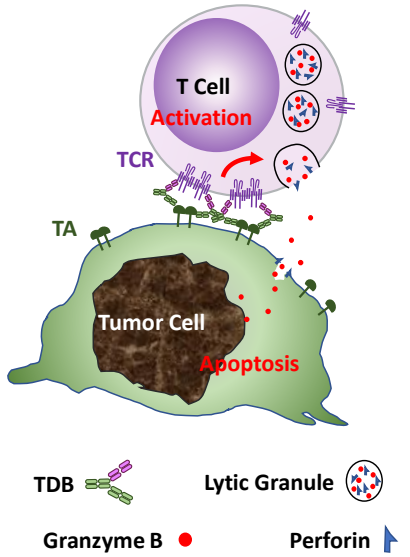
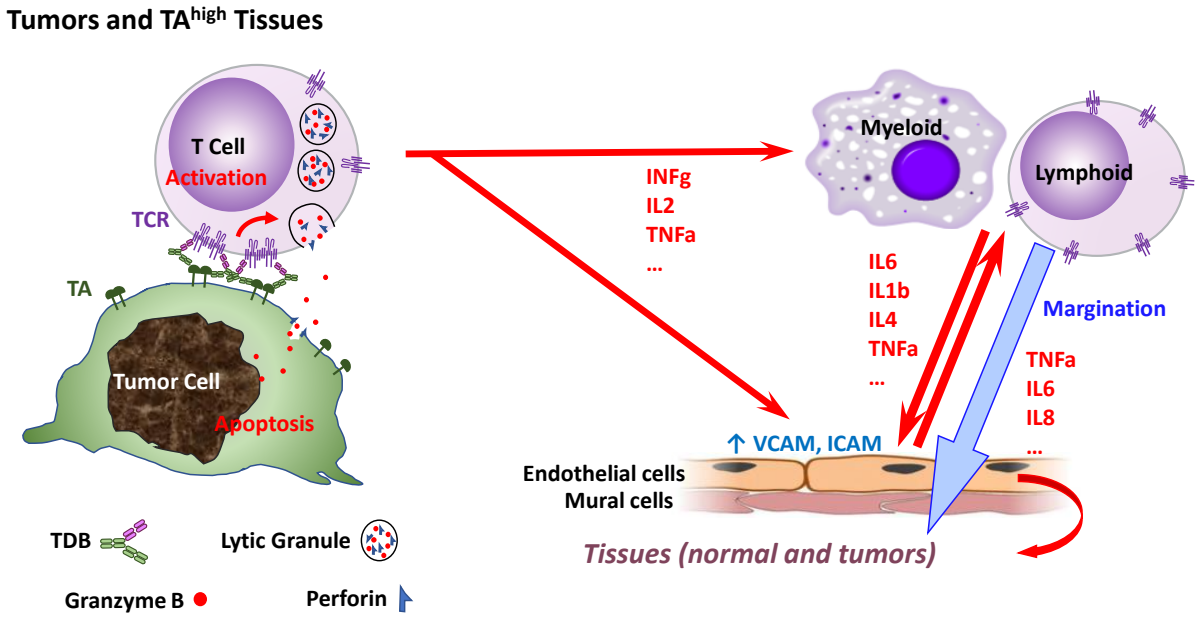


A Cascade of Events Is Triggered by TDBs



Developmental Challenges with T Cell Redirecting Therapies

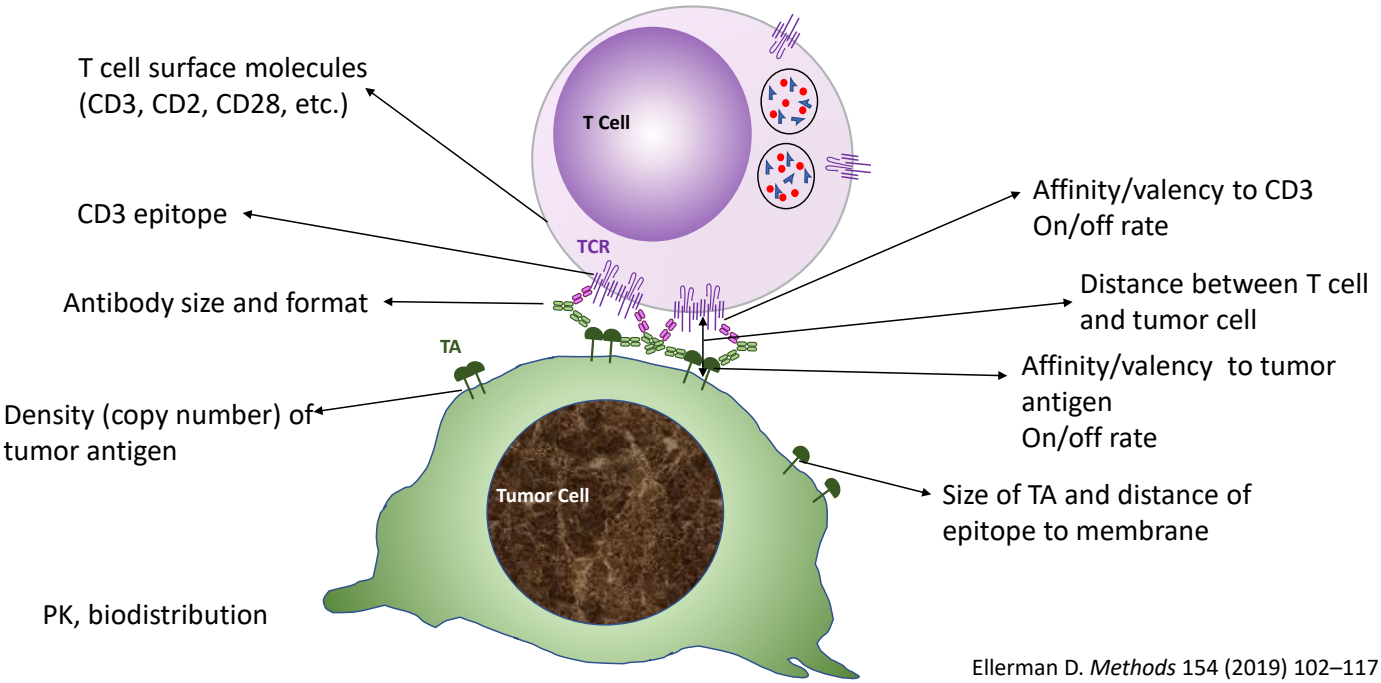
Highly potent	Immunogenicity	Target expression/distribution
Infusion reactions (CRS, acute phase responses) often define MTD	Low doses and immunogenicity may limit the conduct of RD studies	TDB-MOA effective on cells expressing low levels of target

Affinity of each arm	Species differences	Narrow Therapeutic Index (TI)
Impact potency/safety/PK	Affinity/cytokine responses	FIH dose selection

Key Aspects of the T Cell Engaging Modalities

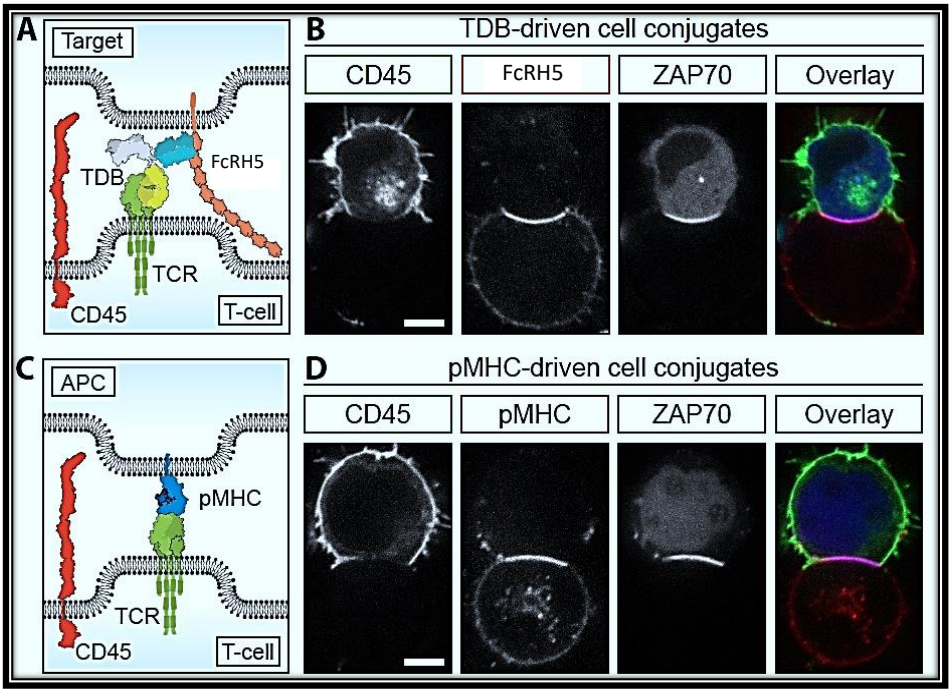
- Strictly target cell–dependent activation of resting T cells
- Highly potent and complete lysis of target cells by engaged T cells
- Lysis of dividing as well as nondividing target cells
- Serial lysis by T cells
- Sustained proliferation of T cells
- Does not require MHC Class I and peptide antigen for recognition by T cell
- Does not require T cell clone with specific T cell receptor

Variables That Impact Potency and Selectivity of T Cell Engagers



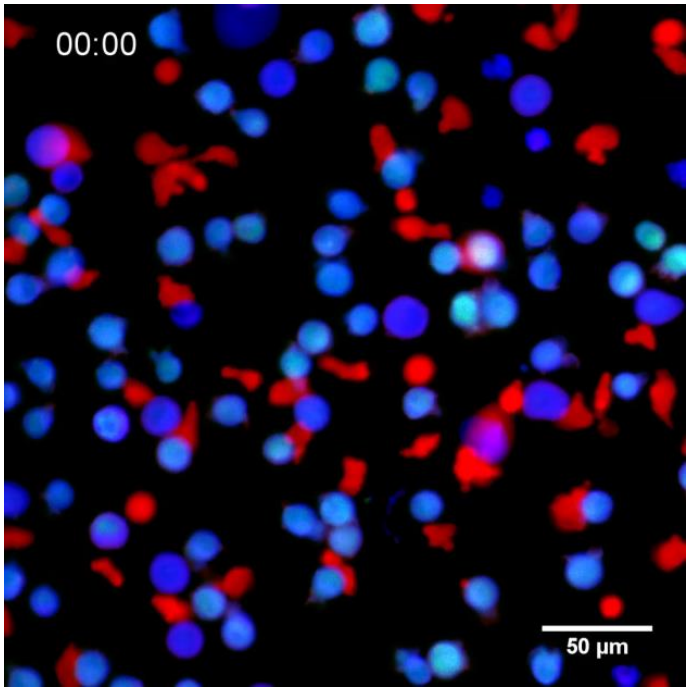
Ellerman D. *Methods* 154 (2019) 102–117

T Cell-Dependent Bispecific MOA Phenocopies Normal pMHC-TCR Immune Synapse



Li J et al., (2017) *Cancer Cell*. 31, 383–395

TDB-Mediated Killing Is Rapid and Progressive

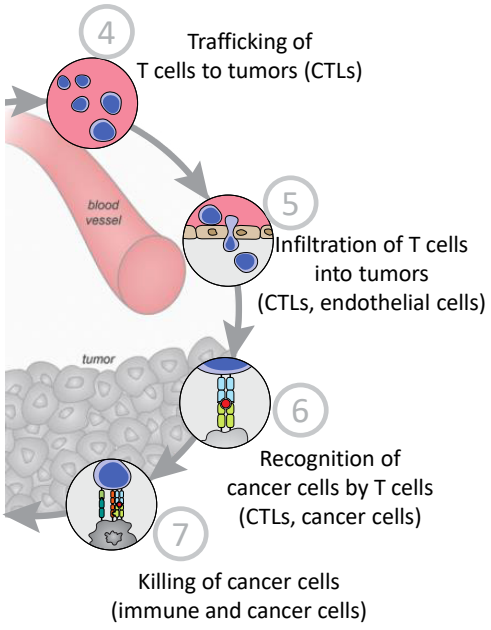


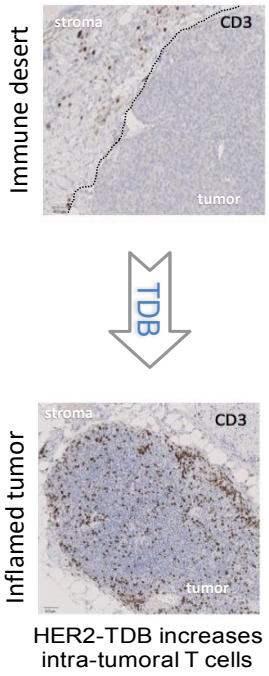
- **TDB leads to rapid change in motility and target cell engagement**
- **TDB-activated T cells are serial killers**

Red: T cells
Blue: tumor cells
Blue flash: tumor cells killed by T cells

Movie courtesy of Alex Ritter, Teemu Junttila, Ira Melman

Mutual Benefit between TDBs and Immune Checkpoint Inhibitors





HER2-TDB increases intra-tumoral T cells

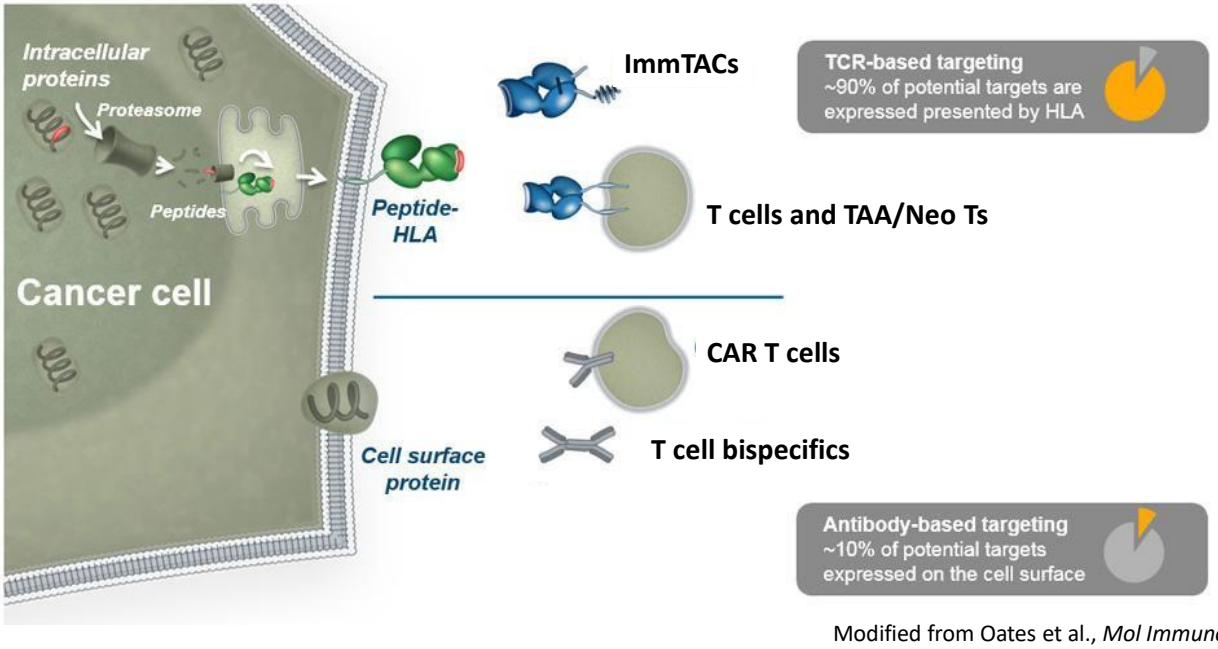
1. Response to checkpoint inhibitors correlates with T cell infiltrate in tumors
2. T cell engaging bispecifics lead to polyclonal T cell **recruitment and activation** within tumors
3. Potential to broaden and enhance activity of immune checkpoint inhibitors
4. Conversely, TDB activity is augmented upon immune checkpoint inhibition

Chen DS, and Mellman I. (2013) *Immunity*, 39(1), 1–10
Junttila T, (2014). *Cancer Research*, 74(19), 5561–71

Next Hurdle for TDBs—Solid Tumor Indications

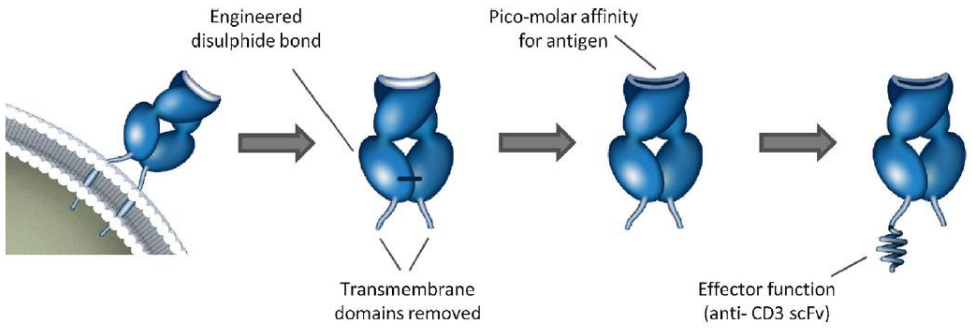
- **TDB success demonstrated for hematologic malignancies**
  - Lineage-restricted expression of targets/antigens
  - Lack of dependency on tumor specificity via TCR engagement
  - High sensitivity to CD3-mediated activation
  - Pharmacology-related safety liability is one of the main intrinsic risks
- **Unmet medical need drives expansion of TDBs to solid tumors**
- **Notable challenges in developing TDBs for solid tumors**
  - Lack of tumor-restricted antigens: most/all solid tumor antigens are expressed in normal tissues
  - Potential on-target off-tumor adverse effects
  - Clinical and nonclinical on-target toxicities are reported (i.e., gp100, EGFR, HER2)
  - Heterogeneity in expression levels of target antigens

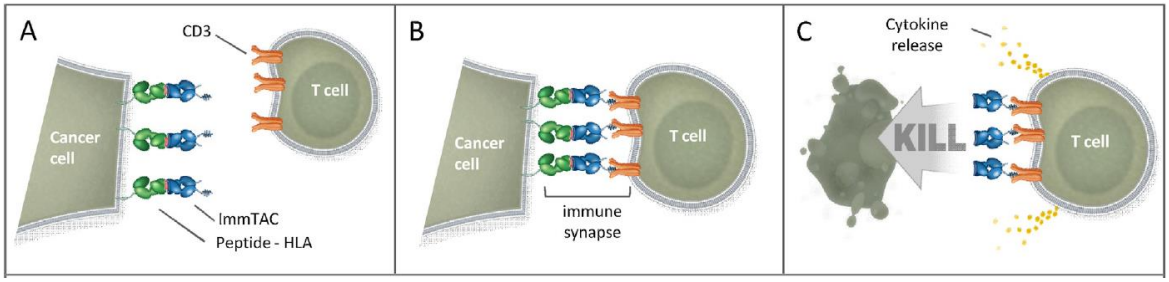
Immune Mobilizing Monoclonal TCR Against Cancer (ImmTACs) Allow Targeting of Intracellular Tumor Antigens



Modified from Oates et al., *Mol Immunol*. (2015)

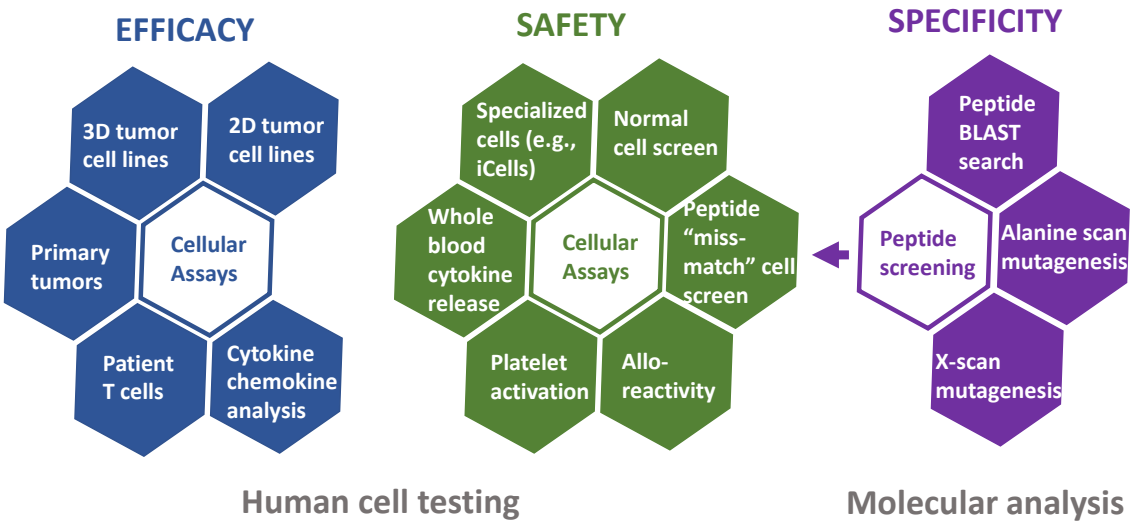
Immune Mobilizing Monoclonal TCR Against Cancer (ImmTAC)





Modified from Oates et al., *Mol Immunol*. (2015)

In Silico/In Vitro Safety Analysis to Support First-in-Human Clinical Trials



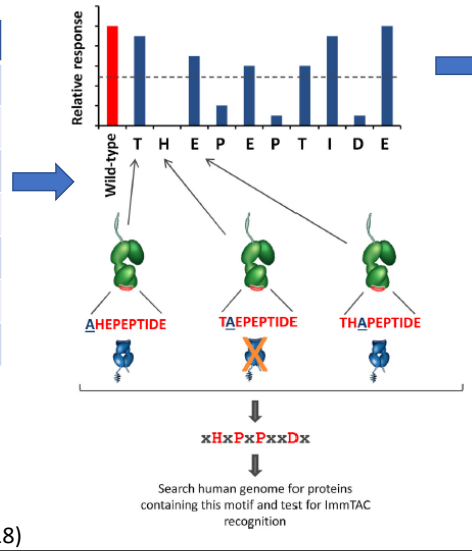
Oates et al., *Mol Immunol*. (2015); Harper et al., *PLoS ONE* (2018)

In Silico and Experimental Analysis

Genome Blast

Antigen	Sequence	Tissue expression
Target	THEPEPTIDE	Prostate
Ag 1	THVPEPNIDE	Heart/lung
Ag2	TIEPEPTSPDE	Various
Ag3	VHKIEPTILE	Brain, pancreas
Ag4	THEPETTFPF	Overexpressed in heart
Ag5	TPIPEGNKDE	Various
Ag6	THEPIPTIFE	Heart, brain, lung

Ala Scan and X Scan



Motif Blast

xHxPxPxxDx

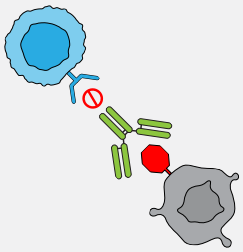
Screen hits for off target activity

Oates et al., *Mol Immunol*. (2015); Harper et al., *PLoS ONE* (2018)

Ways to Raise a Tumor-Specific T Cell Army: Engineered T Cells

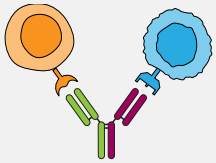
Overcoming Immunosuppression

Immunomodulators

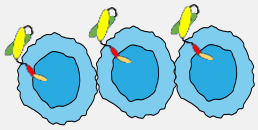


Directed Immunity to Tumor Targets

Tumor-Directed Bispecifics

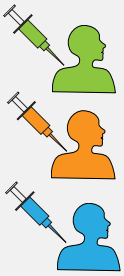


Chimeric Antigen Receptor T Cells

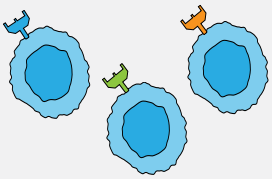


Priming, Activation, and Expansion of Tumor-Specific T Cells

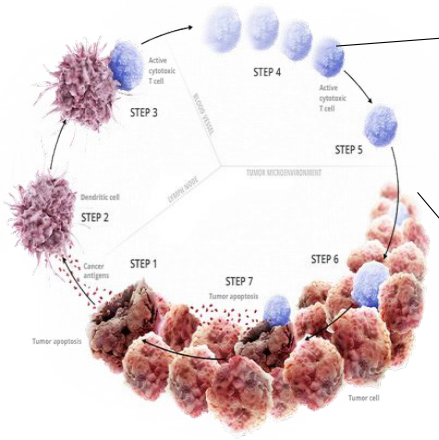
Personalized Cancer Vaccines



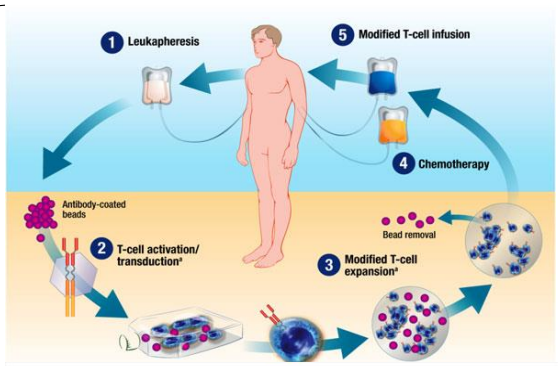
TAA and Neoantigen T Cell Therapies

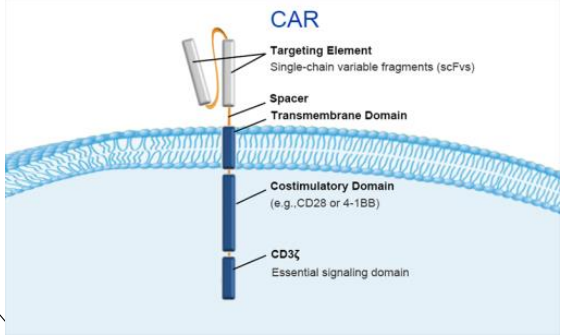


Chimeric Antigen Receptor (CAR) T Cell Therapies



Maus and June, *Clin Can Res* (2016)



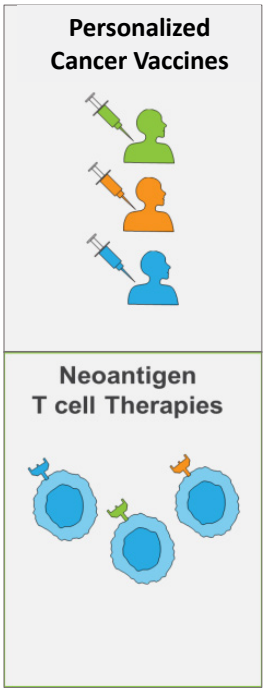


Engineered T cell–based therapies

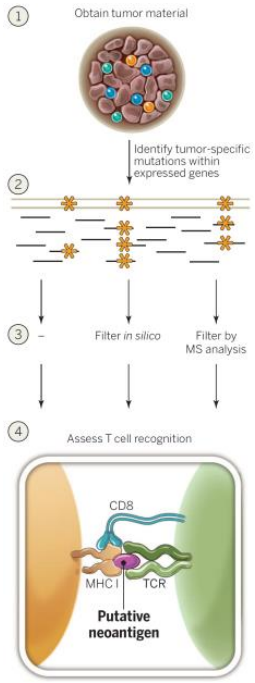
- T cells collected from patient
- Transduce T cells with genetically engineered viral vector to express Chimeric Antigen Receptor (CAR)
- CAR link extracellular domain to intracellular signaling domains
- T cells expanded *ex vivo*
- CAR T cells reinfused into patient; recognize surface antigen (MHC independent); proliferation; and cytolytic activity

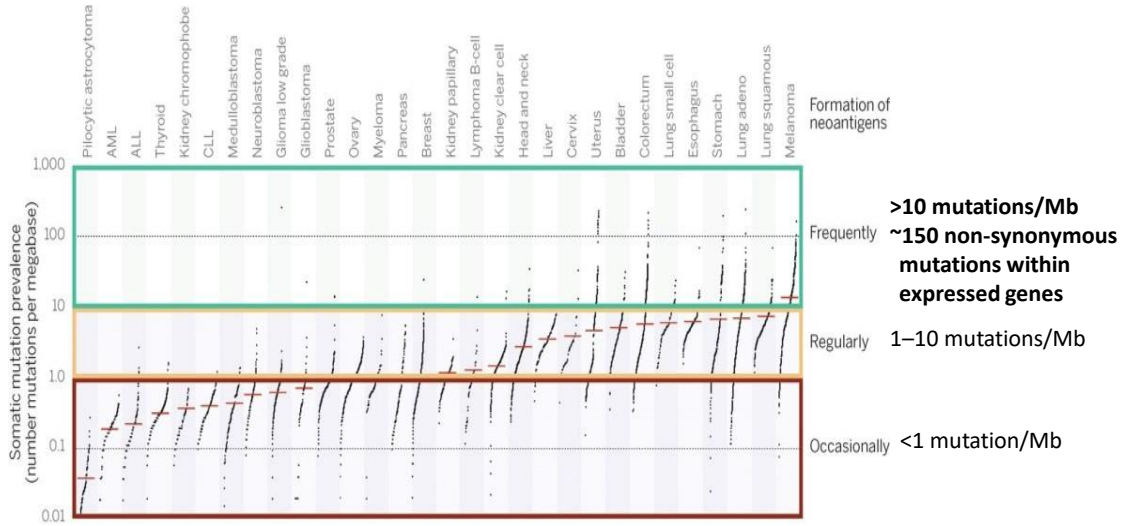
Neoantigen-Based Therapies

- **TAA and Neoantigen T cell therapies differ from CAR Ts**
  - Native TCR recognizes peptide/MHC complex versus engineered CAR
  - Depends on normal co-stimulation versus directly linked to costimulatory domains
- **Neoantigens are created by non-synonymous tumor-specific DNA alterations**
  - Single nucleotide polymorphisms, insertions, deletions, frame shifts, etc.
  - Mutations absent from normal human genome
  - A large fraction of these mutations are patient specific and not shared among patients
- **Tumor-specific neoantigens can be recognized by the immune system and have the potential to drive potent antitumor immune responses**
  - Within tumor infiltrating lymphocytes, only a small fraction of $CD4^+$ or $CD8^+$ T cells are neoantigen specific
  - Next-generation sequencing technologies and MHC class I/II epitope prediction algorithms have made it possible to detect, predict, and prioritize potential neoantigens
  - A vaccine targeting multiple neoantigens may be able to amplify existing $CD8^+$ and $CD4^+$ T cell responses and/or produce responses that might have been silent prior to vaccination



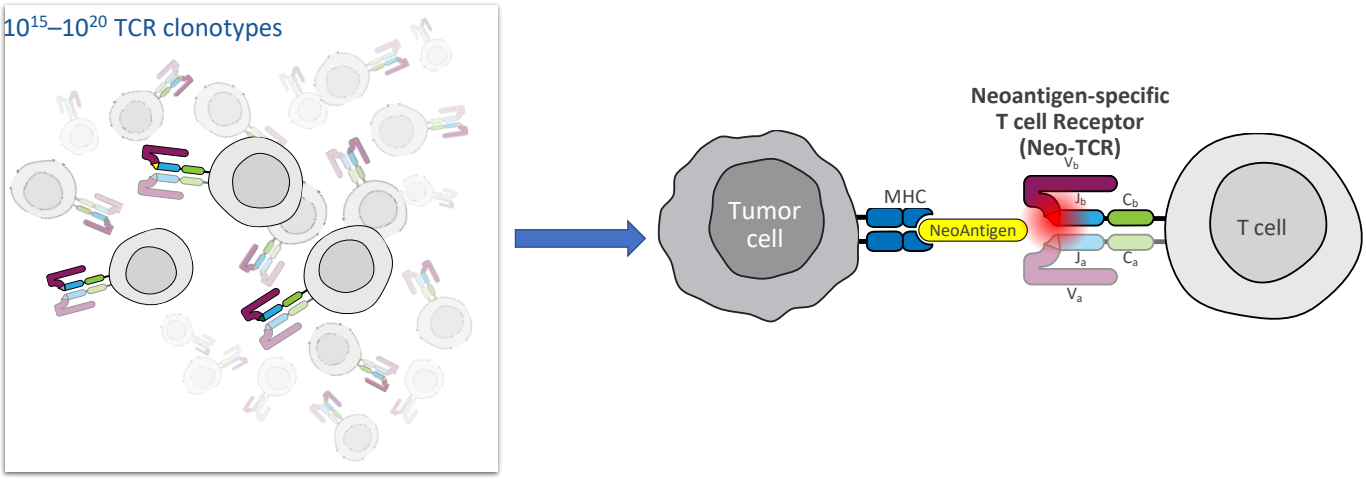
Human Cancers Accumulate Mutations



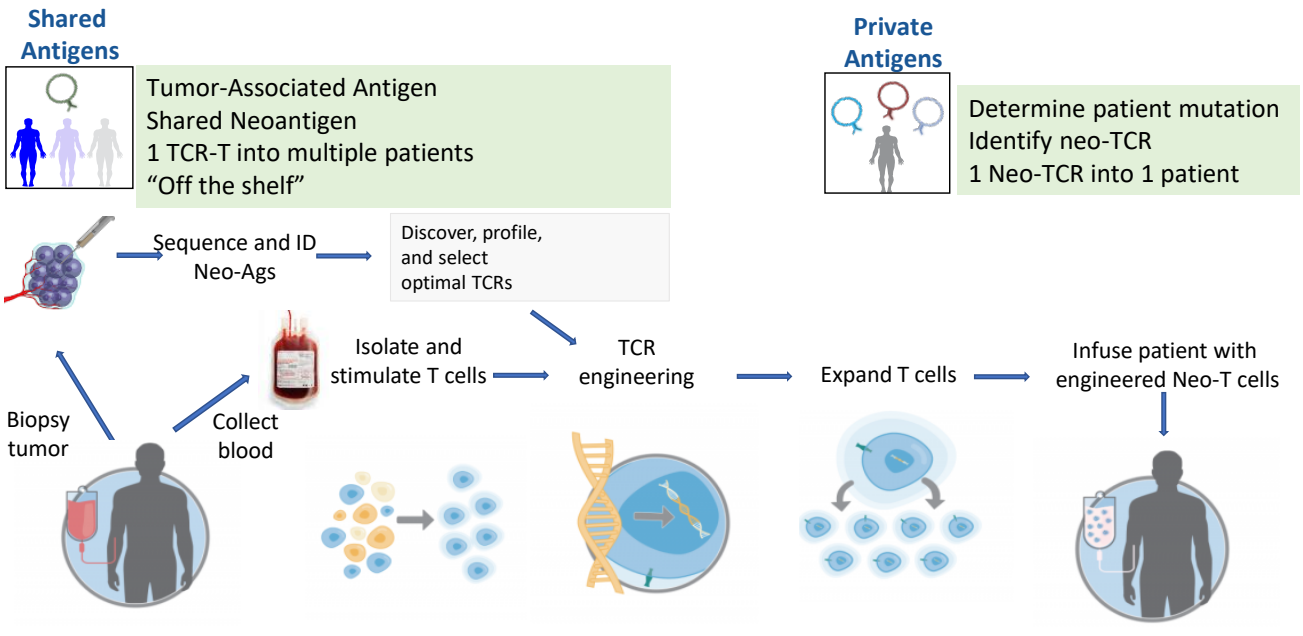


Schumacher and Schreiber (2015)

Technology Now Exists to Identify Neoantigen-Specific TCRs

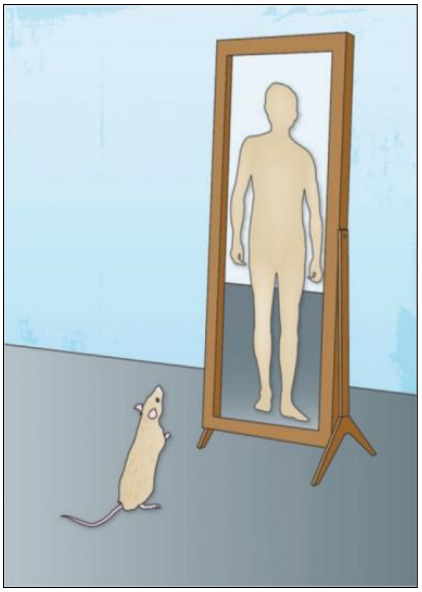


Neoantigen-Specific T Cells Can Be Shared or Private



Shared and Neo-T Nonclinical Safety Assessment Is Limited

- **TAA and Shared Neoantigen TCRs**
  - TCR characterization
    - Avidity
    - Cell reactivity
    - Specificity to mutation versus WT (neo-T)
    - Off-target activity
    - Reactivity to primary cells
    - Allo-reactivity
    - Antigen distribution in normal tissues
- **Neo-Ts**
  - Specificity to mutation versus WT
  - Antigen distribution in normal tissues
  - Genome blast
- **Demonstration of efficacy and safety of Neo-Ts relies heavily on clinical data**



Summary

- T cell redirectors, including CAR Ts, are now established immunotherapeutics
- Optimization of molecular characteristics to maximize benefit/risk will likely depend on the format
- Nonclinical safety assessment to support first-in-human studies must be fit for purpose
- Personalized therapies will require novel safety assessment strategies
- *In silico* and *in vitro* approaches will become an integral part of the nonclinical safety assessment strategy

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Engineered T Cells as Cancer Therapeutics: An Update on Their Design, Manufacture, and Clinical Experience

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Conflict of Interest Disclosure

- The author declares no conflict of interest.

Agenda

- CAR T therapy—evolution, rationale
- Modeling CAR T cell molecular design, manufacture
- Clinical/regulatory experience
- Modeling safety
  - Hypotheses, models

Milestones in Gene Therapy

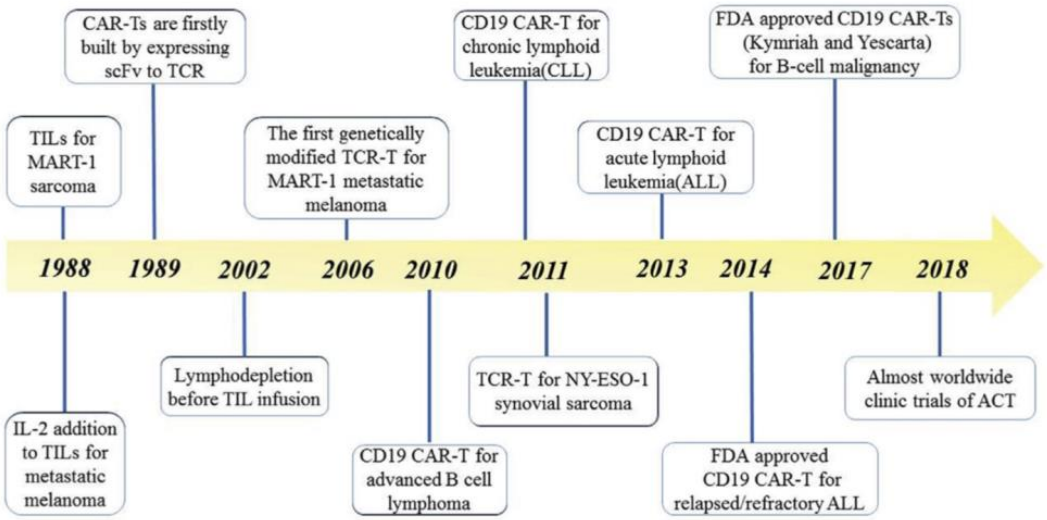
High and Roncarolo (2019)
NEJM 381:455

Table 2. Regulatory Milestones in Gene Therapy.*

Year and Milestone	Regulatory Authority	Indication	Vector†	Route of Administration
2003: approval of recombinant human p53 adenovirus for injection (Gendicine, Sibiono GeneTech)	NMPA	Head and neck squamous-cell carcinoma	Ad–p53	Intratumoral injection; intracavity or intravascular injection
2012: approval of alipogene tiparvovec (Glybera, uniQure)	EMA‡	Lipoprotein lipase deficiency	AAV1–LPL	Intramuscular injection
2015: approval of talimogene laherparepvec (Imlygic, Amgen)	EMA and FDA	Melanoma	HSV–GM-CSF	Intratumoral injection
2016: approval of autologous CD34+ cells encoding adenosine deaminase cDNA sequence (Strimvelis, Orchard Therapeutics)	EMA	Adenosine deaminase–deficient SCID	RV–ADA	Transplantation of autologous gene-modified CD34+ cells
2017				
Approval of tisagenlecleucel (Kymriah, Novartis)	FDA	Patients younger than 25 yr of age with relapsed or refractory ALL	LV–CD19	Intravenous infusion of autologous gene-modified T cells
Approval of axicabtagene ciloleucel (Yescarta, Kite Pharma)	FDA	Certain types of non-Hodgkin's lymphoma	RV–CD19	Intravenous infusion of autologous gene-modified T cells
Approval of voretigene neparvovec-rzyl (Luxturna, Spark Therapeutics)	FDA	Biallelic *RPE65*-associated retinal dystrophy	AAV2–RPE65	Subretinal injection
2018				
Approval of tisagenlecleucel (Kymriah)	EMA	Patients younger than 25 yr of age with relapsed or refractory ALL	LV–CD19	Intravenous infusion of autologous gene-modified T cells
Approval of axicabtagene ciloleucel (Yescarta)	EMA	Certain types of non-Hodgkin's lymphoma	RV–CD19	Intravenous infusion of autologous gene-modified T cells
Review of gene-therapy IND applications in United States streamlined to single reviewing agency, the FDA	FDA and NIH	—	—	—
Approval of voretigene neparvovec (Luxturna)	EMA	Biallelic *RPE65*-associated retinal dystrophy	AAV2–RPE65	Subretinal injection
2019				
Conditional approval of autologous CD34+ cells encoding $\beta^{A\text{-}T87Q}$-globin gene (Zynteglo, Bluebird Bio)	EMA	Patients older than 12 yr of age with transfusion-dependent β-thalassemia without β^0/β^0 genotype	LV–β-globin	Transplantation of autologous gene-modified CD34+ cells
Approval of onasemnogene abeparvovec-xioi (Zolgensma, AveXis)	FDA	Patients younger than 2 yr of age with spinal muscular atrophy	AAV9–SMN1	Intravenous infusion

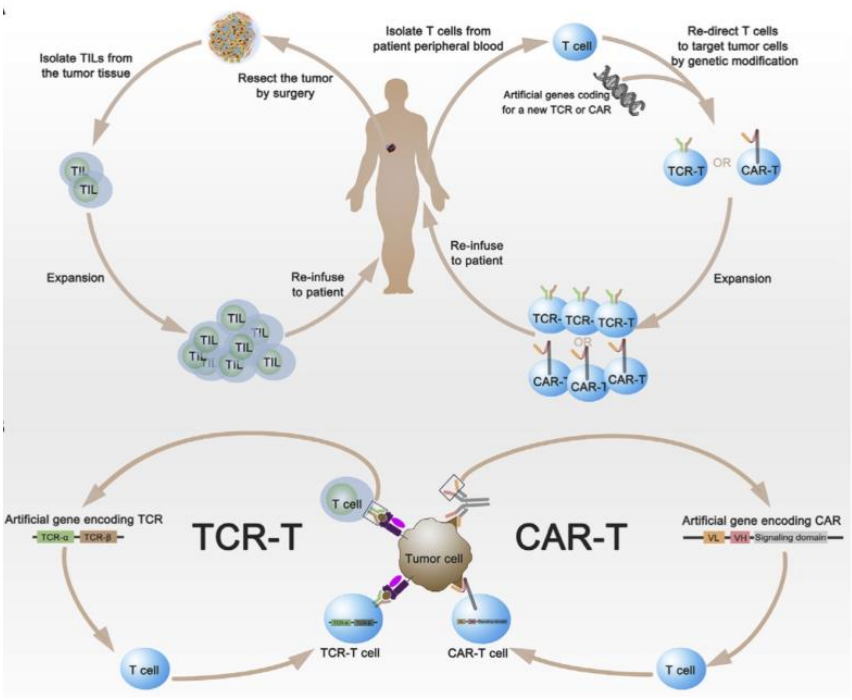
* ALL denotes acute lymphoblastic leukemia, cDNA complementary DNA, EMA European Medicines Agency, FDA U.S. Food and Drug Administration, IND investigational new drug, NIH National Institutes of Health, and NMPA National Medicine Products Administration (China).
† Vector designations indicate the type of vector (adeno-associated viral [AAV], adenoviral (Ad), herpes simplex viral [HSV], lentiviral [LV], or retroviral [RV]) and the gene transduced.
‡ Regulatory approval was allowed to lapse by the sponsor in 2017.

Milestones in Engineered T Cell Development



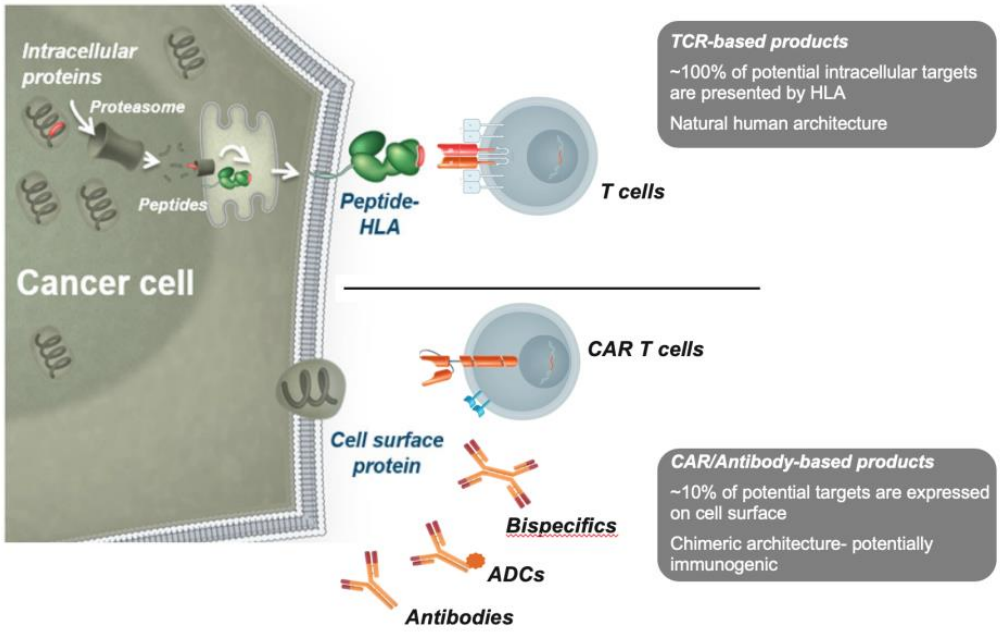
Jiang et al., (2019) *Cancer Letters* 462:23–32

Engineering T Cells:
eTCR T Cells versus CAR T Cells

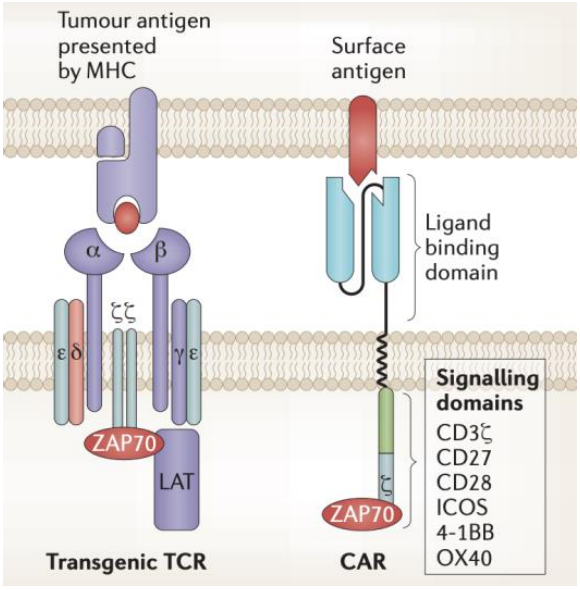


Jiang et al., (2019) *Cancer Letters* 462:23–32

Engineering T Cells: eTCR T Cells versus CAR T Cells



Engineered T Cell Platforms



Chimeric antigen receptors (CAR)

Single chain variable fragment (scFv)

Spacer

Transmembrane domain

Co-stimulatory domain

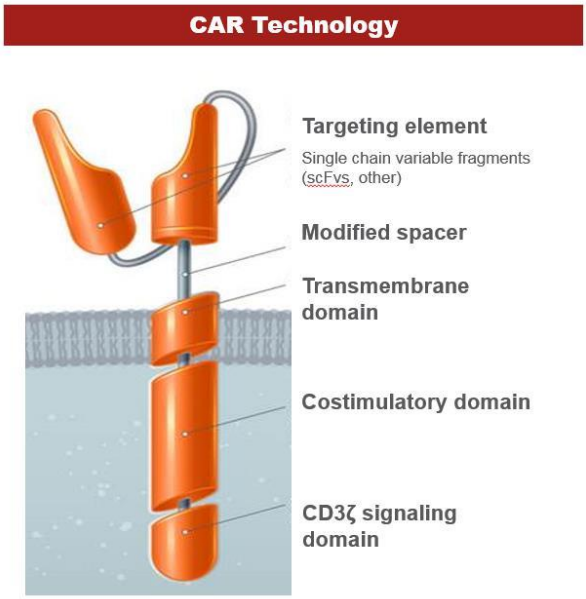
Stimulatory domain

Fesnak et al., *Nat Rev Cancer* (2016) 16:566–581

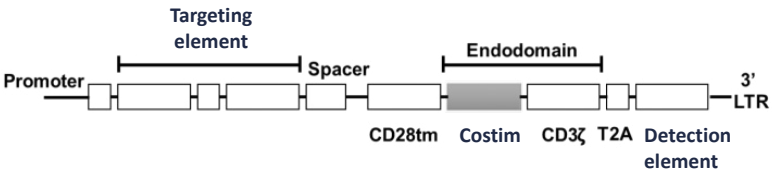
CAR T Cells: Design and Function

Functional impacts on product quality

- Process
  - T cell expansion methodologies
  - T cell subset selection
  - Transduction method
  - Cell handling
- Patient
  - Lymphodepletion (preconditioning)
  - Disease type, prior therapies, age
  - Co-medications
  - Checkpoint overrides
  - Combination targeting



CAR vector construct

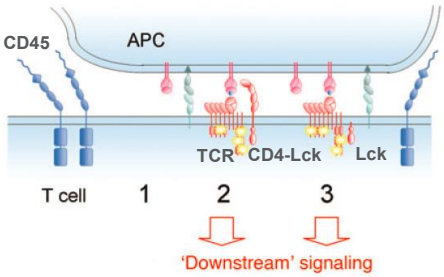


Promoter: CMV or other (stability)
Targeting element: Anti-CD19 scFv (FMC63)
T2A: Ribosomal skip element for bi-cistronic vector
Detection element

Designing a CAR
Optimizing the Extracellular Domain

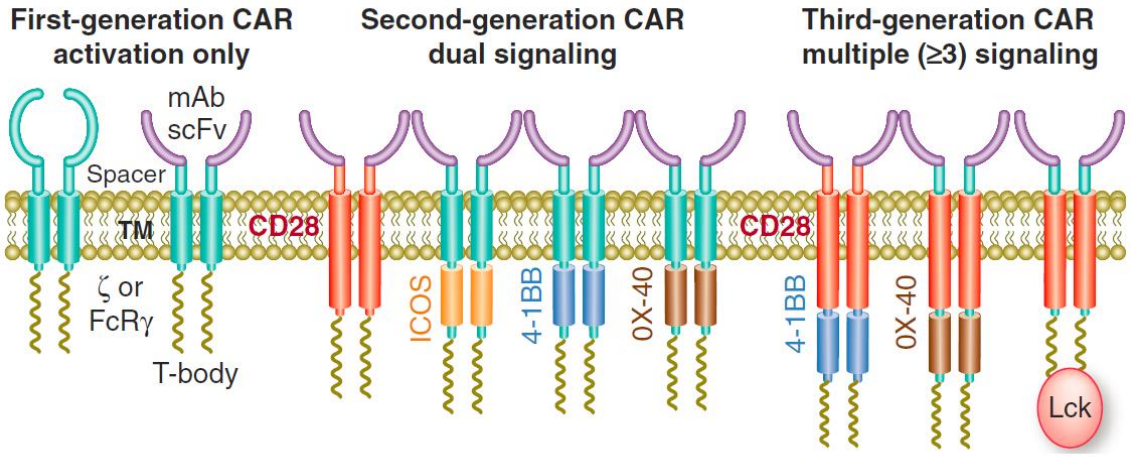
- Considerations
  - Design: ScFv, Fab, ligands (zetakine), other
  - Target specificity, affinity, expression level
  - ECD spacer length (T cell synapse)
  - Clustering (tonic signaling)

Kinetic-segregation model of T cell activation



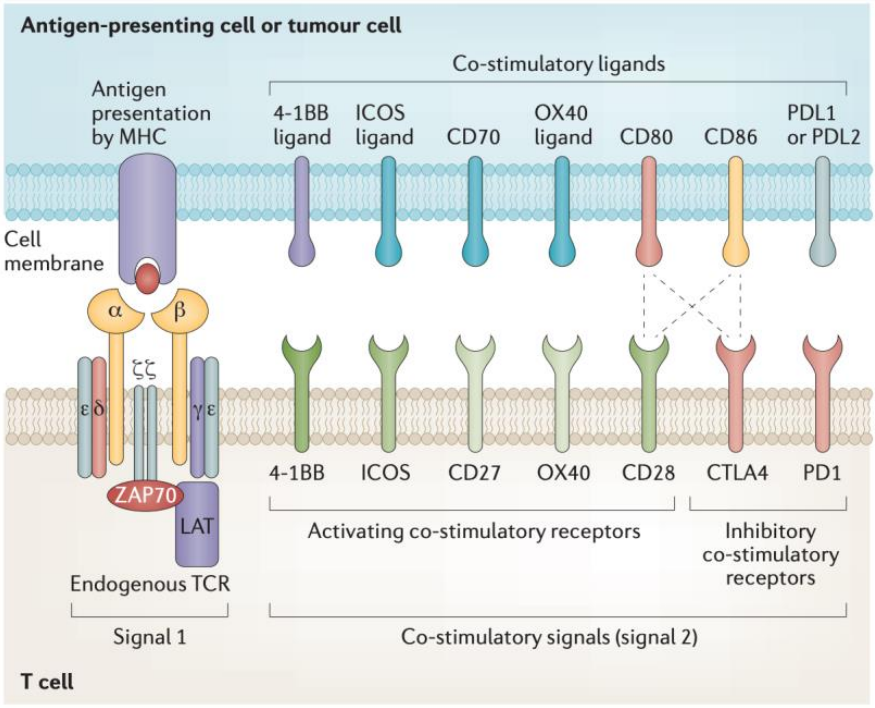
Nat Immunol (2006) 7:803

Designing a CAR
Optimizing the Intracellular Domain



Sadelain et al., (2013) *Cancer Discov* 3(4); 388–98

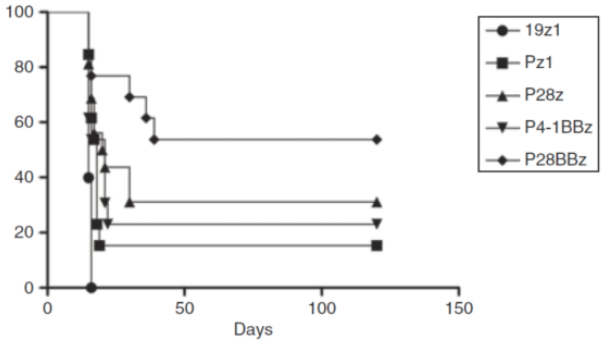
Designing a CAR: Co-stimulation



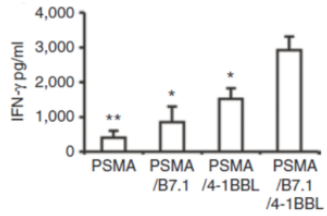
Fesnak et al., *Nat Rev Cancer* (2016) 16:566–581

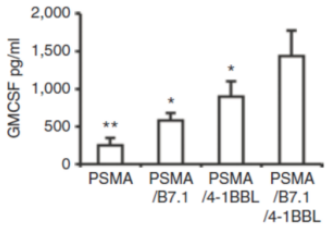
CAR T Cells: Co-stimulation Impacts Cytokine Production

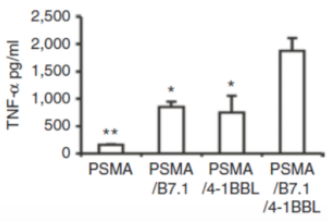
Comparison of the relative potency of PSMA-targeted T cells in the RM1.PGLS pulmonary metastases model.



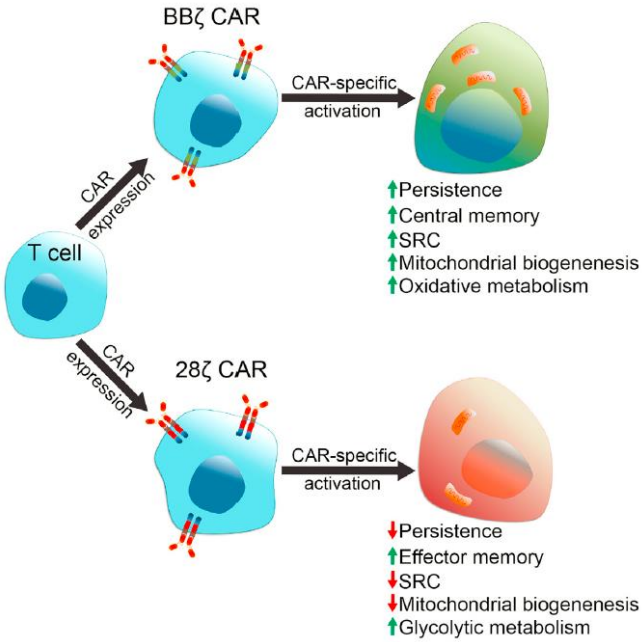
Zhong et al. (2010) Molecular Therapy 18:413







CAR T Cells: Co-stimulation Skews Differentiation Fate



Kawalekar et al., (2016) *Immunity* 44:380

Engineered T Cell Manufacture

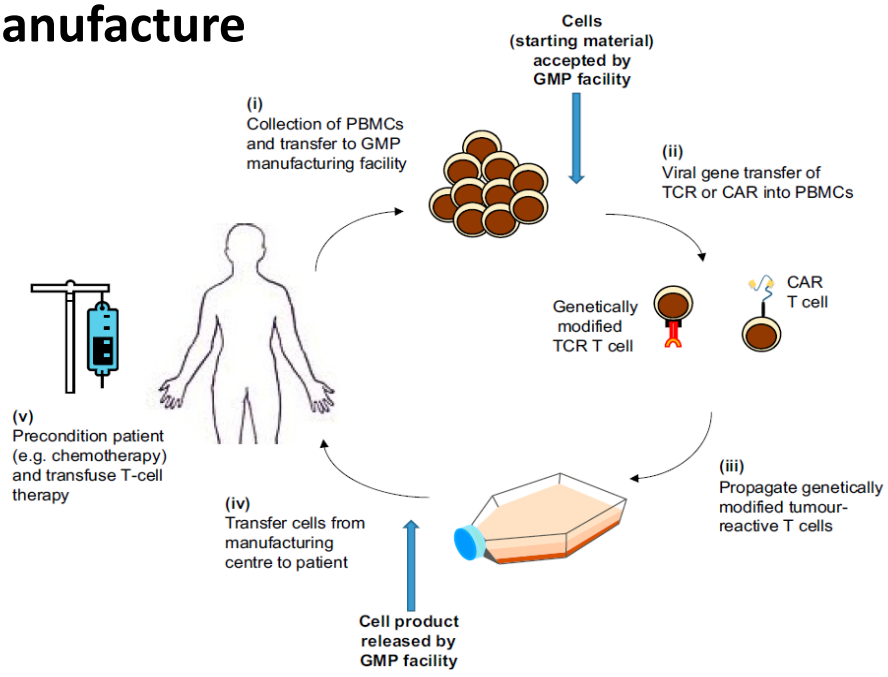


Table 1 | Outcomes after use of CD19-CAR T cells for B cell malignancies

Disease	Site	Population studied; phase	Construct	Vector	Reported CR (MRD⁻ at 28 days for B-ALL; CR at 3 months for NHL)	ITT CR (MRD⁻ for B-ALL)	Median follow-up	T cell persistence	Relapse rate	CD19⁻ relapses	High-grade/ severe CRS[a]	High-grade/ severe neuro-toxicity[a]	Notes and references
B-ALL	Children's Hospital of Philadelphia and University of Pennsylvania	Pediatric and adult; phase I	19.BB.z	Lentiviral	79% (22/28)[b,e]	NR	7 months	68% (PCR) at 6 months	26% (7/27)	43% (3/7)	27% (8/30)	NR[d]	5
B-ALL	Memorial Sloan Kettering Cancer Center	Adult (age 18+); phase I	19.28.z	Retroviral	67% (32/48)[c,e]	39% (32/83)[c,e]	29 months	0% (PCR) at 6 months	61% (25/41)	16% (4/25)	26% (14/53)	42% (22/53)	9
B-ALL	Novartis Global (ELIANA)	Pediatric, AYA; phase II	19.BB.z	Lentiviral	81% (61/75)	66% (61/92)	13 months	83% (BCA) at 6 months	33% (20/61)	94% (15/16)[e]	47% (35/75)	13% (10/75)	6
B-ALL	NCI	Pediatric, AYA; phase I	19.28.z	Retroviral	60% (12/20)	57% (12/21)	10 months	0% (PCR) at 6 months	17% (2/12)	100% (2/2)	29% (6/21)	5% (1/21)	10/12 responders underwent allo-HSCT[7]
B-ALL	Fred Hutchinson Cancer Center	Adult (age 18+); phase I	19.BB.z	Lentiviral	93% (27/29)	84% (27/32)	NR	NR	33% (9/27)	22% (2/9)	23% (7/30)	50% (15/30)	10
B-ALL	Seattle Children's Hospital	Pediatric, AYA; phase I	19.BB.z	Lentiviral	93% (40/43)[e]	89% (40/45)[e]	9.6 months	~30% at 6 months (BCA)[f]	45% (18/40)	39% (7/18)	23% (10/43)	21% (9/43)	8
NHL	Multiple (ZUMA-1)	Adult (age 18+); phase II	19.28.z	Retroviral	54% (55/101)	50% (55/111)	15.4 months	NR	NR	27% (3/11)[g]	13% (13/101)	28% (28/101)	14
NHL	Multiple (JULIET)	Adult (age 18+); phase II	19.BB.z	Lentiviral	40% (37/93)	22–27% (37–45/ 165)[i]	14 months	NR	NR	NR	22% (24/111)	12% (13/111)	13
NHL	University of Pennsylvania	Adult (age 18+); phase I	19.BB.z	Lentiviral	57% (16/28)	42% (16/38)	28.6 months	50% (BCA) at 12 months[f]	0%	NA	18% (5/28)	11% (3/28)	29
NHL	Fred Hutchinson Cancer Center	Adult (age 18+); phase I	19.BB.z	Lentiviral	33% (10/30)	27% (10/37)	NR	NR	11% (1/9)[h]	NR	13% (4/32)	28% (9/32)	Results dramatically improved after adoption of flu/cy preconditioning[12]
NHL	National Cancer Institute	Adult (age 18+); phase I	19.28.z	Retroviral	55% (12/22)	55% (12/22)	NR	NR	8% (1/12)	NR	18% (4/22)	55% (12/22)	31
CLL	Fred Hutchinson Cancer Center	Adult (age 18+); phase I	19.BB.z	Lentiviral	17% (4/24)	13% (4/30)	NR	100% (PCR) at 6 months	NR	NR	8% (2/24)	25% (6/24)	18
CLL	University of Pennsylvania	Adult (age 18+); phase I	19.BB.z	Lentiviral	29% (4/14)	22% (4/18)	19 months	100% (PCR and BCA) in responders at 6 months	0%	0%	43% (6/14)	7% (1/14)	19

NR, not reported; NA, not applicable; MRD, minimal residual disease, defined as <0.01% by flow cytometry; ITT, intent-to-treat response rate; AYA, adolescent and young adult; BCA, B cell aplasia;PCR:, polymerase chain reaction detection of CAR transgene. [a]As defined by the study authors or grade 3 or above. [b]Three patients not assessed for MRD. [c]Five patients not assessed for MRD. [d]High-grade neurotoxicity not reported; overall neurotoxicity 43%. [e]Some patients had no detectable disease at the time of infusion and were considered to be in CR, but whether treatment mediated clinical benefit was unclear. [f]Five patients were not assessed for CD19 expression at relapse. [g]Among responders only. [h]Includes patients with progressive disease after initial infusion; only a limited number of patients were analyzed. Strict cutoff for CD19⁻ <1% by immunohistochemistry. [i]Only reported data for patients receiving lymphodepletion with fludarabine and cyclophosphamide (flu/cy). [j]Inferred from the text; the ITT overall response rate was reported as 34%, but no ITT CR rate was reported.

Majzner and Mackall (2019) *Nat Med* 9:1341

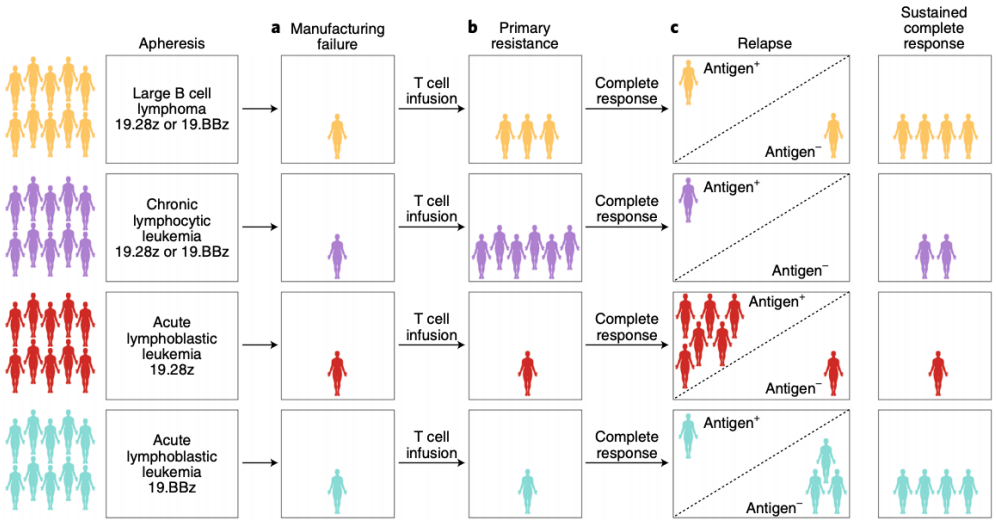
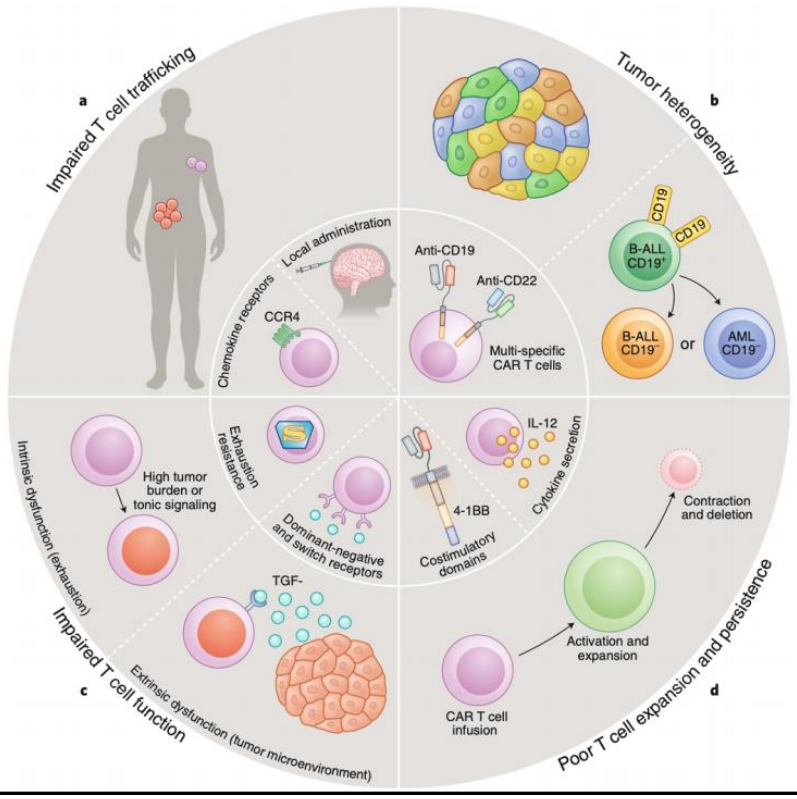


Fig. 1 | Patterns of failure after CD19-CAR T cell therapy and potential causes. Each row depicts mechanisms of failure and relapse for a different disease histology and/or CAR T cell construct. There are ten figures per row; each figure represents approximately 10% of patients, and each figure within a box represents patients in that category of treatment failure or resistance. **a**, In some series, manufacturing failures are an important cause of treatment failure. The rate of manufacturing failure has not been associated with underlying disease or the costimulatory domain. In general, with improved manufacturing processes this failure can be limited to <10% of cases[6–8,13,14]. **b**, Primary resistance is highly associated with underlying disease, with CLL[18–20,27] > LBCL[13,14,29,31] > B-ALL[5–9]. **c**, Among patients with B-ALL, CD19⁺ relapse tends to occur more commonly after treatment with CD19.28.z-CARs, which manifest short persistence[7,9], whereas CD19$^{-/lo}$ relapse tends to occur after treatment with CD19.BB.z-CARs[6], which often induce prolonged immune pressure. The incidence of CD19⁺ versus CD19$^{-/lo}$ relapse in LBCL occurs but remains incompletely characterized[14,95,96]; approximately 50% of relapses have been reported to be due to loss of CD19[136]. Whether CD19 expression at relapse in LBCL correlates with the costimulatory endodomain remains unknown. Credit: Debbie Maizels/Springer Nature

Majzner and Mackall (2019) *Nat Med* 9:1341

Overcoming Barriers to CAR T Activity



Majzner and Mackall (2019) *Nat Med* 9:1341

Anti-CD19 Autologous Chimeric Antigen Receptor T Cell Clinical Trials for Hematologic Cancers: Acute Lymphocytic Leukemia

[A] Occurred in B cell ALL patients with a high tumor burden.
[B] One patient received previous CAR T cell treatment.

Trial and Trial Phase	No. of Patients/Age	Signaling Domain CAR T Cell	Disease	HSCT
NCT01044069[2] Phase 1	n=46 Adult	CD3ζ and CD28	B-cell ALL	18 allo-SCT prior
NCT01626495[3] Phase 1	n=59 Children	CD3ζ and CD137	CD19 (+) ALL	39 allo-SCT prior 5 allo-SCT post
NCT02028455[4] Phase 1	n=36 Children and young adults	CD3ζ, CD137, and EGFRt	CD19 (+) ALL	Not reported
NCT01029366 NCT02030847[5] Phase 1/2	n=27 Adult	CD3ζ and CD137	CD19 (+) ALL	9 allo-SCT prior
NCT01865617[1] Phase 1/2	n=90 Adult	CD3ζ, CD137, and EGFRt	B-cell ALL (n=36) B-NHL (n=41) CLL (n=13)	Not reported
NCT01593696[6] Phase 1	n=21 Young adults and children	CD3ζ and CD28	B-ALL (n=20)[b] B-NHL (n=1)	8 allo-SCT prior 10 allo-SCT post

1. Turtle CJ et al., ASCO Meeting Abstracts 2016;15_suppl:102.
2. Park JH et al., ASCO Meeting Abstracts 2016;15_suppl:7003.
3. Maude SL et al., ASCO Meeting Abstracts 2016;15_suppl:3011.
4. Gardner RA et al., ASCO Meeting Abstracts 2016;15_suppl:3048.
5. Frey NV et al., ASCO Meeting Abstracts 2016;15_suppl:7002.
6. Lee DW et al., *Lancet* 2015;385:517–28.

Shank BR et al., *Pharmacotherapy* 2017

Anti-CD19 Autologous Chimeric Antigen Receptor T Cell Clinical Trials for Hematologic Cancers: Acute Lymphocytic Leukemia

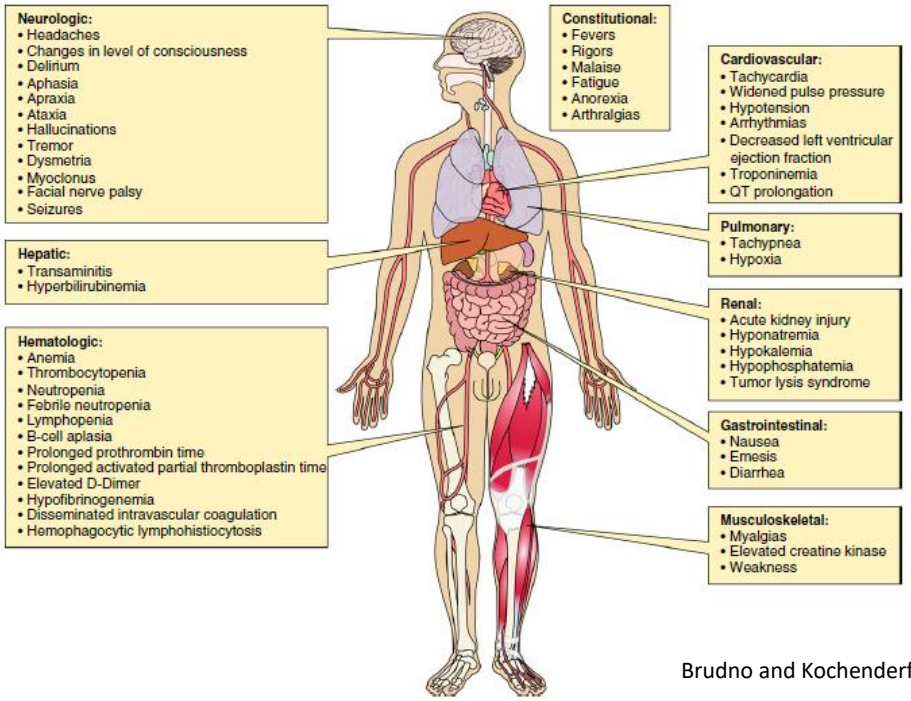
[A] Occurred in B cell ALL patients with a high tumor burden.
[B] One patient received previous CAR T cell treatment.

Trial and Trial Phase	No. of Patients/Age	Signaling Domain CAR T Cell		Outcomes	Toxicity
NCT01044069[2] Phase 1	n=46 Adult	CD3ζ and CD28	B-	CR 83%	Severe CRS in 11 patients Grade 3–4 neurotoxicity in 13 patients
NCT01626495[3] Phase 1	n=59 Children	CD3ζ and CD137	CI	CR 93%	CRS in 52 patients Severe CRS in 16 patients
NCT02028455[4] Phase 1	n=36 Children and young adults	CD3ζ, CD137, and EGFRt	CI	CR 91%	
NCT01029366 NCT02030847[5] Phase 1/2	n=27 Adult	CD3ζ and CD137	CI	CR 56%	CRS grade 3 or greater in 21 patients, 3 died from CRS
NCT01865617[1] Phase 1/2	n=90 Adult	CD3ζ, CD137, and EGFRt	B- B C	ALL: CR 94% NHL: CR 47% CLL: CR 50%	Severe CRS 14 patients, grade 3 or greater neurotoxicity in 27 patients, 4 patients died[a]
NCT01593696[6] Phase 1	n=21 Young adults and children	CD3ζ and CD28	B- B	ALL: CR 70% NHL: PD	CRS Grade 4 in 3 patients

1. Turtle CJ et al., ASCO Meeting Abstracts 2016;15_suppl:102.
2. Park JH et al., ASCO Meeting Abstracts 2016;15_suppl:7003.
3. Maude SL et al., ASCO Meeting Abstracts 2016;15_suppl:3011.
4. Gardner RA et al., ASCO Meeting Abstracts 2016;15_suppl:3048.
5. Frey NV et al., ASCO Meeting Abstracts 2016;15_suppl:7002.
6. Lee DW et al., *Lancet* 2015;385:517–28.

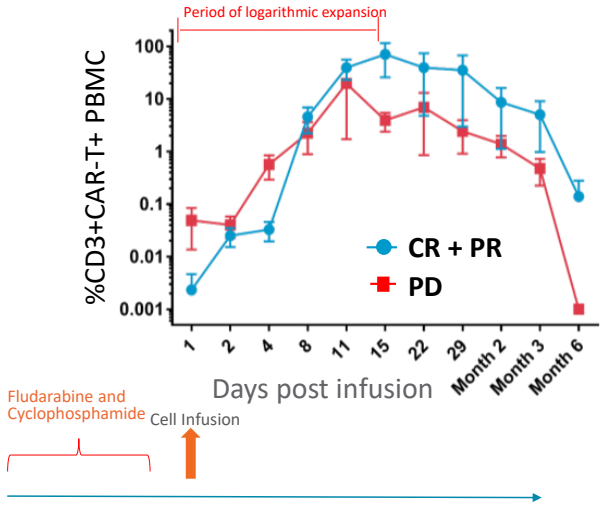
Shank BR et al., *Pharmacotherapy* 2017

Toxicities Associated with CAR T Cell Therapy



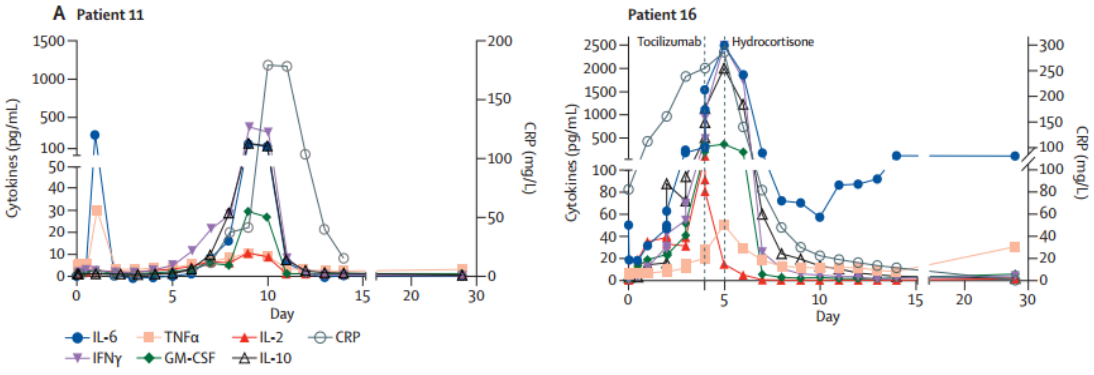
Brudno and Kochenderfer (2016) Blood 127:3321

Antigen-Driven CAR T Cell Expansion Contributes to Both Response and Toxicity Risk



Abramson JS et al., *ASH*, 2016

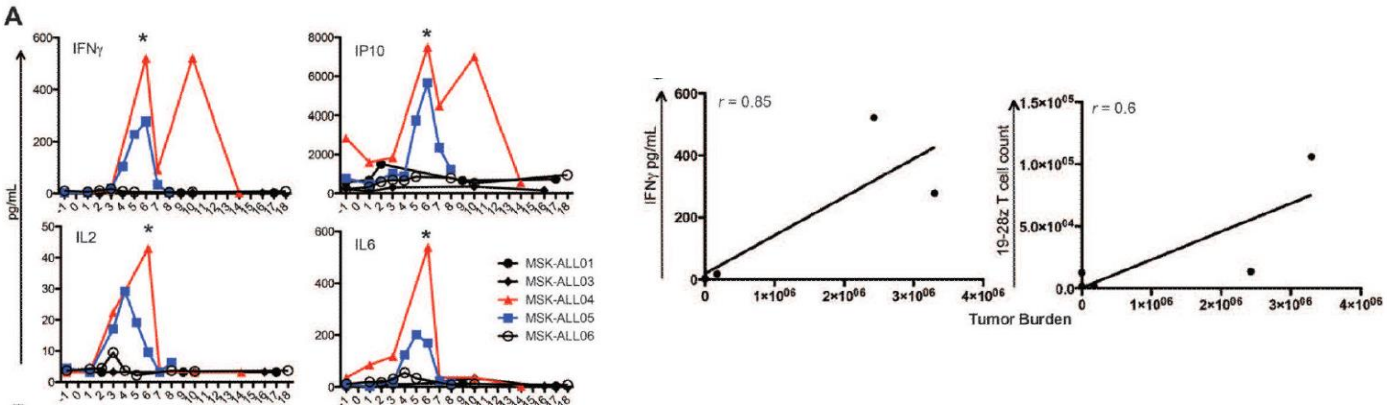
CRS: Cytokinemia Profile Post–CD19 CAR Therapy



A phase 1, dose-escalation trial in children and young adults (aged 1–30 years) with relapsed or refractory acute lymphoblastic leukemia or non-Hodgkin lymphoma. Autologous T cells were engineered to express a CD19-CAR incorporating an anti-CD19 single-chain variable fragment plus TCR zeta and CD28 signaling domains. All patients received fludarabine and cyclophosphamide before a single infusion of CD19-CAR T cells. Patients received either 1×10^6 CAR-transduced T cells per kg (dose 1), 3×10^6 CAR-transduced T cells per kg (dose 2), or the entire CAR T-cell product if sufficient numbers of cells to meet the assigned dose were not generated. ClinicalTrials.gov, number NCT01593696.

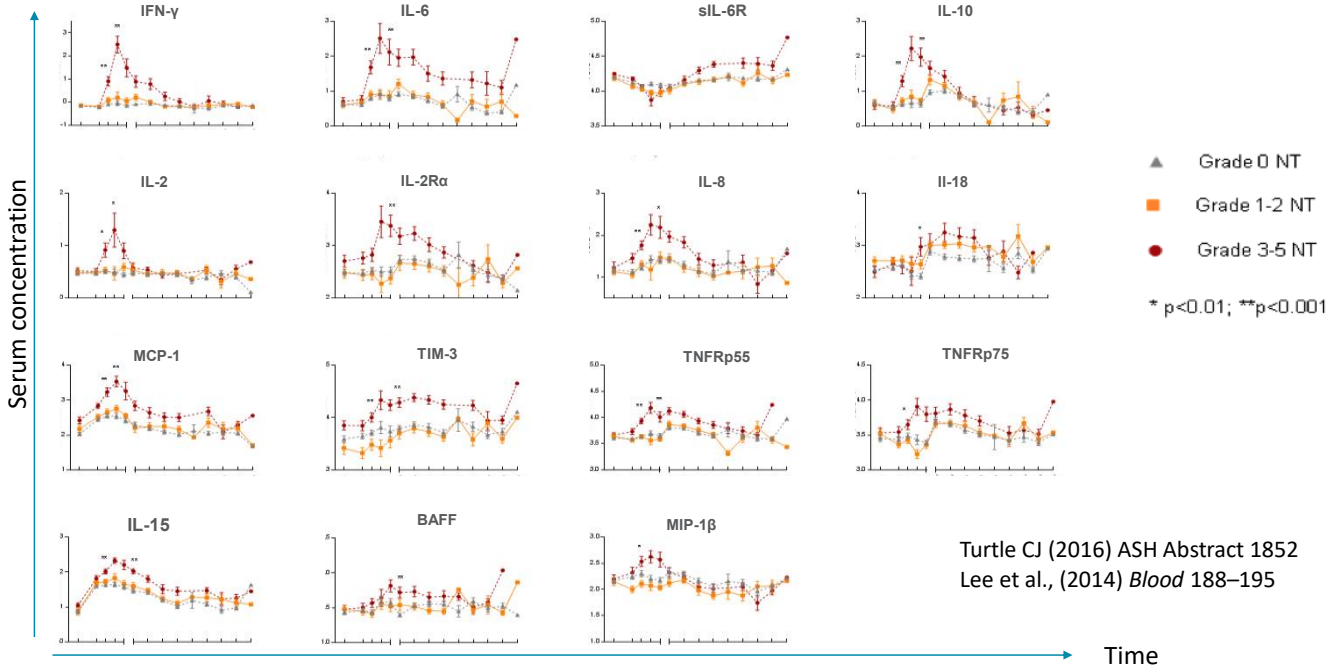
Lee at al., (2015) *Lancet* 385:517

Cytokine Release Is Driven by Tumor Burden



Brentjens et al., (2013) *Sci Transl Med* 20:177ra38

Serum Cytokines Are Associated with Neurotoxicity

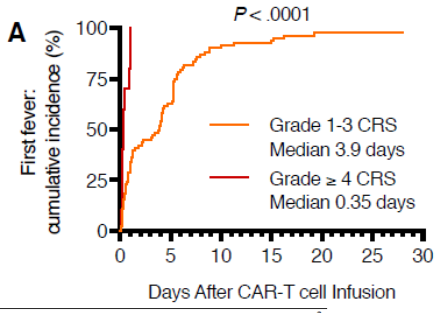


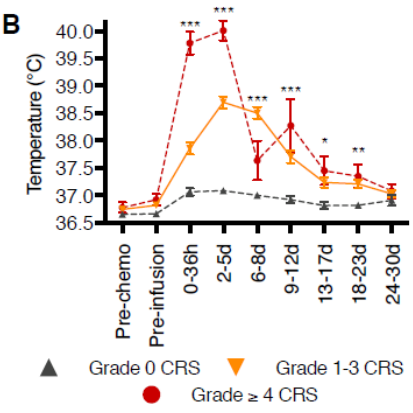
Turtle CJ (2016) ASH Abstract 1852
Lee et al., (2014) *Blood* 188–195

CRS Symptoms and Management

- Cytokine Release Syndrome is the most common side effect of CAR T therapy.
  - Driven by high levels of pro-inflammatory cytokines, including IFNγ, IL-6, IL-1, and IL-2RA.
- CRS presentation is highly individualized and requires personalized patient management. Hypercytokinemia can . . .
  - Activate the prostaglandin system (flu-like symptoms, including fevers, myalgias, and fatigue)
  - Lead to vasodilation with subsequent hypotension, tachycardia, and capillary leak with edema, culminating in organ damage including hepatic, renal, and cardiopulmonary toxicity
  - Lead to severe shock that is fluid refractory and requires high-dose vasoactive support to maintain tissue perfusion
  - Mirror hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome (MAS) and patients can develop similar clinical and laboratory manifestations including cytopenias, hepatosplenomegaly, coagulopathy with marked hypofibrinogenemia, and hyperferritinemia
- Tocilizumab (anti-IL-6R) ± steroids are used to manage emergent CRS
  - Prophylactic CAR T cell dose titration is used by some institutions to reduce risk of CRS with high tumor–burden patients

Early Onset of Fever Associated with Increased Risk of High-Grade CRS





CRS Grade	1-3	4-5	Total	*P* value[a]
Number of Patients, n	83	10	92	
Fever Onset (days after CAR-T cell infusion)				<.0001
Median [IQR]	3.9 [0.8, 5.6]	0.4 [0.3, 0.9]	2.2 [0.9, 5.6]	
Range	0.1, 19	0.2, 1.0	0.1, 19	
Time to Peak Temperature (days after CAR-T cell infusion)				.001
Median [IQR]	5.7 [4.3, 7.6]	2.8 [1.3, 3.2]	5.3 [3.4, 7.3]	
Range	0.2, 30	0.4, 11	0.2, 30	
Maximum Temperature (°C)				<.0001
Median [IQR]	39.4 [39.2, 30.6]	40.4 [40.1, 40.6]	39.5 [39.2, 39.8]	
Range	37.7, 41.3	39.9, 40.9	37.7, 41.3	
Fever Duration (days after first fever)				.03
Median, [IQR]	2.5 [1.2, 4.7]	4.4 [3.6, 5.4]	3.0 [1.2, 4.8]	
Range	0.02, 15	3.1, 6.8	0.02, 15	

[a]Two-sided *P*-values calculated based on Wilcoxon test.

Hay et al., (2017*) Blood* 130(21):2295–2306

Chemotherapeutic Lymphodepletion (Preconditioning)

Protocol name/ institution	Construct name	Patient populat	Lymphodepletion	Responses	Toxicity
NIH/NCI [74,75]	Autologous CD3z-28	Adult B-NHL $n=22$	Cy 60 mg/kg × 1–2 days + Flu 25 mg/ m^2 × 5 days	ORR 73% CR 55% PR 18% 6-month EFS 39% (all 20 pts)	CRS not formally reported Fever 91% Grade 3–4 neurotoxicity 55%
NIH/NCI [76]	Allogeneic CD3z-28	Adult B-NHL $n=10$ (Whole cohort +	None		CRS 55% Neurotoxicity not formally reported Headache in 3 patients
NIH/NCI [77]	HuCAR-19	Advanced NHL $n=9$	Cy 300 mg/m^2 daily for 3 days + Flu 30 mg/m^2 daily for 3 days	ORR 86% CR in 2 pts	CRS 82% Neurotoxicity in 1 patient
Seattle Children's Hospital [78] NCT01318317 (NHL1)	CD19R;ζ 1st generation CD8 + TCM-enriched	NHL $n=9$	CAR T-cells administered after ASCT	50% free of relapse at both 1 and 2 years	None reported
Seattle Children's Hospital [78] NCT01815749 (NHL2)	CD4 (+) and CD8 (+) TCM subsets $CD4^+/CD8^+$ TCM-enriched	NHL $n=9$	CAR T-cells administered after ASCT	75% progression free at 1 year	None reported
Fred Hutch [11] NCT01865617	JCAR014	Adult B-NHL $n=32$	Cy 60 mg/kg × 1 ± etoposide or Cy 60 mg/kg × 1 + Flu 25 mg/m^2 × 3 days	ORR 63% CR 33%	Severe CRS 13% Grade ≥ 3 neurotoxicity 28%
University of Pennsylvania [79]	CTL019	Adult DLBCL, M $n=24$	Bendamustine ($n=6$), cy (11 pts), flu-cy ($n=1$), modified EPOCH ($n=3$), and TBI-Cy ($n=3$)	3-month ORR: 68% (15/22): DLBCL 54%; FL 100%; MCL 50%. 12-month PFS: 62% (DLBCL 43%; FL 100%).	CRS 67% Grade 3–4 83% Neurotoxicity 12%
University of Pennsylvania [80] NCT02030834	CTL019	FL $n=14$	Bendamustine ($n=6$), Cy ($n=2$), Flu-Cy ($n=1$), TBI-Cy ($n=3$), EPOCH ($n=1$), and carboplatin-gemcitabine ($n=1$)	3-month ORR 79% 3-month CR 50%	CRS 43% Grade 3–4 CRS 14% Grade 5 neurotoxicity in 1 patient
ZUMA-1 trial [81]	KTE-C19	Refractory agres lymphomas $n=7$	Cy 500 mg/m^2 and Flu 30 mg/m^2 for 3 days	ORR 71% CR 57% Ongoing CR in 3 (> 12 months of follow-up)	Grade 3–4 CRS 14% Neurotoxicity 57%
JULIET trial [19] NCT02445248 Updated Lugano 2017	CTL019	DLBCL $n=141$	Flu 25 mg/m^2 + Cy 250 mg/m^2/day × 3 days or bendamustine 90 mg/m^2/day × 2 days	ORR 59% CR 43% PR 16% 3-month CR 37% 3-month PR 8%	CRS 57% Grade 3–4 neurotoxicity 13%
TRANSCEND trial [20] NCT02631044 Updated from Juno Therapeutics' website	JCAR017	R/R agressive N $n=67$	Flu + Cy	Best ORR 76% Best CR is 52% 3-month ORR 51% (21/41) 3-month CR 39%	Grade 3–4 CRS 2% Grade 3–4 Neurotoxicity 16%

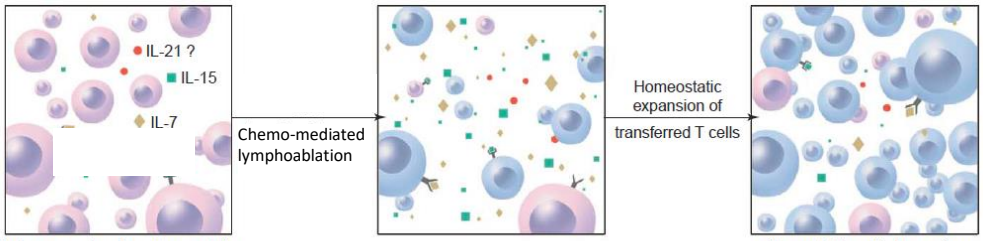
Gauthier and Yakoub-Agha (2017) *Curr Res Transl Med* 65: 93–102

Chemotherapeutic Lymphodepletion

Hypotheses/rationale

- Establish permissive niche for engraftment/survival of CAR T cells
- Deplete Treg population
- Suppress/kill tumor cells, enhance adaptive immune response

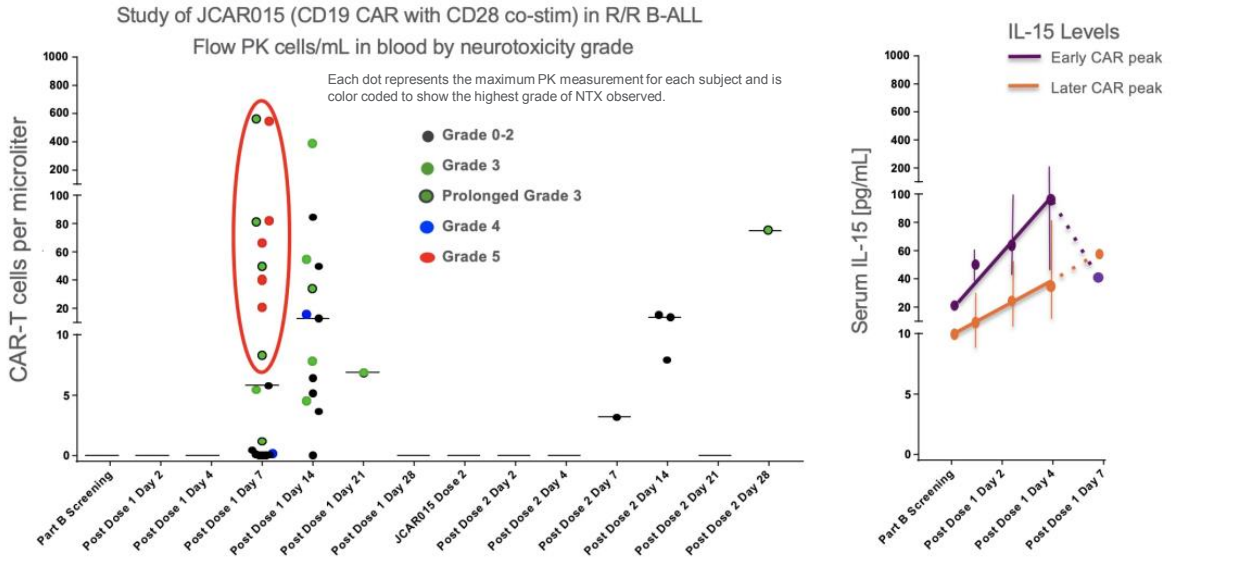
Klebanoff et al., (2005) *Trends Immunol* 26:111



- Lymphodepletion with cyclophosphamide and fludarabine improved CD19 CAR-T cell <u>expansion, persistence, and disease-free survival</u>
  - 29 adults with B-ALL (median age 40, range 22–73 years; median 175 marrow blasts, range 0%–97%), including 10 patients who had relapsed after allogeneic transplantation, who received one prior CAR T cell infusion

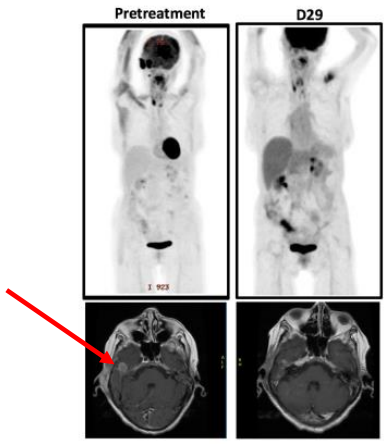
Turtle et al., (2015) *Blood* 126:3773

Early, Rapid Peak Expansion of CAR T Cells Correlates with Severe Neurotoxicity, Including Cerebral Edema



Gilbert M (2017) *SITC*

Example of Complete Response in DLBCL with CNS Involvement without Neurotoxicity in Clinical Trial



In addition . . .

In patients with Acute Lymphoblastic Leukemia treated with CD19+ CAR T cells, there is not a clear *correlation* between neurotoxicity and either CAR T cells or the presence of CNS leukemia, which typically responds to CAR T

Mechanistic Inferences . . .

1. CAR T cells can readily access the CNS and safely exert effector function
2. CNS neurotoxicity is unlikely to be the result of target expression in the brain or CAR T "on-target" toxicity

Product Characteristics Associated with Neurotoxicity (Rocket)

Univariate Logistic Regression of CMC Variables Identified CD8 Dose & Cytokine Expression Correlated With Cerebral Edema

Results of root cause investigation, ROCKET (JCAR015 treatment in R/R B-ALL)

Attribute Category	Attribute	P Value
Dose	Annexin V– CD8+CAR+ dose[1]	< .001
	Annexin V– CD8+CAR+ dose/kg[1]	.001
	Annexin V– CD3+CAR+ dose[1]	.012
	Annexin V– CD3+CAR+ dose/kg[1]	.015
Function	% of CD4+CAR+ producing IL-2	.019
	% of CD8+CAR+ producing TNFα	.044
	% of CD4+CAR+ producing TNFα	.044

No association was identified between fatal NTX and T cell differentiation state or other phenotypes

Gilbert M (2017) *SITC*

Factors That May Be Associated with Neurotoxicity (JCAR015 R/R B-ALL)

Early, rapid expansion of CAR T cells appears to correlate with risk of severe neurotoxicity. Multiple factors may be associated with the etiology of early, rapid expansion:

- Patient-specific factors
  - Age, prior therapies, comorbidities
  - Baseline cytokines
  - Disease
    - ALL versus others
    - Impacts on T cell function/dysfunction
    - Disease burden (extent of marrow involvement)
- Intensity of lymphodepletion
- Product-related factors
  - Cell health/viability
  - Cell ability to produce inflammatory cytokines
  - Dose regimen
  - Co-stimulation

Neurotoxicity with Immune Agonism

	IL-2 (Proleukin) Package insert	IFN-a (Pegasys, Intron-A, Roferon) Package insert	TGN-1412 NEJM (2006) 355:1018)	Blinatumomab (anti-CD19xCD3 BiTE) Package insert	CAR T cells (anti-CD19) Blood (2016) 127:3321
Mood	Irritability/Anxiety (19-38%)	Depression (18-28%) Mood alteration (2-6%)			
Behavior	Confusion (34%; Gr 4: 1%) Anxiety (12%)		Aphasia	Other ≥GR 3: Speech disorders, confusion and disorientation	Aphasia, Apraxia
Physical effects			Weakness	Tremor (7-19%; ≥GR 3: 1%) Convulsions: ≥GR 3: ≥2% Other ≥GR 3: encephalopathy, convulsions	Tremor, Myoclonus, Seizures
Proprioception	Dizziness (11%)	Dizziness (12-16%)		Dizziness 5-13%; ≥GR 3: <1%) Other ≥GR 3: Coordination and balance disorders	Ataxia, Dysmetria
Pain, Perception		Headache (43-58%)	Headaches (early and late); Paresthesia or localized numbness, Hyperalgesia	Headache (28-36%; ≥GR 3: 2-4%)	Headache, Hallucinations, Facial nerve palsy
Consciousness	Coma (Gr 4: 2%) Somnolescence (22%)	Insomnia (19-37%)	Delirium	Altered consciousness (7-10%; ≥GR 3: 1-4%)	Changes in levels of consciousness
Memory, Concentration	Memory impairment (4-6%)	Concentration impairment (8-13%)	Partial amnesia; Difficulty concentrating (late)		

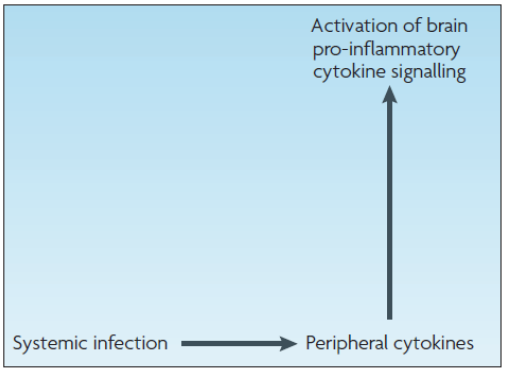
Peripheral Inflammation Is Interpreted and Propagated within the Brain

Key facilitators

- Afferent nerves (e.g., vagus, trigeminal)
- "Neurovascular unit": the cerebral vascular endothelium
  - Establishes the blood-brain barrier
- Circumventricular organs
- Brainstem

Key pro-inflammatory mediators

- IL-1α and IL-1β
- TNF-α
- IL-6

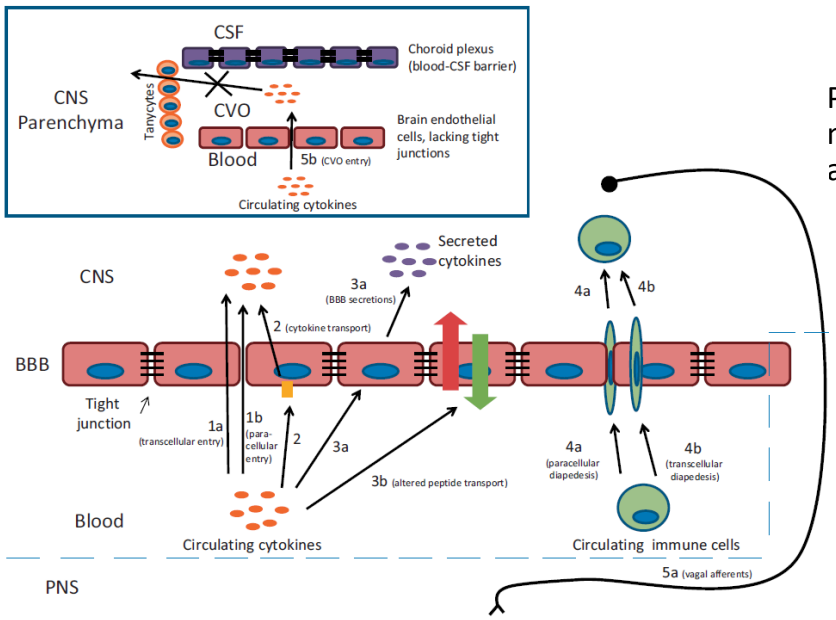


Changes in neuronal function

Sickness behaviour
- Attenuation of parasympathetic tone
- Activation of HPA axis
- Reduced appetite
- Altered thermoregulation and energy metabolism
- Flattening of diurnal rhythms
- Decreased social and physical activity
- Increased SWS and reduced REM
- Impaired learning and memory
- Pain
- Fatigue

Dantzer et al., (2008) *Nat Rev Neurosci* 9:46

Neuroinflammation at the BBB



Peripheral cytokines have various modes of interaction with the CNS at the blood-brain barrier

Erickson et al., (2012) *Neuroimmunomodulation* 19:121

Differences in CRS Grading Challenges Cross-Study Comparisons

TABLE 2 CRS grading scales

Institution	Grade 1	Grade 2	Grade 3	Grade 4
CHOP/PENN	Mild symptoms (supportive care only)	Moderate symptoms with some signs of organ dysfunction (need for hospital admission for some IV antibiotics and monitoring)	Moderate to severe symptoms with increased organ dysfunction (interventions for hypotension, coagulopathy & hypoxia)	Life threatening complications requiring aggressive interventions (high dose vasoactives, mechanical respiratory support)
Lee Criteria	Mild flu-like symptoms (supportive care only)	Moderate symptoms requiring intervention: hypotension responsive to fluids or low dose vasoactive, hypoxia responsive to supplemental O_2, mild organ dysfunction	Increasing symptomatology: Hypotension necessitating multiple vasoactives, hypoxia necessitating increasing O_2 supplementation (>40%), grade 4 transaminitis	Life-threatening symptoms (mechanical respiratory support, grade 4 organ toxicity)
MSKCC	Mild symptoms (observation and supportive care)	Hypotension requiring vasoactive <24 h or Hypoxia requiring supplemental O_2 <40%	Hypotension requiring vasoactive >24 h. Hypoxia requiring supplemental O_2 >40%	Life threatening: Hypotension refractory to vasoactives. Hypoxia necessitating mechanical respiratory support
CARTOX	Temperature ≥38°C. Grade 1 organ toxicity	Hypotension responsive to fluids or low dose vasoactives. Hypoxia requiring supplemental O_2 <40%. Grade 2 organ toxicity	Hypotension requiring multiple vasoactives. Hypoxia requiring >40% O_2. Grade 3 organ toxicity or Grade 4 transaminitis	Life threatening hypotension. Hypoxia requiring mechanical respiratory support. Grade 4 organ dysfunction
ASBMT	Temperature ≥38°C	Temperature ≥38°C with hypotension and/or hypoxia (not requiring additional interventions other than supplemental O_2 by NC)	Temperature ≥38°C with hypotension requiring 1 vasoactive (±vasopressin) or hypoxia requiring high flow or other non-positive pressure intervention	Temperature ≥38°C with hypotension requiring multiple vasoactives or hypoxia requiring positive pressure

Abbreviations: ASBMT, American Society for Blood and Marrow Transplantation; CARTOX, CAR-T therapy associated toxicity working group; CHOP, Children's Hospital of Philadelphia; MSKCC, Memorial Sloan Kettering Cancer Center.

Oved et al., (2019) *Immunol Rev* 290:1114–126

Cell-Related Encephalopathy Syndrome/Immune Effector Cell-Associated Neurotoxicity Syndrome

TABLE 3 Modified summary of CRES/ICANS grading scales

Institution	Grade 1	Grade 2	Grade 3	Grade 4
CTCAE 5.0	Mild symptoms (brief partial seizure w/o LOC)	Symptoms limiting instrumental ADL (brief generalized seizure)	Symptoms limiting self-care ADL (multiple seizures despite medical care); New onset cerebral edema	Life threatening symptoms
CARTOX	7-9 CARTOX score (mild impairment)	3-6 CARTOX score (moderate impairment)	0-2 CARTOX score (severe impairment); seizures responsive to benzodiazepine; stage 1-2 papilledema	Critical/obtunded condition; Generalized seizures or non-convulsive status epilepticus; stage 3-5 papilledema or cerebral edema
ASBMT Adult	7-9 ICE score; awaken spontaneously	3-6 ICE score; awaken to voice	0-2 ICE score; awaken to tactile stimulus; seizure that resolves with intervention; focal edema on neuroimaging	Life threatening symptoms
ASBMT Pediatric[a]	CAPD 1-8	CAPD 1-8	CAPD ≥9	Unable to perform CAPD

The CTCAE scale incorporates encephalopathy, seizure, dysphagia, tremor, headache, confusion, depressed level of consciousness and cerebral edema; CARTOX criteria incorporates Neurologic Assessment Score (CARTOX-10 score), elevated ICP, seizure or motor weakness. ASBMT criteria are based on ICE score (modified CARTOX-10 evaluation), level of consciousness, seizures, motor findings, and elevated ICP.
Abbreviations: ADL, activities of daily living; LOC, loss of consciousness.
*ASBMT pediatric criteria are for children <12 and include all of the adult criteria with the addition of the CAPD score based off the Cornell delirium assessments.

Oved et al., (2019) *Immunol Rev* 290:114–126

Relevant Regulatory Guidances

- US FDA Guidance—Gene therapy
- US FDA Preclinical assessment of investigational cellular and gene therapy products
- US FDA Supplemental guidance on testing for replication competent retrovirus and retroviral vector-based gene therapy products
- US FDA Potency tests for cellular and gene therapy products
- US FDA Content and review of CMC information for human gene therapy INDs
- US FDA Determining the need for and content of environmental assessments for gene therapies, vectored vaccines, and related recombinant viral or microbial products
- CHMP ATMP risk-based assessment
- CPMP Gene transfer guidance
- EMEA Guideline to mitigate risks from novel investigational therapies

(Unique) Considerations for Nonclinical Safety of Engineered T Cells

- Genotoxicity, mutagenicity associated with gene insertion into cell product
  - Gamma-retroviral
  - Lentivirus

  Vector insertion mapping
  In vitro *transformation (IL-2 independent growth)*
  In vivo *transformation*
  - Nonviral transduction (e.g., transposons)
  - Other (e.g., CRISPR editing)
  - Transduction of non–T cells
- Binder specificity
  - *In vitro* cell/tissue screening (use scFv-Fc or T [CAR±] cells)
  - Retrogenix platform (use scFv-Fc or T [CAR±] cells)
- Nonclinical (animal) model development
  - Mouse
  - NHP

(Unique) Considerations for Nonclinical Safety of Engineered T Cells

Third-generation lentiviral vectors have several built-in safety features that minimize the risk of generating replication-competent wild-type HIV-1 recombinants.

- Typically, lentiviral vectors are generated by trans-complementation whereby packaging cells are co-transfected with a plasmid containing the vector genome and the packaging constructs that encode only the proteins essential for lentiviral vector assembly and function.
- The vector contains the structural and packaging gag, pol, and rev genes from HIV-1. However, the vector lacks the necessary HIV-1 genes (Tat, Nef, Vif, Vpr, and Vpu) to result in formation of a replication competent virus.
- The lentiviral vector uses a split-genome third-generation system wherein the plasmids encoding the segments and genes required to form the viral vector are segregated onto separate plasmids:
  - The envelope glycoprotein (not derived from a lentivirus) is on one plasmid,
  - The gag and pol genes on second plasmid (derived from HIV-1),
  - The rev gene on a third plasmid (derived from HIV-1), and
  - The transfer genome encoding the transgene on a fourth plasmid (derived from HIV-1 but self-inactivating due to a deletion in the 3’ long-terminal repeat, LTR).
- The development of self-inactivating vectors markedly improves lentiviral safety.
  - Reduces the likelihood that replication competent virus will originate in the vector producer and target cells, and reduces the likelihood that cellular coding sequences located adjacent to the vector integration site will be aberrantly expressed by abolishing the intrinsic promoter/enhancer activity of the HIV-1 LTR.

Insertional Mutagenesis

The risk of insertional oncogenesis in human cells for virally transduced cells has been established in the context of *gamma retroviral vector*-based gene therapy of hematopoietic stem cells for X-linked severe combined etroviral vector insertion near the Lim domain only 2 (LMO-2) oncogene was immunodeficiency [Hacein-Bey-Abina et al., (2003) *Science* 302:415 and (2008) *J Clin Invest*. 118(9): 3132].

However, the LMO-2 oncogene is silent in T cells making this site an unlikely locus of retroviral (or lentiviral) integration [Bonifant et al., (2016) *Mol Ther Oncol* 3:16011], and mature T cells appear to be resistant to retrovirally mediated transformation as compared to hematopoietic stem cells [Newrzela (2008) *Blood* 112(6):2278].

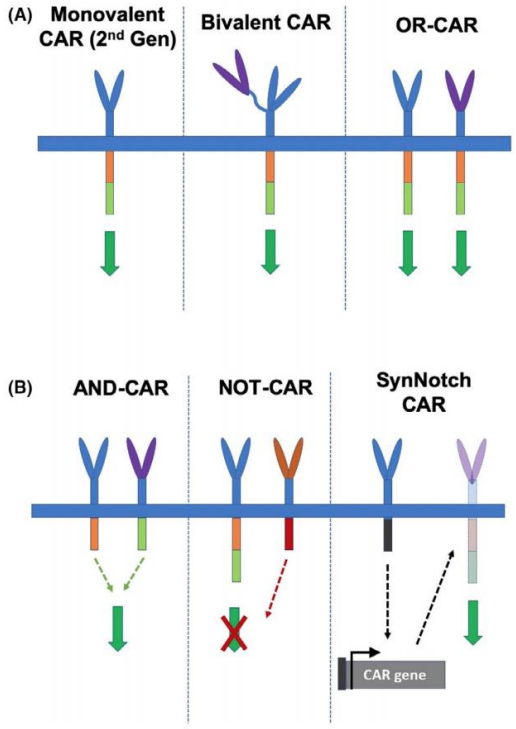
To date, no cases of malignant transformation have been reported following infusion of genetically modified T cells. A decade-long review of retroviral CAR T cell safety and function revealed no evidence of vector-induced immortalization of cells, no evidence of clonal expansion, and no enrichment for integration sites near genes implicated in growth control or transformation [Scholler et al., (2012) *Sci Transl Med*. 4(132):132ra53].

Because HIV is a prototypical lentivirus that infects human T cells, it is useful to understand insertional oncogenesis outcomes among HIV-infected humans to assess the likelihood that lentiviruses can transform T cells. A review of the general literature suggests this is theoretical risk, but not observed in HIV patients.

Engineered T Cells: A Look at the Future

- Novel cell types, novel constructs
- Address mechanisms of resistance

	Primary resistance	Acquired resistance
T cell-intrinsic	i. Insufficient breadth of TCR repertoire ii. Insufficient affinity of TCR repertoire	i. T cell functional incapacitation / exhaustion ii. T cell senescence iii. T cell apoptosis
T cell-extrinsic	i. Failure to prime cancer-specific T cell response ii. Clonal heterogeneity of cancer cells	i. Loss of heterozygosity at HLA loci ii. Transcriptional suppression of HLA class I loci iii. Mutations in antigen processing and presentation genes



Walsh et al., (2019) *Immunol Rev* 290:100
Chandran and Klebanoff (2019) *Immunol Rev* 290:127

Conclusions

- Engineered T cells have arrived as a potent therapeutic modality to treat hematological malignancies
  - Effects in solid tumors ongoing
- Experience to date has revealed "class-specific" toxicities, and classification/treatment paradigms are emerging to identify and manage these effects
- The field is emerging with generation of novel synthetic receptor/cell-type constructs
- Modality- and target-related safety assessment tools will need to evolve to allow prediction and mechanistic understanding of emergent toxicity

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Preclinical Safety Assessment of CAR and TCR T Cell Therapies

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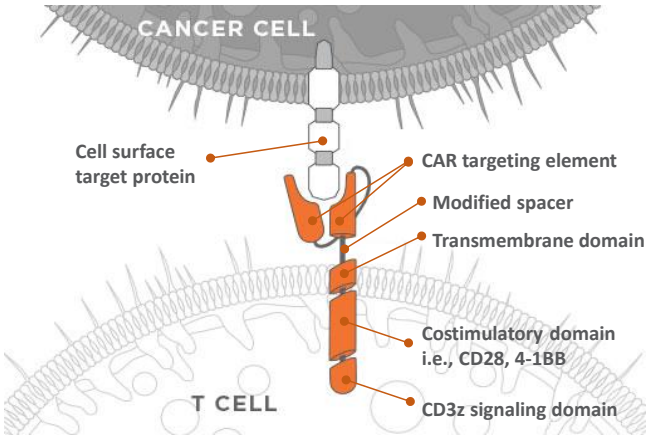
Conflict of Interest Statement

Thomas Long is an employee and equity shareholder of Bristol-Myers Squibb

Abbreviations

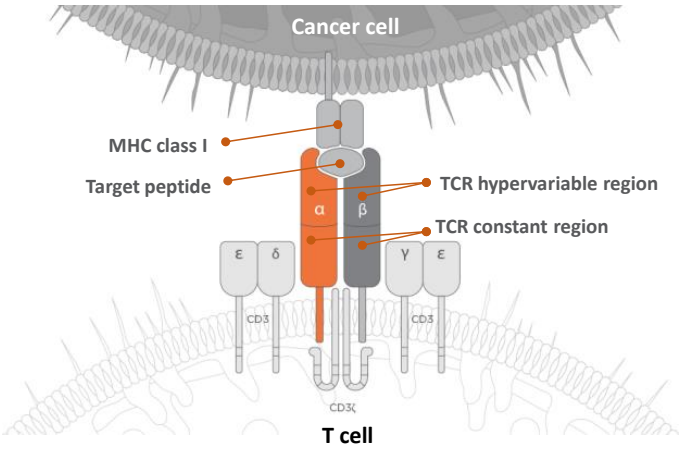
- APC: antigen-presenting cell
- BCMA: B cell maturation antigen
- BM: bone marrow
- CAIX: carbonic anhydrase IX
- CAR: chimeric antigen receptor
- CBC: complete blood count
- CD19: cluster of differentiation 19
- CRS: cytokine release syndrome
- CSF: cerebrospinal fluid
- GI: gastrointestinal
- GvHD: graft versus host disease
- HER2: human epidermal growth factor receptor 2
- HLA: human leukocyte antigen
- iPSC: induced pluripotent stem cell
- LN: lymph node
- MAGE A: melanoma-associated antigen
- MHC: major histocompatibility complex
- NHP: nonhuman primate
- NT: neurotoxicity
- PK: pharmacokinetics
- ROR1: receptor tyrosine kinase-like orphan receptor 1
- scFv: single chain variable fragment
- tAPC: T cell antigen-presenting cell
- TCR: T cell receptor
- TLA: target liability assessment
- US FDA: US Food and Drug Administration

CAR T Cells



- CAR recognizes cell-surface protein on cancer cells
- Targeting element is often a single chain variable fragment (scFv)
- Demonstrated clinical efficacy for CD19-directed CAR T cells in B cell malignancies
- Two US FDA–approved products (Kymriah™ and Yescarta™)

TCR T Cells



- TCR recognizes target peptide presented on MHC class I
- Utilizes endogenous TCR signaling
- Can target peptides derived from intracellular or cell-surface proteins
- Most often applied to solid tumors
- HLA-restricted therapy

Safety of Engineered T Cells

- Platform risks
  - Cytokine release syndrome (CRS)
  - Neurotoxicity (NT)
  - Lentiviral insertion-mediated oncogenesis
- Binder-mediated liabilities (scFv or TCR)
  - Specificity: Can we predict potential off-target activity?
- Target-mediated liabilities (CAR target or MHC-peptide)
  - Cancer selectivity: Can we predict potential on-target, off-tumor activity?

Clinical Examples of Target and Binder-Associated Toxicities with Engineered T Cells

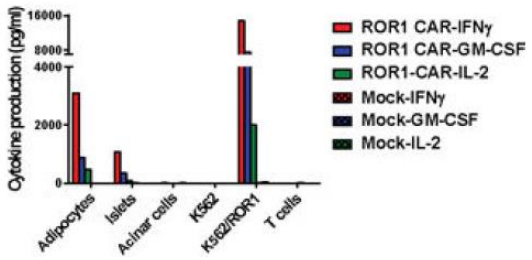
Target/Modality	Notes	References
Carbonic anhydrase IX (CAIX) CAR T cells	• CAIX expressed on bile duct epithelium • CAR T cells cased transient elevations in liver enzymes	Lamers et al., *J Clin Oncol* (2006) 24;e20–e22
HER2 CAR T cells	• HER2 expressed in the lung; CAR T cells caused fatal pulmonary toxicity • HER2 CAR T cells have also been delivered safely indicating importance of binder and clinical protocols	Morgan et al., *Mol Ther* (2010) 18;843–851 Ahmed et al., *J Clin Oncol* (2015) 33;1688–96
MAGE A3 TCR T cells	• TCR recognized MAGE A family proteins in the brain leading to fatal encephalopathy • A different TCR against the same target cross-reacted with Titin peptide on cardiomyocytes and caused fatal cardiac toxicity	Morgan et al., *J Immunother* (2013) 36;133–151 Cameron et al., *Sci Trans Med* (2013) 5;197ra103

CAR T Target Liability Assessment (TLA)

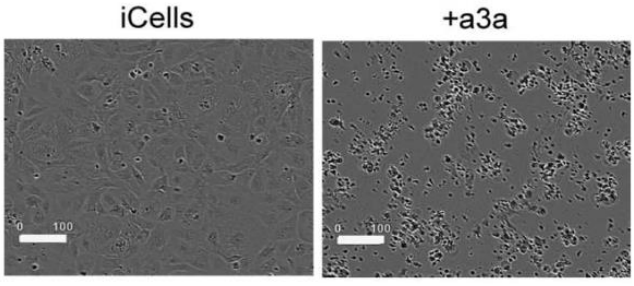
- Serves as a risk assessment for a potential CAR target based on available literature reports, expression databases, and preliminary internal data
- Used to formulate de-risking/toxicology strategy for on-target liabilities
- Components
  - Target structure and homology (extracellular domain)
    - Are there similar proteins with potential for off-target cross-reactivity?
  - Target normal function and function in disease
  - Target expression and tissue distribution
    - Cancer versus normal
    - RNA and protein
    - Similarity in expression profile between species (i.e., mouse, nonhuman primate)
  - Nonclinical and clinical safety data from all groups targeting same protein
    - Care should be taken to distinguish general target risks from modality-specific risks

In Vitro Approaches for Predicting Engineered T Cell Activity

- Normal human primary cells and/or tissues can be co-cultured with engineered T cells to understand potential on- or off-target risks, but caution is warranted when interpreting results.
- Because CARs and TCRs generally require low antigen counts to stimulate function, these assays are very sensitive to target expression.
- Example 1 (top): *In vitro* assays can overpredict risks. ROR1-CAR T cells secrete cytokines against adipocytes and pancreatic islets, which are ROR1+, but no clinical on-target toxicities were observed.
- Example 2 (bottom): *In vitro* assays can predict risk with the right model system. TCR a3a T cells kill iPSC-derived cardiomyocytes expressing titin peptide, but do not kill primary cardiomyocytes under standard culture conditions because titin peptide is not expressed.



Balakrishnan et al., *Clin Cancer Res* (2017) 23;3061–71



Cameron et al., *Sci Trans Med* (2013) 5;197ra103

In Vivo Toolkit for CAR T Cell Toxicology Studies

Species	Target cells	Effector cells	Advantages	Disadvantages
Immuno-compromised mice	Human tumor xenograft	Human CAR T cells	• Typical CAR T pharmacology model • Activation and expansion of CAR T cells against target-expressing human tumor • Simple CAR T functional readout: PK, tumor burden, survival • Uses human T cell product	• Requires CAR cross-reactivity with murine target and similar target expression profile between human and mouse • Human T cells can have xeno-reactivity to mouse tissues (GvHD) that can influence pathology • Human T cell donor can influence activity

Syngeneic mice	Mouse target-expressing tumor	Mouse CAR T cells	• No human T cell xeno-reactivity to mouse tissues
			• Simple CAR T functional readout: PK, tumor burden, survival
			• Mouse strain-derived T cells—no donor dependency
				• Requires CAR cross-reactivity with murine target and similar target expression profile between human and mouse
				• Mouse T cell surrogate
				  • Differences in manufacturing with human process
				  • Potential differences in activity compared with human product
				  • Mouse strain-dependent CRS

In Vivo Toolkit for CAR T Cell Toxicology Studies

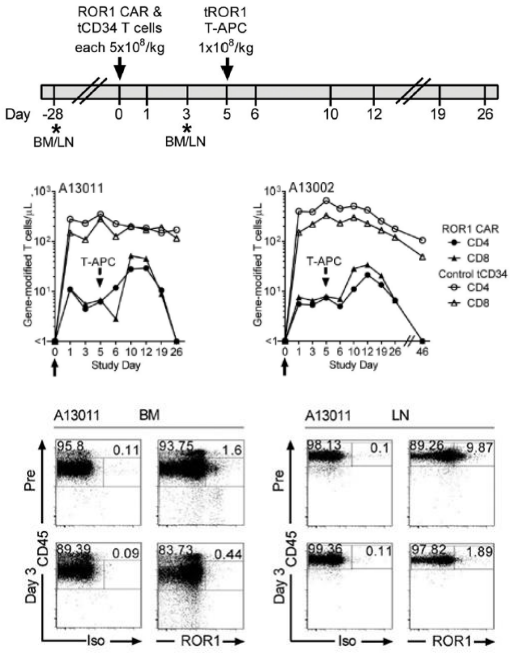
Species	Target cells	Effector cells	Advantages	Disadvantages

Nonhuman primate (NHP)	No on-board tumor; can engineer autologous antigen-presenting cells	Autologous NHP CAR T cells	• Closer approximation to human than mouse
			  • Target expression and homology are more likely to be conserved
			  • CAR cross-reactivity is more likely
			• NHP is immune-competent
			• Can use relevant lymphodepletion regimen
				• Requires CAR cross-reactivity with NHP target and similar target expression profile between human and NHP
				• NHP T cell surrogate
				  • Differences in manufacturing compared with human process
				  • Potential differences in activity compared with human product
				  • Autologous test article—NHP T cell donor can influence activity
				• Likely requires external contract research organization
				• Expensive

Note: no *in vivo* toxicology studies can be performed for TCR T cells in any species because of the species-specific nature of the target, MHC complexes, and the proteome they present

Example: Nonhuman Primate ROR1 CAR T Toxicology Study

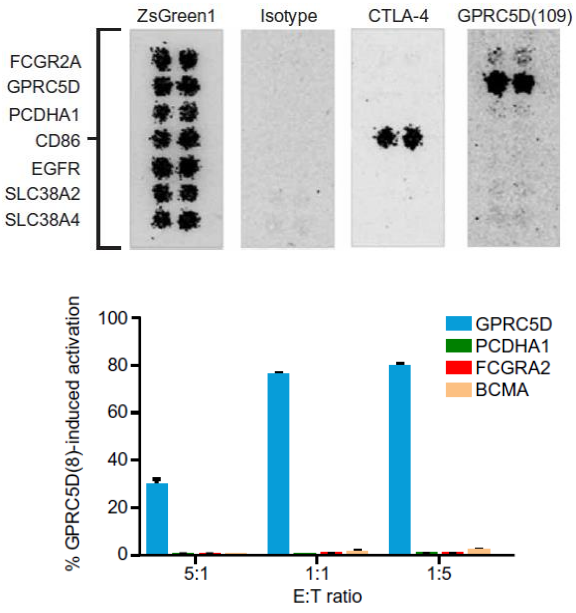
- ROR1 is homologous and expression is largely conserved between human and NHP; off-tumor risks include pancreas, parathyroid, and GI tract
- High-dose (500 million cells/kg) ROR1 CAR T cells delivered to Rhesus macaques along with T cell antigen-presenting cells (tAPC) to stimulate CAR T expansion
- ROR1+ population in the BM and LN reduced along with expansion in peripheral blood showing CAR T functionality
- No clinical symptoms or changes in CBC/serum chemistry indicating off-tumor toxicity



Berger et al., *Cancer Immunol Res* (2015) 3;206–16

Off-Target Assessment of CAR T Cells

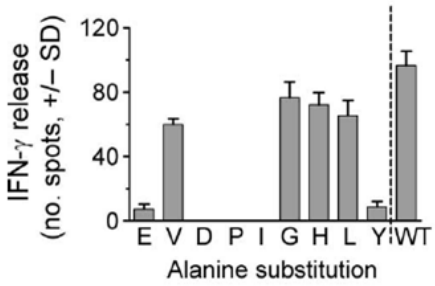
- Tissue cross-reactivity studies: traditional GLP IHC assay measuring scFv-Fc binding to a frozen human tissue microarray
  - Caution: scFv-Fc can make a poor IHC reagent and tissue cross-reactivity studies often yield false positives
  - Can be useful if you want to query an on-target tissue concern
- Retrogenix plasma membrane protein array: can identify specific potential off-target binding against >4500 extracellular proteins
  - Amenable to screen scFv-Fc or whole CAR T binding
  - Need to follow up any hits with CAR T functional assay



Smith et al., *Sci Trans Med* (2019) 11;eaau7746

Off-Target Assessment of TCR T Cells

- Central tolerance selects for TCRs without strong cross-reactivity to self MHC/peptide complexes
- Mutating TCR sequences to increase target affinity may result in increased risk for off-target cross-reactivity
- TCRs naturally have some level of promiscuity to provide immunity
- Peptide scan can generate a TCR binding motif for a given MHC; involves querying TCR function against APCs pulsed with peptide library where amino acids at every position along the target peptide are replaced with other amino acids
- Allopanel can inform on MHC-specific cross-reactivity risks for patient exclusion criteria; assay involves querying TCR function against panel of HLA-typed cells with population-level HLA representation

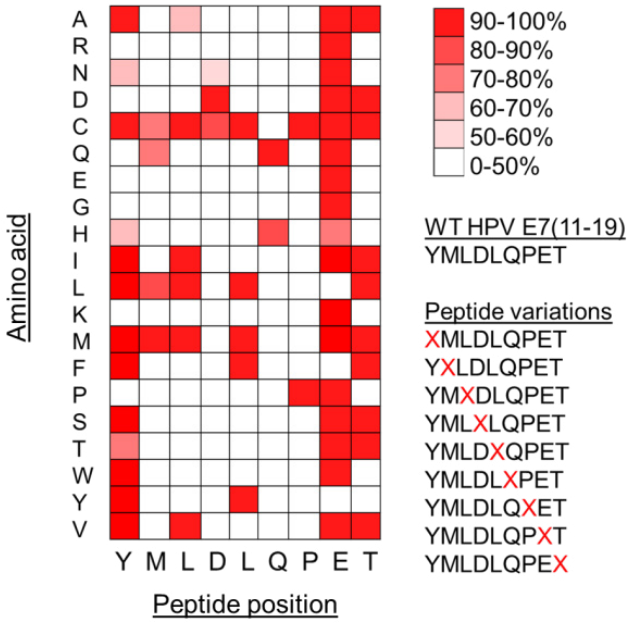


Cameron et al., *Sci Trans Med* (2013) 5;197ra103

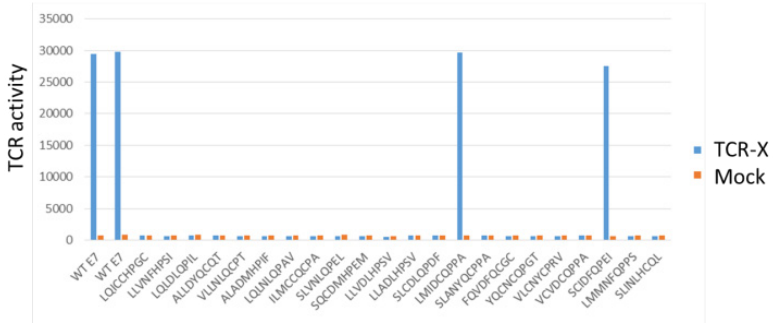
Example: alanine scan identifies TCR motif that captures titin peptide, which led to off-target toxicity

MAGE A3 target peptide: EVDPIGHLY
Titin peptide: ESDPIVAQY

Off-Target Assessment of TCR T Cells



- Peptide scan identified TCR-X binding motif (left)
- Genome-wide screening indicated 22 potential off-target peptides containing that motif
- Functional analysis showed TCR activity against two off-target peptides when pulsed onto APCs (bottom)
- Follow-up studies indicated no T cell activity against cells endogenously expressing or over-expressing off-target proteins despite low-level peptide presentation (not shown)



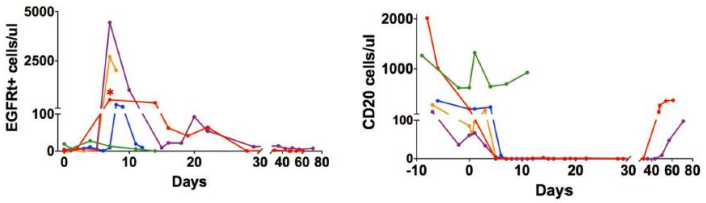
Long et al., Keystone Emerging Cellular Therapies (2020) Abstract 2021

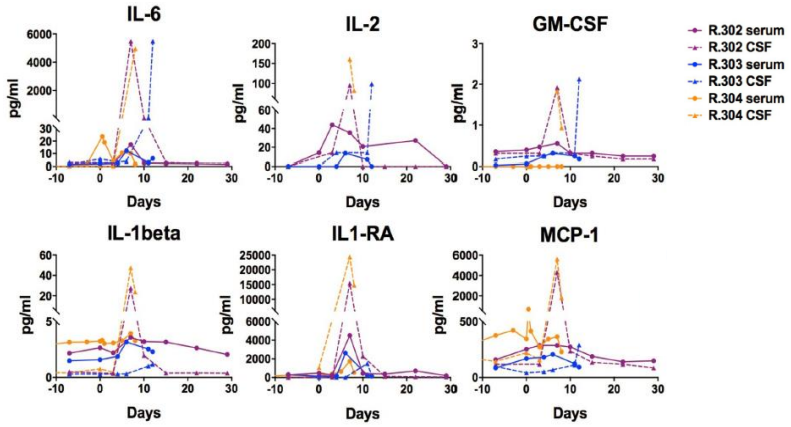
Preclinical Models of CRS and Neurotoxicity

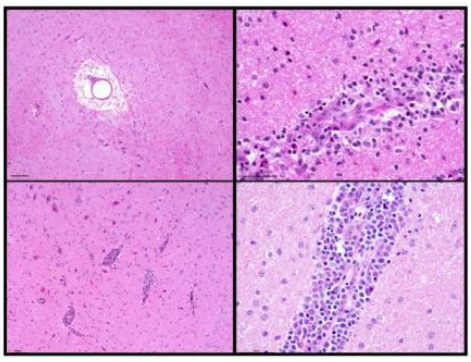
- CRS and neurotoxicity are platform risks of engineered T cell therapies independent of target
  - Have been observed for both CD19 and BCMA-directed CAR T cells
- In general, preclinical models are not used to predict these risks or inform on clinical dose selection
- CRS and NT are hallmark responses observed with other immune-modulating agents
  - Etiology of neurotoxicity is not fully understood
  - Animal models are in development to refine mechanistic understanding and clinical management strategies

Preclinical Models of CRS and Neurotoxicity

- Anti-CD20 CAR T cells expand in Rhesus macaques and produce B cell aplasia
- Cytokines upregulated in blood and CSF
- Pathology shows perivascular inflammation
- Neurotoxicity symptoms observed: lethargy, tremor, ataxia, seizure







Taraseviciute et al., *Cancer Discov* (2018) 8;750–63

Summary and Conclusion

- Preclinical safety assessment of engineered T cell therapies provides unique challenges
- Assembling IND-enabling toxicology packages for CAR and TCR T cell modalities requires careful consideration of on-target/off-tumor and off-target liabilities
- No preclinical model system fully recapitulates the clinical situation
- It is critical to understand the utility and limitations of the available preclinical models to make risk/benefit decisions for novel engineered T cell therapy programs

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- Ahmed et al., *J Clin Oncol* (2015) 33;1688–96.
- Balakrishnan et al., *Clin Cancer Res* (2017) 23;3061–71.
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- Cameron et al., *Sci Trans Med* (2013) 5;197ra103.
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- Morgan et al., *Mol Ther* (2010) 18;843–851.
- Smith et al., *Sci Trans Med* (2019) 11;eaau7746.
- Taraseviciute et al., *Cancer Discov* (2018) 8;750–63.

Getting the Most Out of Your Nonclinical Safety Studies for Antibody-Based CD3 Redirectors to Inform Deselection or Enable First-in-Human Clinical Trials

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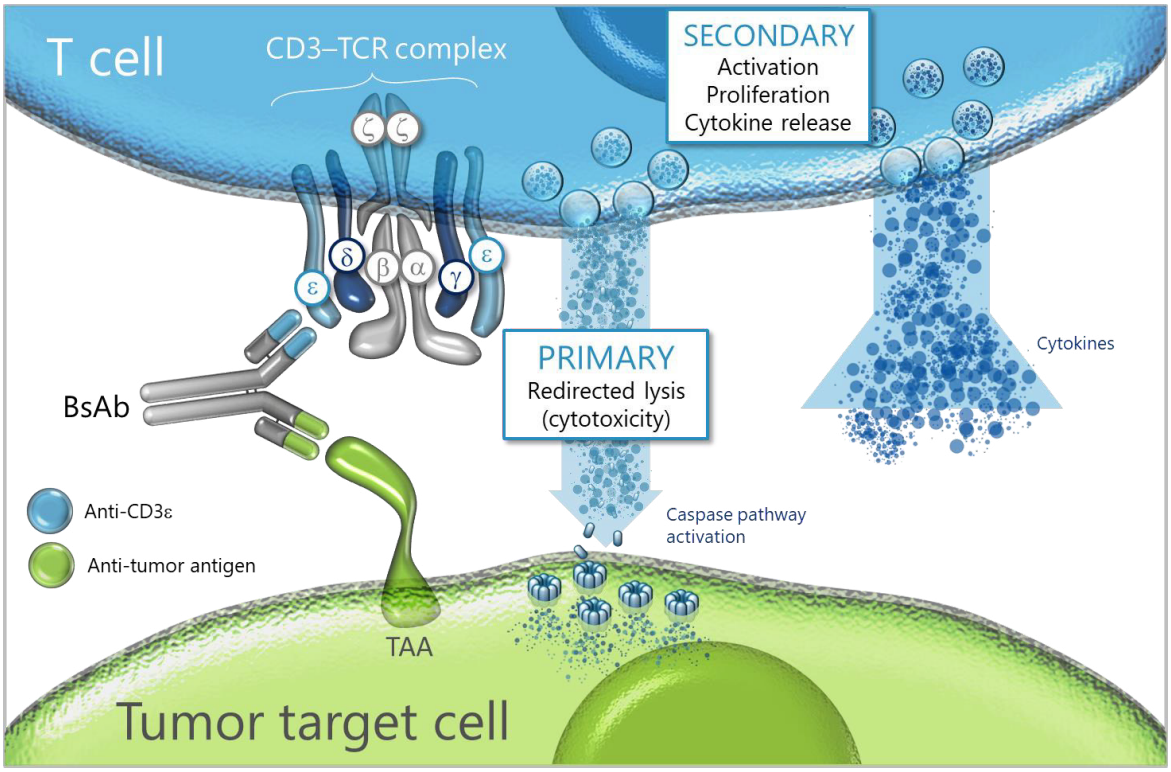
Conflict of Interest Statement

Jacintha Shenton is a paid employee of Janssen Research and Development, a pharmaceutical company of Johnson & Johnson.

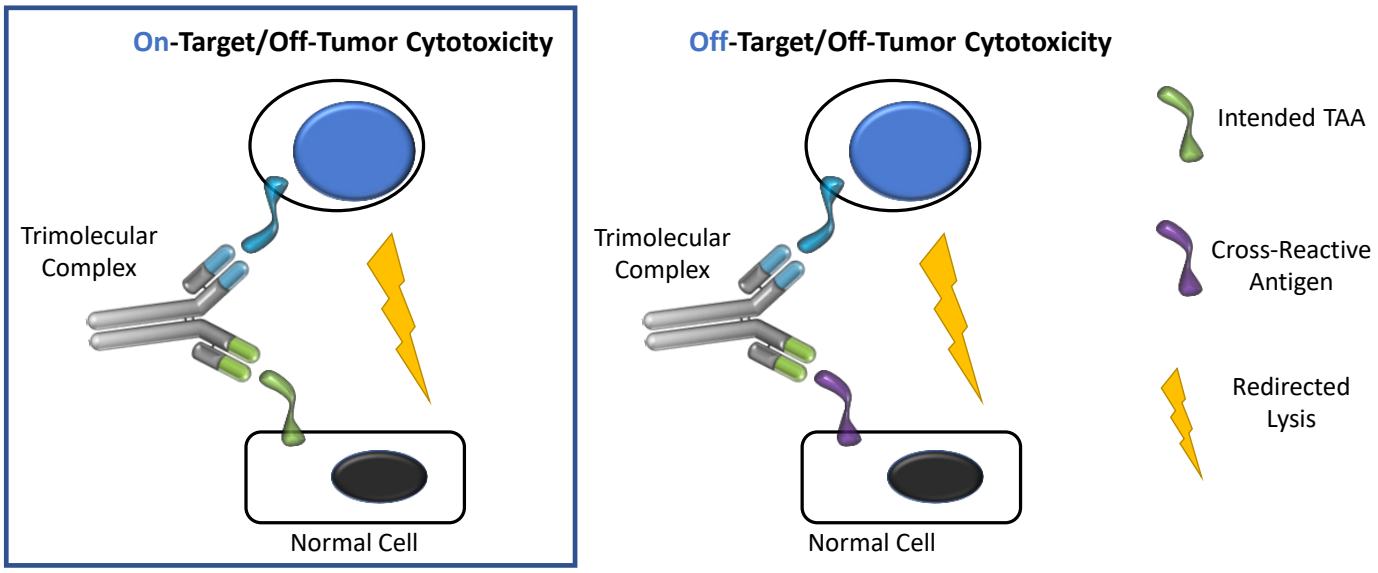
Abbreviations

- Ang2: Angiopoetin 2
- BLAST: basic local alignment search tool
- CHO: Chinese Hamster ovary
- CRS: cytokine release syndrome
- DRF: dose-range finding
- FIH: first-in-human
- GFAP: glial fibrillary acidic protein
- GLP: good laboratory practice
- H&E: Hematoxylin & Eosin
- HNSTD: highest non-severely toxic dose
- IBA1: ionized calcium binding adaptor molecule 1
- IND: Investigational New Drug
- MABEL: Minimal Anticipated Biological Effect Level
- MoA: mechanism of action
- MTD: maximum tolerated dose
- NME: new molecular entity
- NOAEL: No-Observed-Adverse-Effect-Level
- PD: pharmacodynamics
- RNA-seq: RNA sequencing
- TAA: tumor-associated antigen
- vWF: von Willebrand Factor



CD3 = cluster of differentiation 3; TCR = T cell receptor; BsAb = bispecific antibody; TAA = Tumor-Associated Antigen

MoA-Based Liability: Off-Tumor Cytotoxicity



Target-related liabilities are dependent on which normal cells express the intended TAA

Target Liability Assessment

TAA normal tissue versus target-tumor tissue expression profile (e.g., RNA-seq databases, immunohistochemistry)

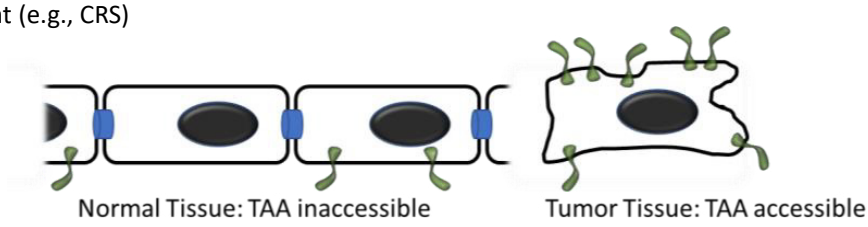
- TAA restricted to tumor
- TAA inaccessible on normal tissues
- TAA expression on nonvital cells/tissues (e.g., B cells)
- TAA expression on tissues with regenerative capabilities
- TAA expression on nonregenerative or vital cells/tissues
- TAA upregulated during other disease processes (e.g., comorbidities) and/or in a pro-inflammatory environment (e.g., CRS)

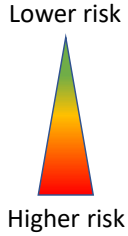
Nonclinical and clinical precedence

- Internal or publicly available data describing safety profile

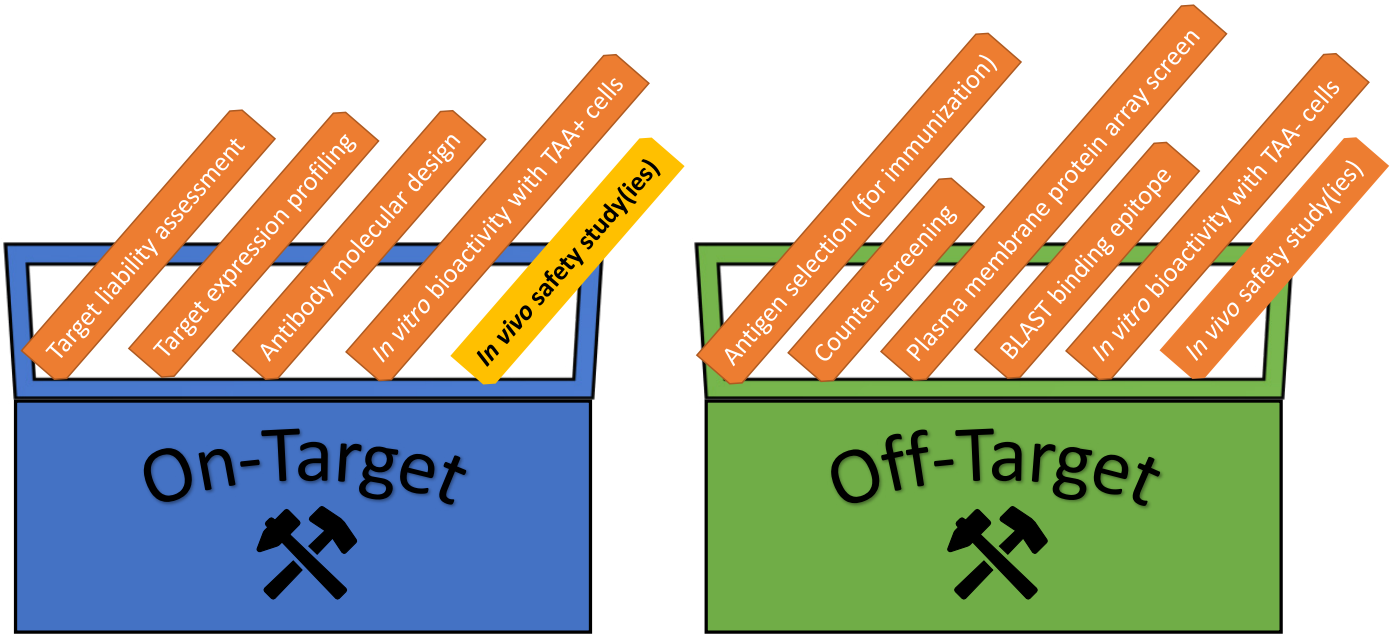
TAA biology

- Soluble or shed TAA can compete for CD3 bispecific (sink); impact steepness of dose-response
- Potential impact of agonism or antagonism (phenotype of knockout or transgenic mice)



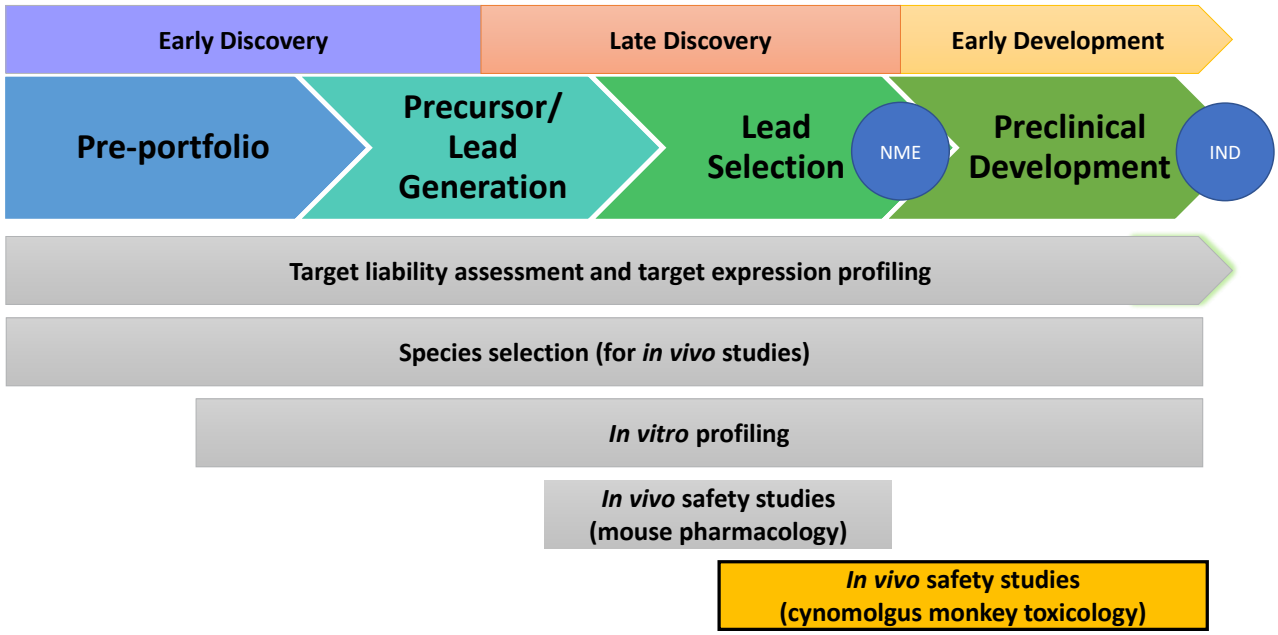


Toolboxes for Addressing Liabilities

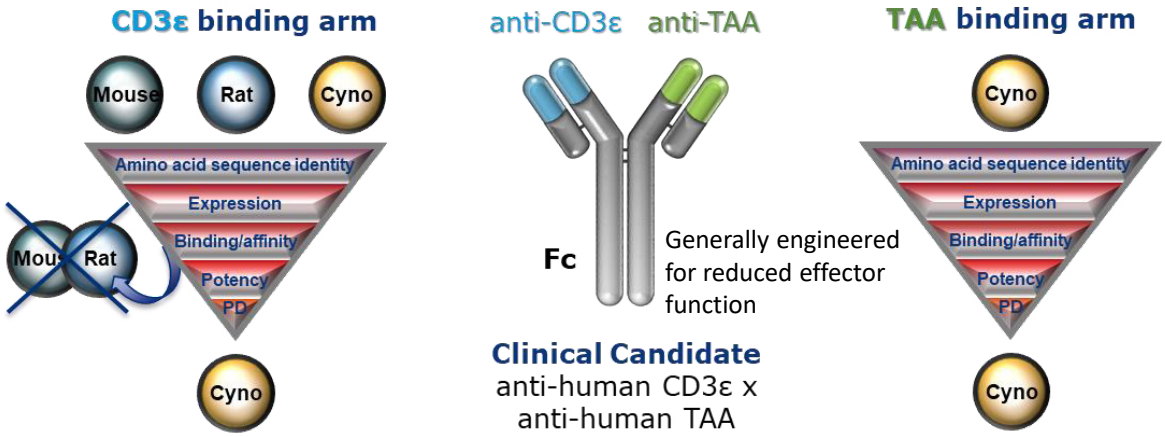


Bioactivity = cytotoxicity, T cell activation, cytokine release

Timing of Nonclinical Safety Assessments



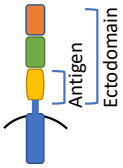
Why Cynomolgus Monkey?
αCD3 Binder Only Binds Human and Cyno CD3



Early CD3 bispecifics (e.g., blinatumomab) did not bind cyno CD3ε; used chimp and/or mouse cross-reactive tool CD3 bispecific for *in vivo* safety assessment

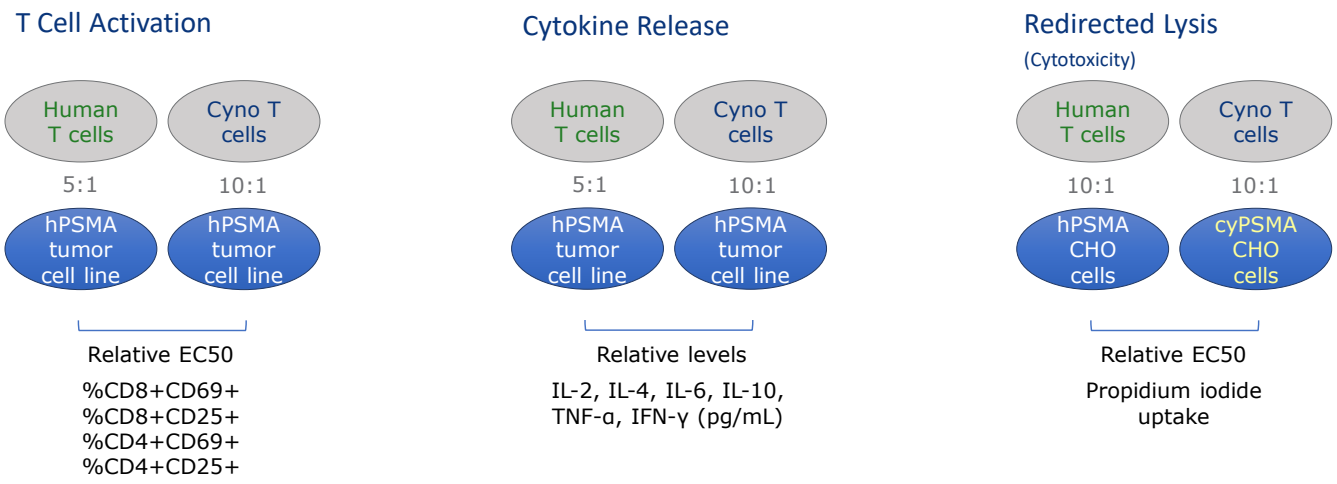
de Haan et al., (2011) *European Biopharmaceutical Review*. 12(155)

Data Package to Evaluate Species Relevance for Human Risk Assessment



Data Packet	Rationale	Timing
Amino acid sequence identity of animal versus human TAA (ectodomain or targeted domain; if known)	Higher % sequence identity is the first indication of whether αhuman antibodies may bind animal ortholog	Pre-portfolio; further refinement once amino acid sequence of αTAA antibody binding epitope is characterized
TAA expression in animal and human tissues by immuno-histochemistry or other method(s)	Critical to evaluate species relevance for human on-target/off-tumor cytotoxicity	Pre-portfolio and/or Precursor/Lead Generation; prior to designing *in vivo* studies
Binding/binding affinity of lead antibodies to animal ortholog relative to human	Antibody needs to bind to the animal ortholog and ideally with similar binding affinity to human TAA	Lead selection; once a series of monospecific leads is available
Ex vivo cell-based functional assay with animal versus human	To demonstrate that human T cells can be redirected to animal ortholog	Lead selection; once pharmacologically active (*in vitro*) bispecific leads are available

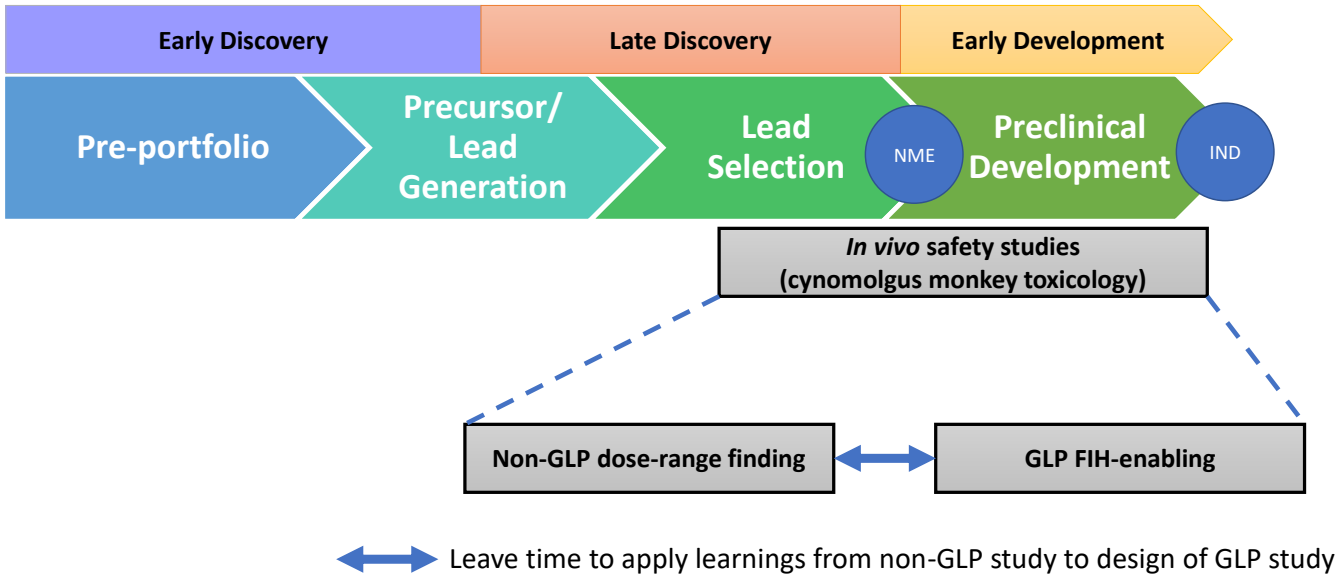
Human/Cynomolgus Monkey Cross-Reactivity of AMG212 (BAY2010112; PSMAxCD3 BiTE)



Bioactivity was not observed in the absence of PSMA-expressing target cells

Friedrich et al., (2012)

Timing of Cynomolgus Monkey Toxicology



Non-GLP DRF Study Objectives

Primary Objectives:

- Evaluate overall tolerability; guide dose setting for GLP study
- Explore effects on normal TAA+ cells and tissues
- Evaluate PK profile*

Secondary Objectives:

- Explore potential PD effects

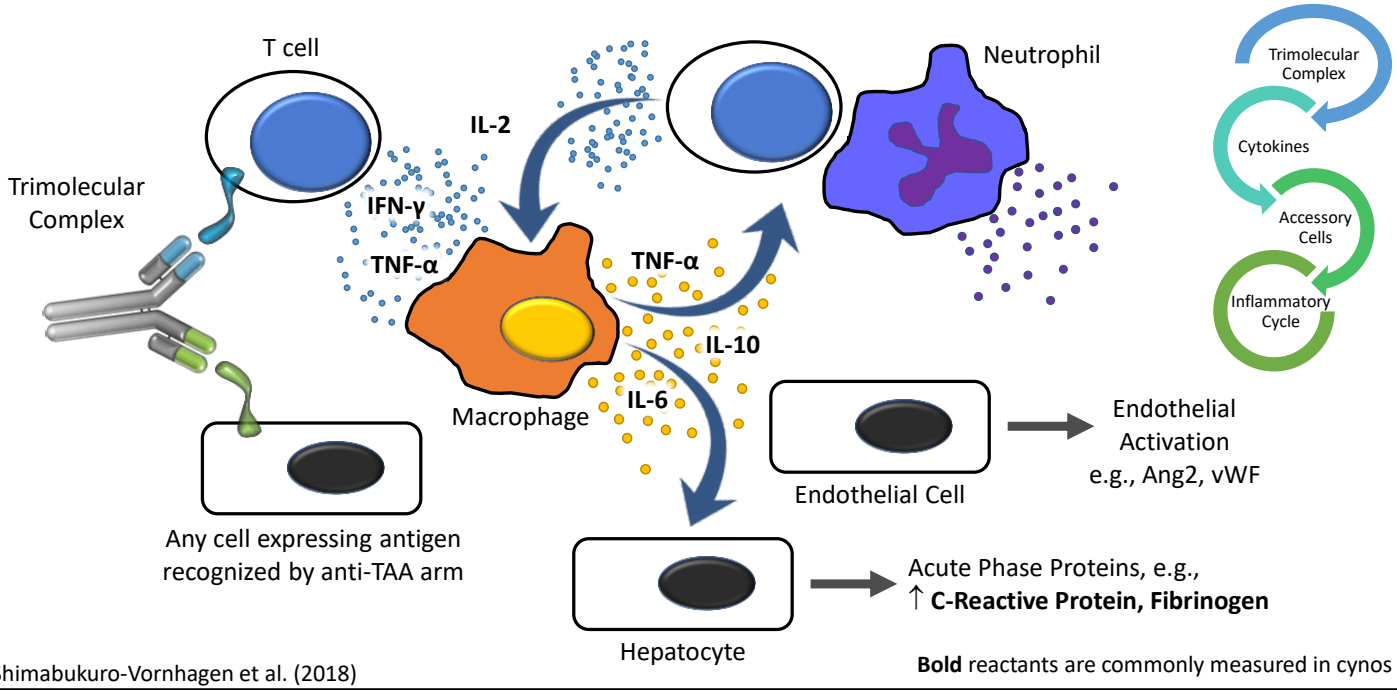
Criteria for Evaluation:

- **Standard clinical assessments**
- **Laboratory investigations:**
  - Standard: clinical pathology, PK
  - Specialized: circulating cytokines, possibly peripheral blood immunophenotyping
- **Terminal investigations:**
  - Organ weights, gross pathology
  - Microscopic pathology (often limited to gross findings, critical organs, and TAA+ tissues)

Additional assessments considered on a case-by-case basis
(e.g., functional observational battery if TAA expressed in brain tissues)

*Suggest conduct of dose-range finding study prior to any potential stand-alone PK/PD study in cynomolgus monkeys due to risk of exceeding maximum tolerated dose

MoA-Based Liability: Cytokine Release Syndrome



Shimabukuro-Vornhagen et al. (2018)

Bold reactants are commonly measured in cynos

Non-GLP DRF Study Potential Design

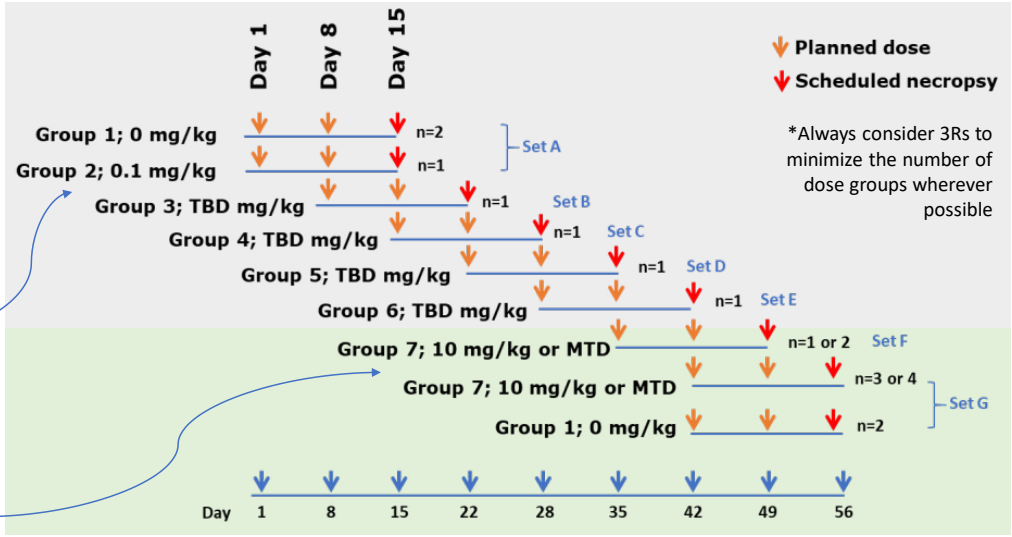
Antibody-Based CD3 Bispecific

Design Notes:

- ❑ Dose on Monday or Tuesday
- ❑ IV bolus dosing

Starting dose informed by cyno *in vitro* bioactivity data, data from other CD3 bispecifics against the same TAA

Top dose (for test article requirements) estimated to saturate CD3



TBD = to be determined by amendment based on survival, clinical signs, and clinical pathology findings at the prior dose level

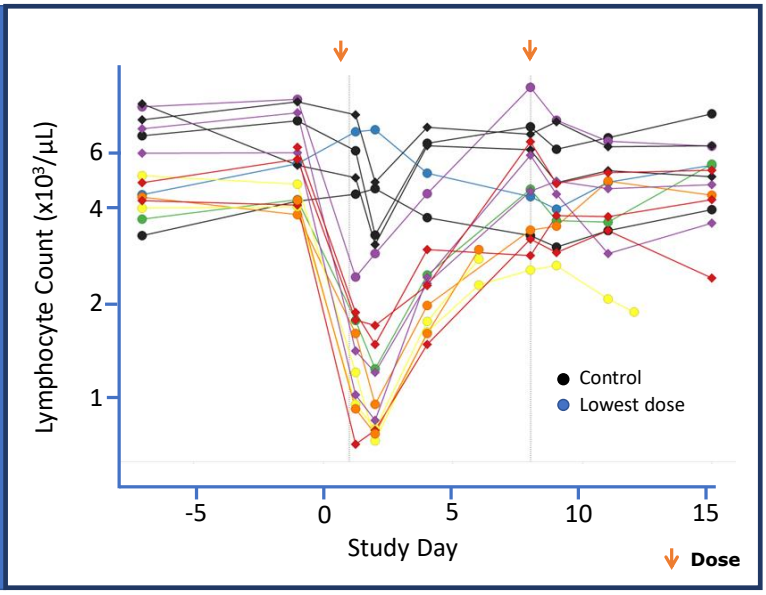
Dose Escalation Decision-Making: Evidence of Lymphocyte Redistribution

Lowest Dose

- ➢ **Clinical signs:** none
- ➢ **Clinical pathology:** no changes

Next Dose

- ➢ **Clinical signs:** none
- ➢ **Clinical pathology:** moderate ↓ lymphocytes, minimal ↑ CRP



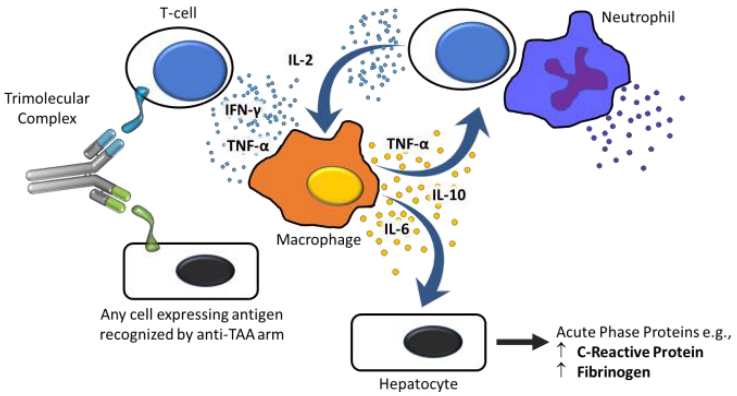
Lowest dose not active—no evidence of redistribution—continue to dose escalate

Dose Escalation Decision-Making: Evidence of Cytokine Release

Clinical signs: Emesis, diarrhea, lethargy, hunched posture, lateral recumbency, erythema (face, body), changes in body temperature, tremor.

Clinical pathology: Acute phase response evident (e.g., ↑ CRP and fibrinogen) with greatest magnitude after the first dose. ↑ APTT and/or PT.

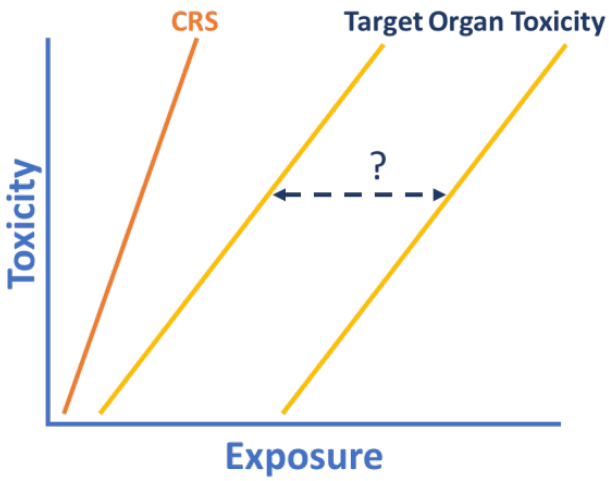
First Dose Effects

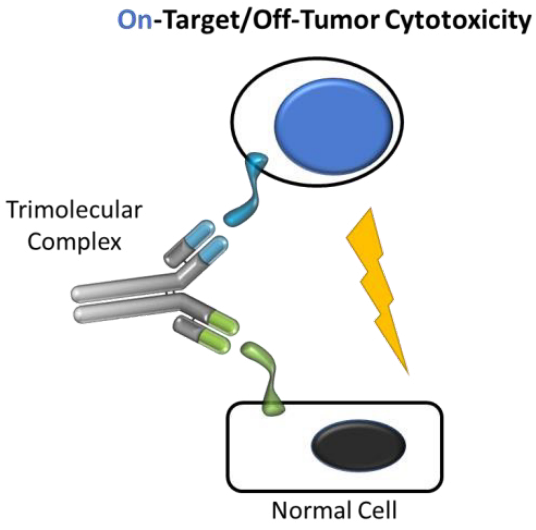


Circulating cytokines: ↑ IL-2, IL-6, IL-10, TNF-α, IFN-γ generally of peak magnitude within first 24 hours post-dose and lower magnitude with subsequent doses (except IL-10)

Severity of clinical signs and clinical pathology changes informs next dose-level

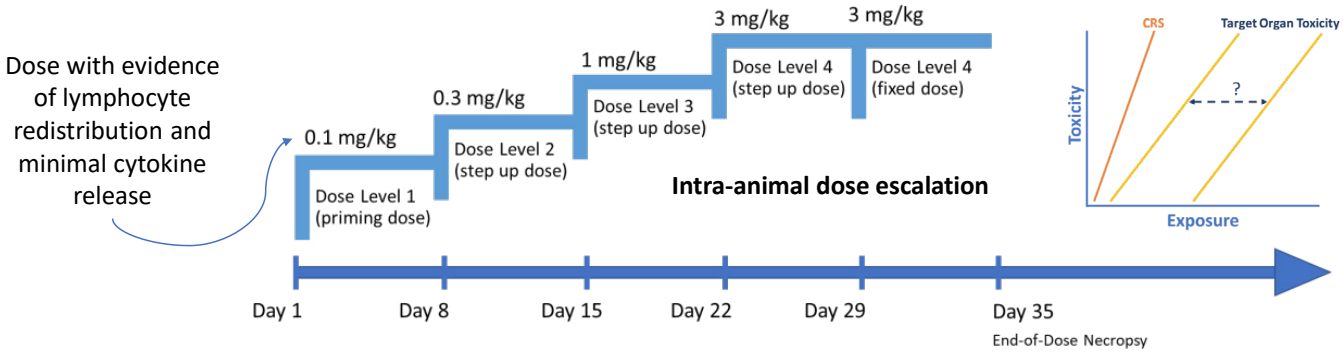
Cytokine Release Can Be Dose-Limiting in Toxicity Studies





Adaptive Study Designs

Consider step-up dosing if findings suggest dose escalation is limited by cytokine release (challenge: clearing anti-drug antibodies)



Chichili et al., (2019)

Histopathologic Findings

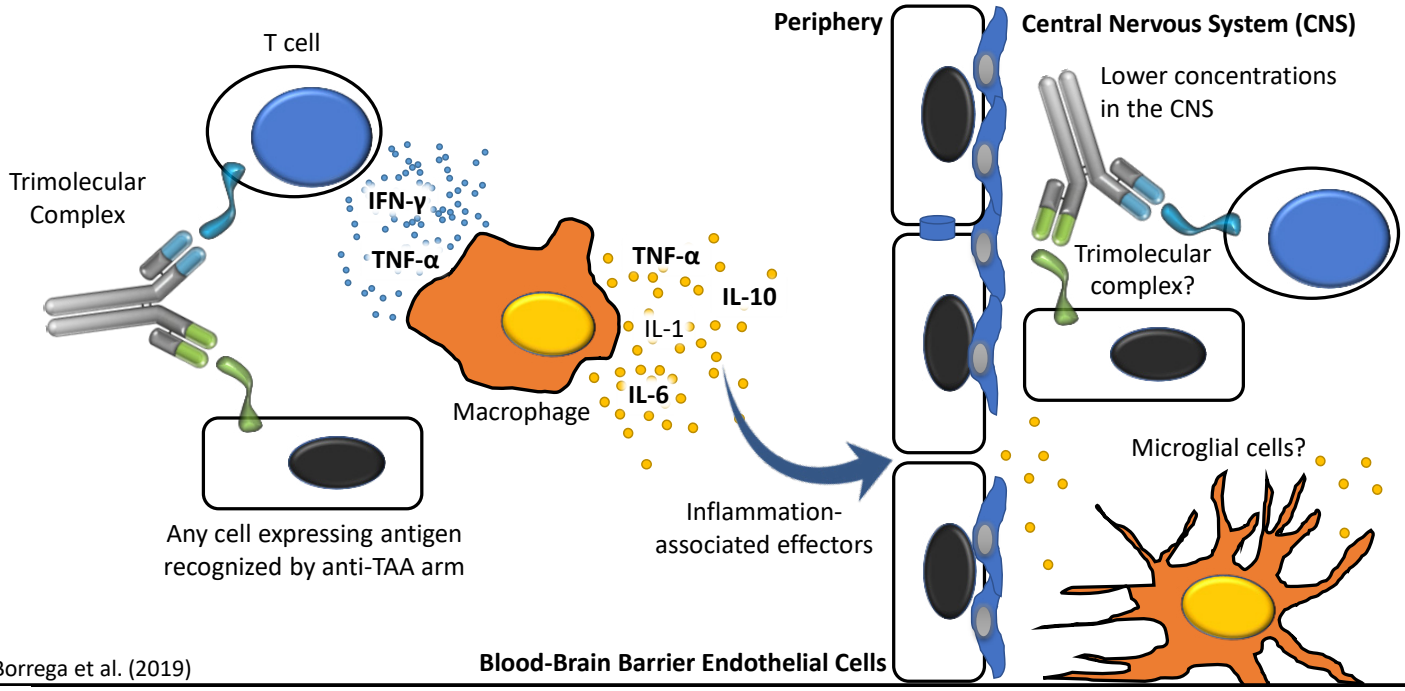
- Multi-organ mononuclear cell infiltrates in various tissues consistent with an inflammatory state
- Degeneration may occur in tissues, which may be coincident with TAA expression
- Based on 10 INDs examined, Saber et al. (2017) also reported observations of:
  - GI toxicities (e.g., epithelial degeneration)
  - Hepatotoxicity (potentially secondary to inflammation)
  - Neurotoxicity (axonal degeneration in the spinal cord and sciatic nerve; vasculitis in the CNS characterized by mononuclear cell infiltrates, and accompanied in some animals by minimal microgliosis)

GLP Study Design

- Design dependent on learnings from non-GLP study(ies)
- Generally 1 month in duration; evidence of clearing anti-drug antibodies* may result in shorter duration (e.g., 2 weeks)
- May include fixed dose level(s) and step-up dosing
- Larger number of animals than in non-GLP study
- Include safety pharmacology, ophthalmology
- Include full tissue list for histopathology
- Informs HNSTD or NOAEL
- May inform on clinical dose-escalation strategy and clinical monitoring plan

*FIH-enabling study could be the registrational study if immunogenicity impacts exposure

MoA-Based Liability: Neurotoxicity



Borrega et al. (2019)

Addressing Neurotoxicity in Nonclinical Studies

When TAA is expressed in brain, additional assessments can be conducted

Functional observational battery

Drug levels in brain

Special processing of brain—intravascular perfusion

More intense sectioning of brain

Histologic Stains

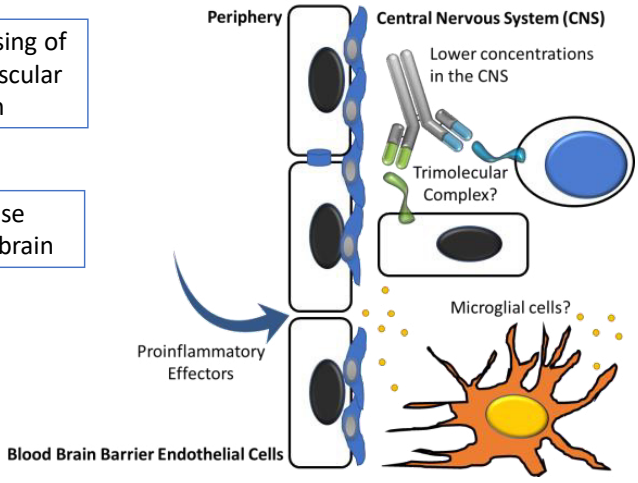
H&E (for general morphology)

Fluoro Jade B (for neuronal necrosis)

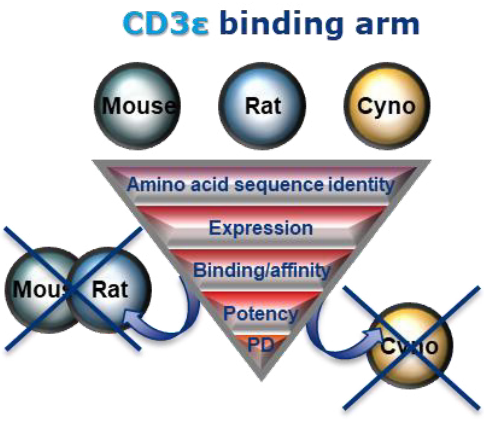
Immunohistochemistry

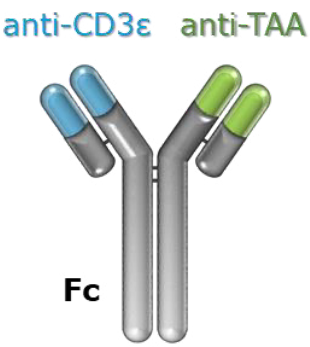
IBA1 (microglial activation)

GFAP (astrocyte activation)

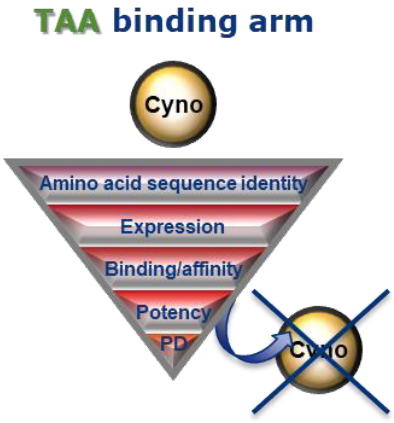


What if Cynomolgus Monkey Is Not a Relevant Species?





Clinical Candidate
anti-human CD3ε x anti-human TAA



Leverage a Tool Molecule Cross-Reactive to Cynomolgus Monkey

Clinical Candidate Is Pharmacologically Active in Cyno	**Traditional Toxicity Study** ❖ May inform on potential target organ toxicity due to normal tissue TAA expression* ❖ May inform on potential for cytokine release ❖ May inform on potential off-tumor/off-target toxicity ❖ Can be used to determine HNSTD and/or NOAEL
Tool Molecule Is Pharmacologically Active in Cyno	**Hazard Identification Study Only** ❖ May inform on potential target organ toxicity due to normal tissue TAA expression* ❖ Does not inform on potential for cytokine release ❖ May inform on potential off-tumor/off-target toxicity if clinical candidate anti-TAA binder is tested ❖ Cannot be used to determine HNSTD or NOAEL

*Can inform go/no-go decisions and/or clinical monitoring

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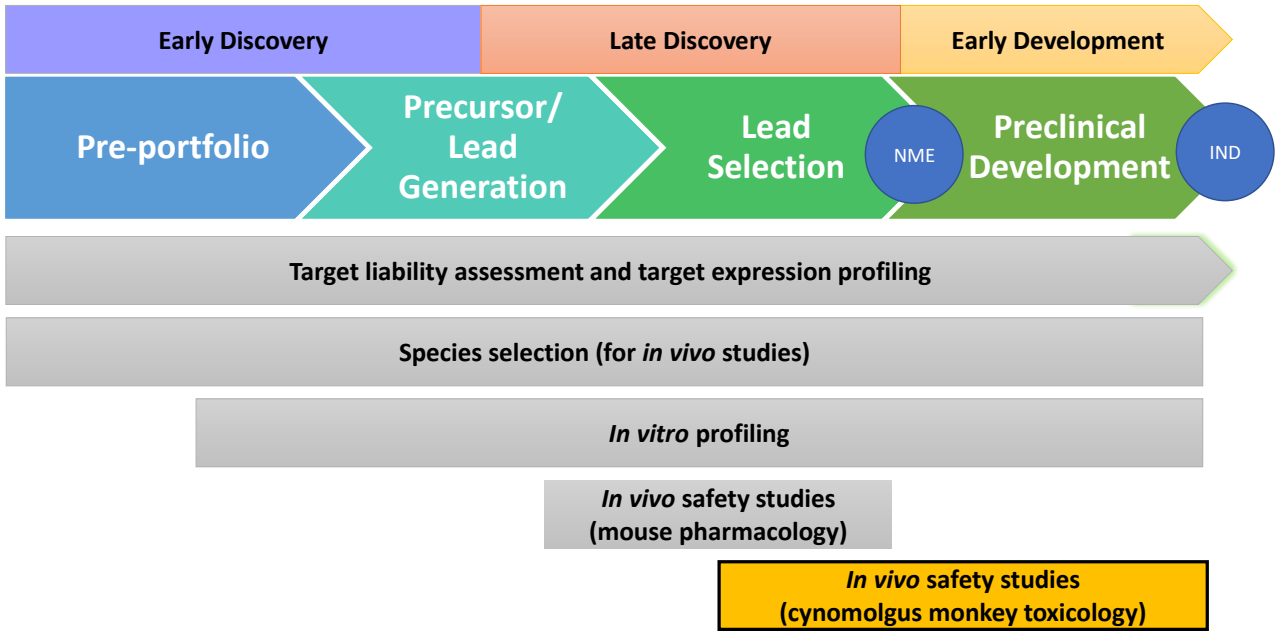
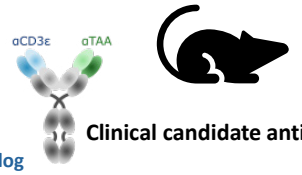
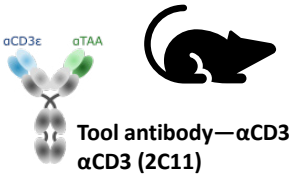
Leverage Mouse Pharmacology Studies



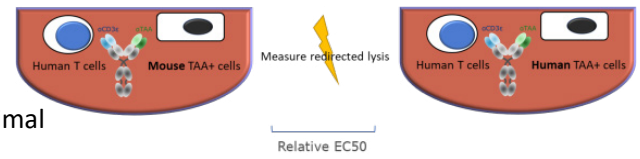
Leverage Mouse Pharmacology Studies?

Immunodeficient mouse model	Mouse genetically engineered to express human CD3 and human TAA	Mouse genetically engineered to express human TAA
		
Clinical candidate antibody αTAA needs to cross-react with mouse ortholog	**Clinical candidate antibody**	**Tool antibody—αCD3 is mouse αCD3 (2C11)** αTAA needs to cross-react with mouse ortholog
Human T cell with **human** CD3 **Human** tumor cell with **human** TAA **Mouse** normal cell with **mouse** TAA	**Mouse** T cell with **human** CD3 **Mouse** tumor cell with **human** TAA **Mouse** normal cell with **human** TAA	**Mouse** T cell with **mouse** **Mouse** tumor cell with **human** TAA **Mouse** normal cell with **human** TAA
	Crawford et al. (2019)	Li et al. (2019)

Opportunities and Challenges with Pharmacology Models for Safety Assessment

Opportunities

- Investigate potential toxicology earlier in development
- Evaluate effects on normal tissues versus tumor in same animal



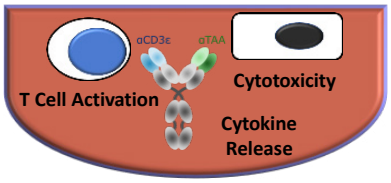
Challenges

- Duration of study may be limited by growth of tumor and/or development of GvHD (with human tumor)
- Genetically engineered models require time to develop and characterize
- Genetically engineered models may not have normal immunophenotype/immune function
- Limited availability of mouse tumors for immunocompetent mouse studies
- May not be able to test the clinical candidate
- Pharmacological relevance of the model must be established (species selection considerations)
- Translatability to human not only dependent on formation of trimolecular complex
  - E.g., T cell trafficking, T cell modulation (e.g., checkpoint inhibitors)

In Vitro Only Package

- Likelihood of success increases with a lower-risk TAA and an established CD3 bispecific platform
- Heavy reliance on normal human tissue expression profile (versus tumor) and clinical monitoring plan
- Confirm specificity of αTAA binder for TAA only
- *In vitro* bioactivity assays are used to address safety concerns
  - **On-target/on-tumor cytotoxicity**: TAA+ tumor cells versus TAA- cells (including cytokine release)
  - **On-target/off-tumor cytotoxicity**: TAA+ normal primary cells versus TAA+ tumor cells
  - **Cytokine release**: TAA+ normal cells (for heme target) ± TAA+ tumor cells
  - **Off-target/off-tumor cytotoxicity**: TAA- cells versus TAA+ cells

Bioactivity Assay

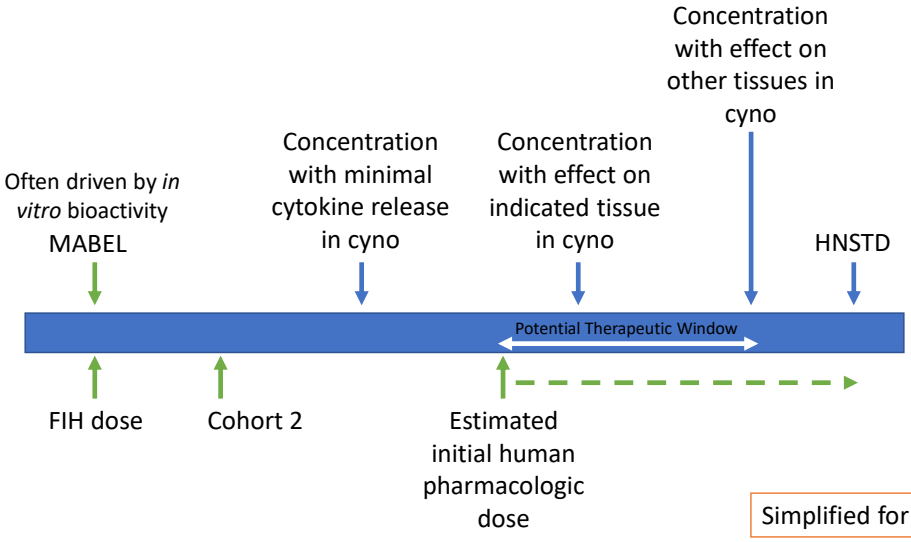


Establish effective concentration (EC)20 or 50

Confirm lack of activity with appropriate controls e.g., CD3xnull or nullxTAA

Cyno Study Findings in FIH Dose Setting and Clinical Trial Design Considerations

- Starting Dose: MABEL
- Dose Escalation: nonclinical safety findings



Simplified for illustrative purposes!

FIH Nonclinical Safety Package

Build the appropriate package based on the available tools in the toolboxes and the level of risk associated with the TAA

- ❑ Human Target Expression Profile
- ❑ Species Selection Justification
- ❑ *In vitro* assessments (including cytokine release)
- ❑ Mouse pharmacology studies (describe why relevant or irrelevant)
- ❑ Cynomolgus monkey safety studies (including safety pharmacology)*
- ❑ First-in-human dose selection and escalation plan
- ❑ Clinical monitoring plan (captured in Investigator's Brochure)

For IND, nonclinical safety data are presented in pharmacology, pharmacokinetic, and toxicology sections of Module 2

*FIH-enabling study could be the registrational study if immunogenicity impacts exposure

Summary and Conclusions

- CD3 bispecifics offer a rare opportunity in which standard toxicology studies in cynos can inform human risk assessment for an immuno-oncology agent.
- Build the appropriate nonclinical safety package based on the available tools in the toolboxes and the level of risk associated with the TAA. This includes cyno studies but also mouse pharmacology studies and *in vitro* data.
- CD3 bispecifics require formation of a trimolecular complex to exert their effects.
- The potential for on-target/off-tumor cytotoxicity can be evaluated in cyno toxicity studies.
- Be ready to adapt the design(s) of your cynomolgus monkey study(ies) in the event of cytokine release or evidence of clearing anti-drug antibodies.
- Cytokine release—especially following the first dose—may be dose limiting in cynomolgus monkey given a CD3 bispecific. Intra-animal dose escalation may mitigate the cytokine release and allow for higher exposure to the CD3 bispecific.
- FIH dose set by MABEL but cyno data inform on the potential for a therapeutic window, aggressiveness of dose escalation, and clinical monitoring plan.

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- ICH S6(R1) Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals
- ICH S9 Nonclinical Evaluation for Anticancer Pharmaceuticals
- ICH M3(R2) Guidance of Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals
- EMA Strategies to Identify and Mitigate Risks for First-in-Human and Early Clinical Trials with Investigational Medicinal Products
- FDA Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

Regulatory Perspective on the Preclinical Development of T Cell Immunotherapies

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Conflict of Interest Disclosure

Nothing to disclose

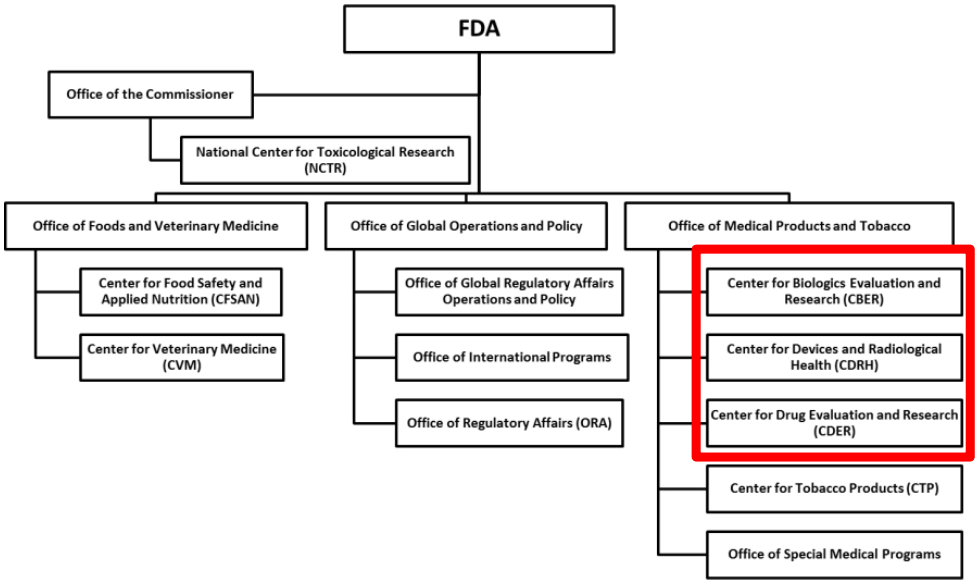
List of Abbreviations

- CAR: chimeric antigen receptor
- CBER: Center for Biologics Evaluation and Research
- CMC: chemistry, manufacturing, and controls
- DCEPT: Division of Clinical Evaluation and Pharmacology/Toxicology
- MSC: mesenchymal stem cell
- NK cell: natural killer cell
- OTAT: Office of Tissues and Advanced Therapies
- POC: proof of concept
- TCR: T cell receptor
- Treg: regulatory T cell
- US FDA: United States Food and Drug Administration

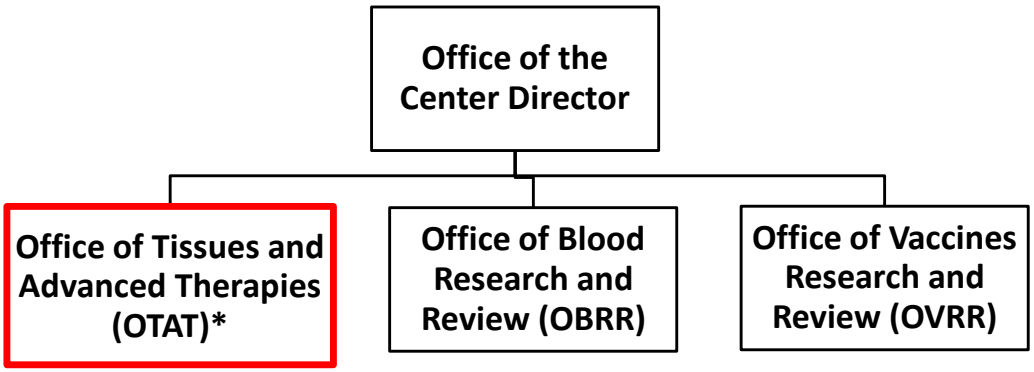
Overview

- US FDA/CBER/OTAT (Office of Tissues and Advanced Therapies) organizational overview
- Preclinical regulatory review principles
- Preclinical development of T cell–based products
  - Example 1: CAR T cells
  - Example 2: engineered T cell receptor products
- Potential preclinical pitfalls and resources
- Summary

US Food and Drug Administration



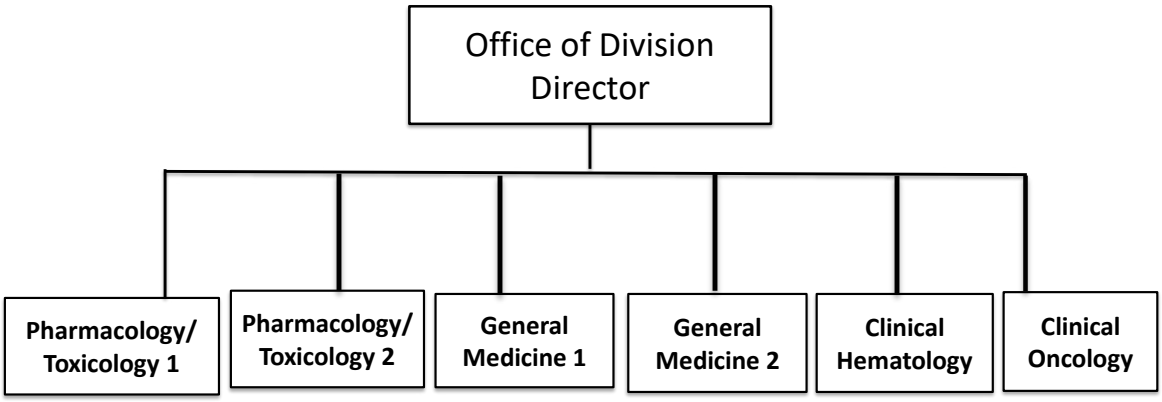
Center for Biologics Evaluation and Research (CBER)—Product Review Offices



**Formerly the Office of Cellular, Tissue, and Gene Therapies (OCTGT)*

Office of Tissues and Advanced Therapies (OTAT)

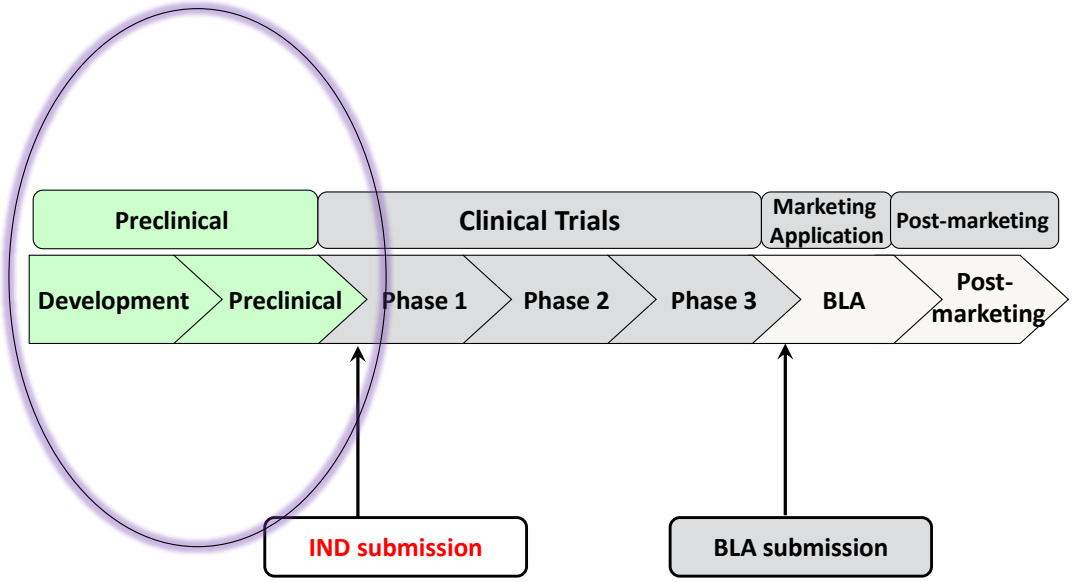
Division of Clinical Evaluation and Pharmacology/Toxicology (DCEPT)



Examples of Cell-Based Immunotherapy Products Regulated in OTAT

- Chimeric antigen receptor (CAR) T cells
- T cell receptor (TCR) modified T cells
- Non-T cell CARs (e.g., NK cells, etc.)
- Regulatory T cell (Treg) products
- Mesenchymal Stem Cells (MSCs)
- Cell-based therapeutic vaccines (e.g., dendritic cells, irradiated tumor cells)

Product Life Cycle for Biologics



What Regulations Govern Preclinical Testing?

Pharmacology and Toxicology Studies

"... adequate information about the pharmacological and toxicological studies ... on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations. The kind, duration, and scope of animal and other tests required varies with the duration and nature of the proposed clinical investigations."

IND Regulations [21 CFR 312.23 (a)(8) - Pharmacology and Toxicology]

Expectations from Preclinical Data

Support a rationale for the first-in-human clinical trial
- For cell and gene therapy products, the trial is usually conducted in the disease population, not in healthy volunteers

Make recommendations regarding the proposed clinical trial
- Initial safe starting dose, dose-escalation scheme, dosing schedule, organ toxicity, clinical monitoring

Meet regulatory requirements
- 21 CFR 312.23 (a)(8) (Pharmacology and Toxicology information)
- 21 CFR 58 (Good Laboratory Practice (GLP) compliance)

Potential Safety Concerns for Cell-Based Products

- Risks of the delivery procedure
- Potential immune response to the administered cellular product
- Inappropriate cell proliferation (i.e., tumor formation)
- Inappropriate cell differentiation (i.e., ectopic tissue formation)
- Cell migration to nontarget areas/tissues
- Interactions with concomitant therapies
- For vector-transduced cells:
  - Vector insertion/integration/transformation
  - Unintended immune responses to vector or transgene
  - Transgene effects

Potential Safety Concerns for T Cell–Based Products

- Specificity:
  - On-target, off-tumor toxicity
  - Off-target toxicity
- Cytokine release, tumor lysis, macrophage activation syndromes
- Vector concerns—insertional mutagenesis, immunogenicity, etc.
- Genome-editing concerns—off-target activity, cellular transformation, etc.
- Novel suicide genes—effects of expressed gene + novel drug inducer

Sources of Data to Support an IND

- GLP-compliant toxicology studies conducted by a qualified testing facility
- Well-controlled studies conducted in-house
- *In silico* studies with supporting qualification
- Published data in peer-reviewed journals
- Cross-reference to similar product(s) in previously submitted files to US FDA
- Detailed clinical study reports from clinical trials

General Guidelines for T Cell–Based Immunotherapy Preclinical Development

- Preclinical data may consist of *in vivo*, *in vitro*, and *in silico* studies to support the safety and activity of the product
- The most informative preclinical development program may vary greatly from product to product based on availability of animal model(s), surrogate product(s), and clinical experience with similar products
- Clinical dose level rationale may be supported by previous clinical experience with similar products

General Guidelines for T Cell–Based Immunotherapy Preclinical Development

- *In vivo* studies
  - When animal model(s) reactive to the product are available/applicable (e.g., HLA transgenic animals, humanized mice)
  - Animal model(s) of disease may be informative for toxicology studies
  - In some cases, a surrogate product may be appropriate
- *In vitro* studies
  - Controlled screening of on-target and off-target activity, cytokine independent growth, etc.
- *In silico* studies
  - High-throughput TCR cross-reactivity screening, presence of homologous proteins/peptides, etc.

Example 1: Preclinical Data Used to Support a CAR T Cell Product Using a Single-Chain Variable Fragment (scFv)

In vitro and in silico

- **On-target/off-tumor toxicity:** expression profile of target (e.g., RT-PCR, immunohistochemistry, flow cytometry, published data)
- **Off-target toxicity:** off-target activity of final CAR T cell product against various cell lines, primary cells, and/or iPSC-derived 3D cell cultures from various tissue sources, tissue cross-reactivity, plasma membrane protein array, etc.
- **Proof of concept:** antigen-dependent activity using final CAR T cell product (e.g., cytokine release assays, cytotoxicity, T cell proliferation)
- **Vector-related:** cytokine-independent growth assays

Example 1: Preclinical Data Used to Support a CAR T Cell Product Using a Single-Chain Variable Fragment (scFv)

In vivo

- POC anti-tumor response in human tumor xenograft models
- POC/safety studies in appropriate animal models, if available
- Studies using surrogate CAR T cell product in animal models, if available
- Any additional product- and indication-specific testing (e.g., novel suicide gene, combined with drug)

Example 1: Preclinical Data Used to Support a CAR T Cell Product Using a Single-Chain Variable Fragment (scFv)

Clinical experience

- Previous clinical experience with similar CAR T cell products (e.g., same scFv)
- Previous experience with investigational or approved monoclonal antibody with identical specificity
- Published experience with same target

Example 2: Preclinical Data Used to Support a TCR-Modified T Cell Product

In vitro and in silico

- **On-target/off-tumor toxicity:** expression profile of target (e.g., RT-PCR, immunohistochemistry, flow cytometry, published data, etc.)
- **TCR cross-reactivity:**
  - Evaluate noncritical positions for TCR reactivity (e.g., alanine scan)
  - Off-target activity of final product against various cell lines, primary cells, and/or iPSC-derived 3D cell cultures from various tissue sources
- **TCR alloreactivity:** *in vitro* co-culture
- **Proof of concept:** peptide-MHC-dependent activity using final product
- **Vector-related:** cytokine independent growth

Example 2: Preclinical Data Used to Support a TCR-Modified T Cell Product

In vivo

- POC antitumor response in human tumor xenograft models
- POC/toxicology studies in appropriate HLA-transgenic animal models, if available
- Any additional product- and indication-specific testing (e.g., novel suicide gene, combined with drug)

Example 2: Preclinical Data Used to Support a TCR-Modified T Cell Product

Clinical experience

- Previous clinical experience with similar TCR-modified T cell products (e.g., same TCR)
- Published experience with same target

Potential Preclinical Pitfalls When Submitting an IND

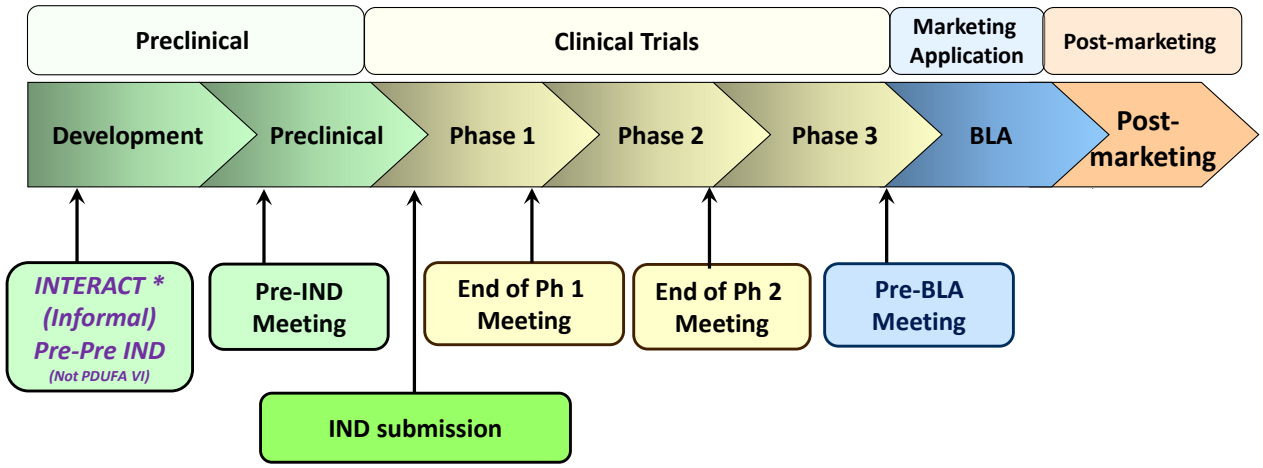
Insufficient information to assess subject risk

- Insufficient product characterization
- Lack of preclinical safety data for intended product
- Incomplete study reports

Inadequate preclinical study design

- Differences between preclinical and clinical products
- Animal species/model
- Study evaluations

Opportunities for Interaction with US FDA during Preclinical Development



Early Communication with OTAT: INTERACT

INitial Targeted Engagement for Regulatory Advice on CBER producTs *(previously known as pre-pre-IND interactions)*

- **Goal:** to obtain early feedback on a product development program for a novel investigational agent
- **Purpose:**
  - A mechanism for early communication with OTAT
  - Nonbinding, informal scientific discussion between CBER/OTAT review disciplines and the sponsor
  - Initial targeted discussion of specific issues
- **Timing:** when you have generated preliminary preclinical data (POC and some safety) and are facing unique challenges with a novel product development program, but are not yet at the pre-IND stage

https://www.fda.gov/vaccines-blood-biologics/industry-biologics/interact-meetings-initial-targeted-engagement-regulatory-advice-cber-products

Early Communication with OTAT: INTERACT

- **Scope (pharmacology/toxicology)**

  Overall pharmacology/toxicology advice related to the design of proof of concept or other pilot safety/biodistribution studies necessary to support administration of an investigational product in a FIH clinical trial

  - Adequacy of the selected animal species and animal models of disease/injury
  - Study designs (e.g., endpoints, dose levels, route of administration, dosing regimen)
  - Acceptability of innovative preclinical testing strategies, products, and/or delivery modalities
  - Advice on modification of a preclinical program or study design, as applicable, to ensure judicious use of animals

Early Communication with OTAT: Pre-IND

A nonbinding, formal scientific discussion between all CBER/OTAT review disciplines (CMC, P/T, and Clinical) and the sponsor

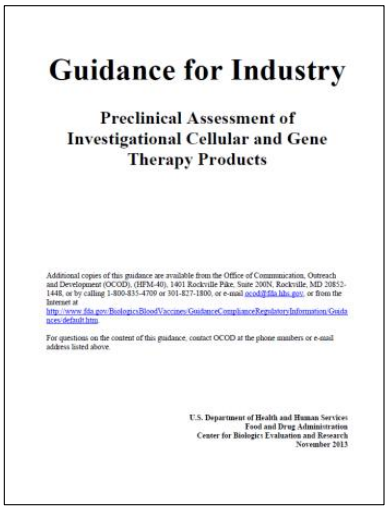
- **Goal:** to achieve a successful IND submission
- **Purpose:**
  - To allow early communication between the sponsor and CBER/OTAT
  - To comprehensively communicate the product/clinical development plan
    - Product characterization issues
    - Preclinical testing program
    - The scope and design of the planned clinical trial
  - To discuss the format for the IND submission
- **Timing:** prior to the conduct of the definitive preclinical safety studies

Early Communication with OTAT: Pre-IND

- **Scope (pharmacology/toxicology)**
  - **A comprehensive summary of all completed preclinical studies**
    - *In vitro* and *in vivo* studies
    - Animal species/models
    - Study designs
    - Product manufacturing and formulation
    - Resulting data and interpretation
  - **Discussion of the planned preclinical program** (e.g., animal species/models, product manufacturing and formulation, study designs)

Selected Guidance Documents

- Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products (November 2013)
  https://www.fda.gov/regulatory-information/search-fda-guidance-documents/preclinical-assessment-investigational-cellular-and-gene-therapy-products
- Guidance for Industry: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products (June 2015)
  https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considerations-design-early-phase-clinical-trials-cellular-and-gene-therapy-products
- Guidance for Industry: Clinical Considerations for Therapeutic Cancer Vaccines (October 2011)
  https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-considerations-therapeutic-cancer-vaccines


Guidance for Industry

Preclinical Assessment of Investigational Cellular and Gene Therapy Products

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or e-mail ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or e-mail address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
November 2013

Summary and Key Points

- It is important to keep US FDA/CBER/OTAT involved at an early phase of the product development program
- The preclinical study designs should be supported by scientific rationale/data
- Novel therapies mean novel testing paradigms

Contact Information

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  Email: OTATRPMS@fda.hhs.gov and

  Lori.Tull@fda.hhs.gov

US FDA Headquarters

- **OTAT Learn Webinar Series:**

  http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm
- **CBER website:** www.fda.gov/BiologicsBloodVaccines/default.htm
- **Phone:** 1.800.835.4709 or 240.402.8010
- **Consumer Affairs Branch**: ocod@fda.hhs.gov
- **Manufacturers Assistance and Technical Training Branch**: industry.biologics@fda.hhs.gov
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Guidances and References

- Draft Guidance for Industry: Formal Meetings Between the FDA and Sponsors or Applicants of PDUFA Products (December 2017)
  https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM590547.pdf
- Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products (November 2013)
  https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM376521.pdf
- Guidance for Industry: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products (June 2015)

  https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considerations-design-early-phase-clinical-trials-cellular-and-gene-therapy-products
- Draft Guidance for Industry: Long Term Follow-Up After Administration of Human Gene Therapy Products (July 2018)
  https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM610797.pdf

Guidances and References (Continued)

- Draft Guidance for Industry: Human Gene Therapy for Rare Diseases (July 2018)
  https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM610802.pdf
- Draft Guidance for Industry: Human Gene Therapy for Retinal Disorders (July 2018)
  https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM610803.pdf
- Draft Guidance for Industry: Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) (July 2018)
  https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM610795.pdf

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