HESI Distance Learning Course: CAR Therapy

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Conflict of Interest and Disclaimer Statement

The authors have no actual or potential conflicts of interest to declare.

The views expressed in this presentation are those of the authors.

Expected Learning Outcomes of This Course

- At the end of this course, attendees will be able to:
  - Describe the fundamental concepts of how a CAR T cell works
  - Understand the basic nonclinical assessments of CAR T therapy in support of clinical development
  - Understand considerations applied for clinical trial design of cell therapies
  - Recognize the gaps and needs for future CAR T development in the field of Oncology

Overview

PART I:

- **Background on CAR Therapy**
- **Considerations for Nonclinical Development of CAR Therapies (example autologous αβ CAR T)**

PART II:

- **Considerations for Clinical Study Design**
- **Future of Cell Therapy as a Treatment Modality for Oncology**

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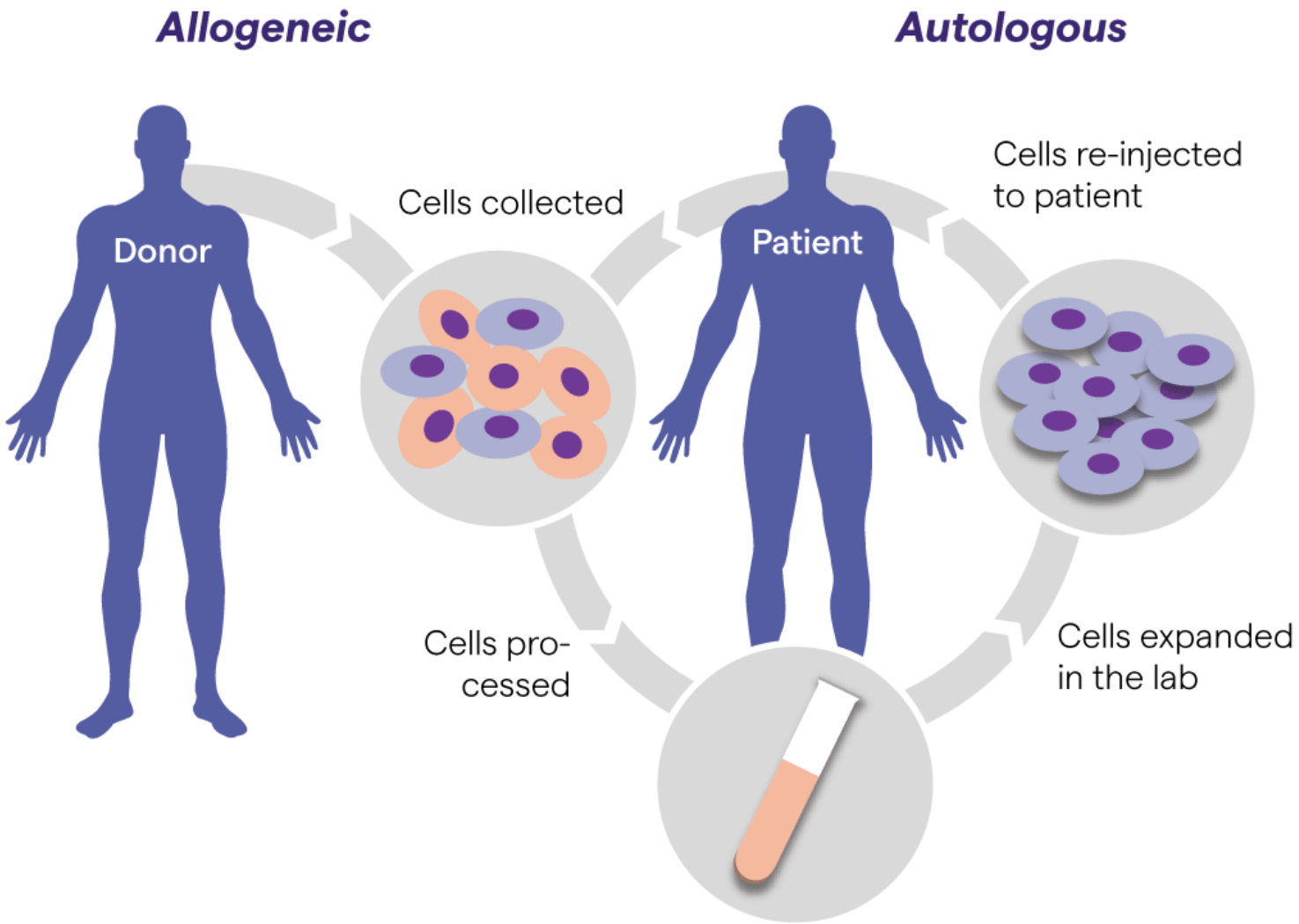
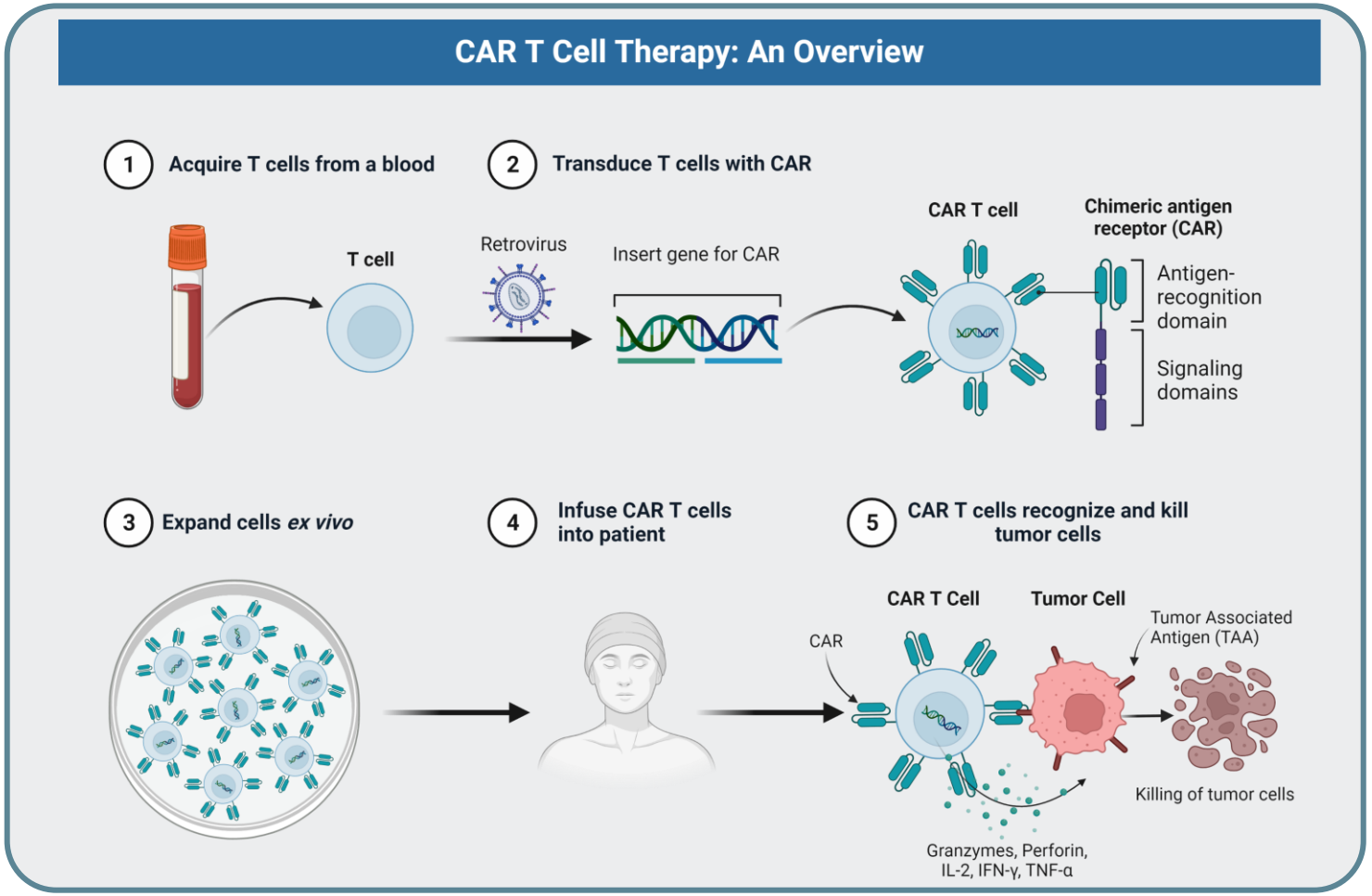
Cell Therapies

An evolving field with unique nonclinical and clinical challenges

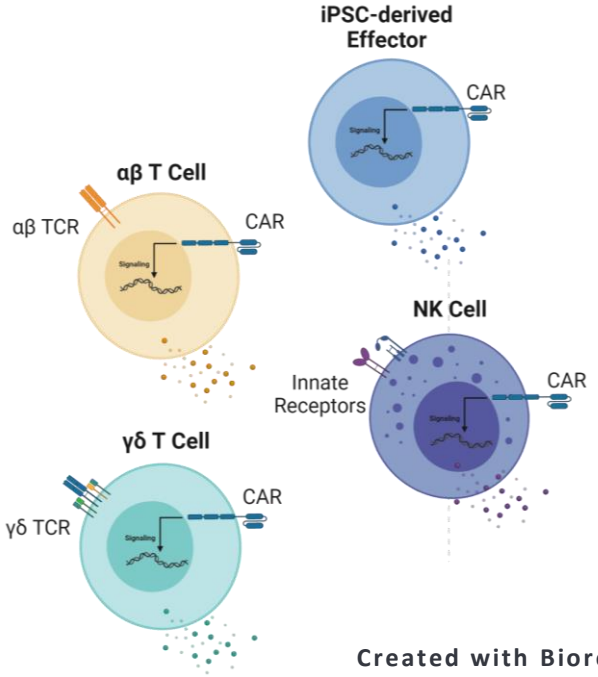


https://bioscience.lonza.com/lonza_bs/US/en/cell-and-gene-therapy

Chimeric Antigen Receptor (CAR) Immune Cell Therapies



- Autologous (patient-derived) or allogeneic (healthy donor) effector cells engineered to recognize and kill tumor cells via a chimeric antigen receptor
- Recognition of tumor associated antigen (TAA) via antigen recognition domain
- Hematological cancers and solid tumors
- Variety of cellular platforms including conventional T cells and innate lymphocytes



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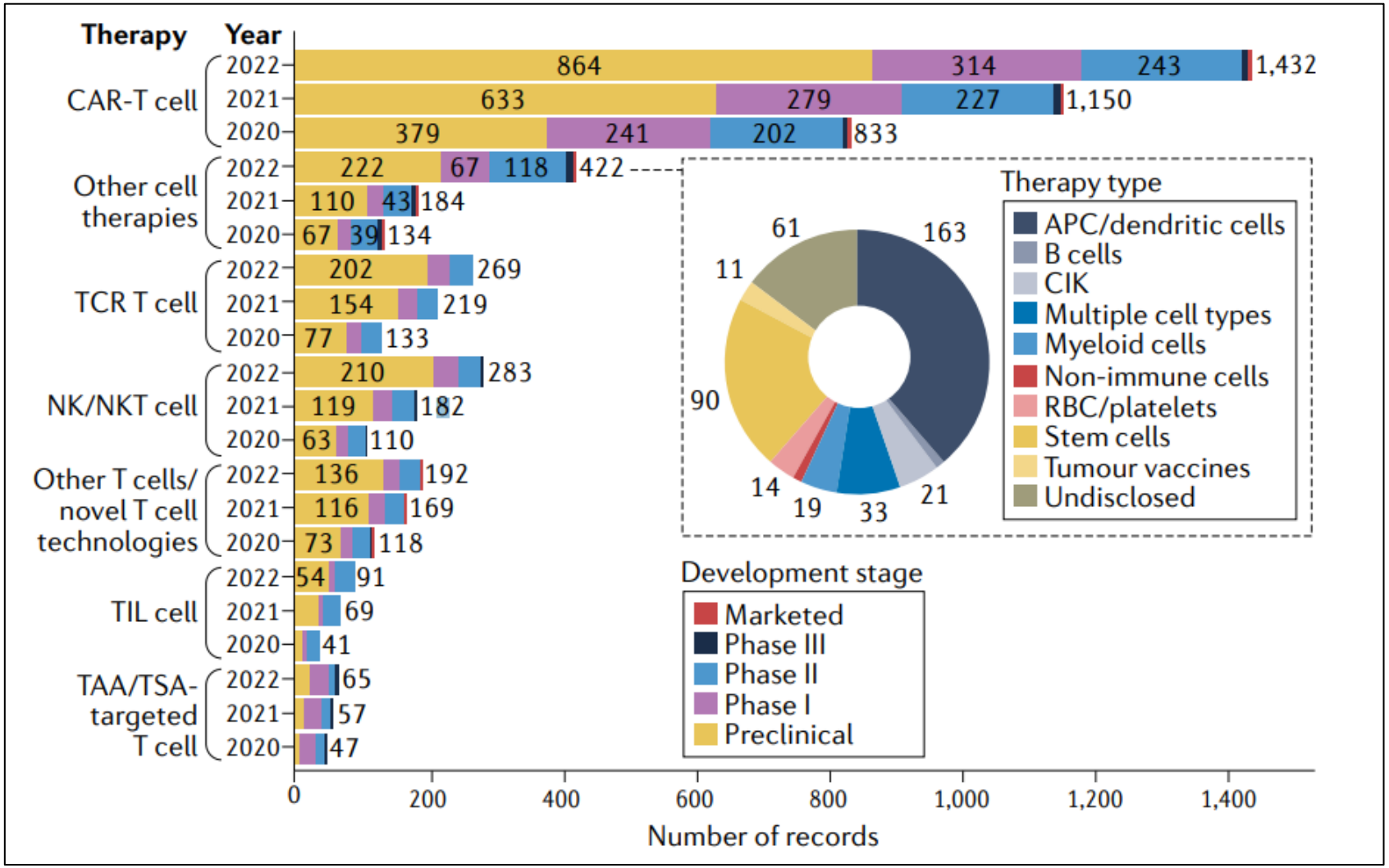
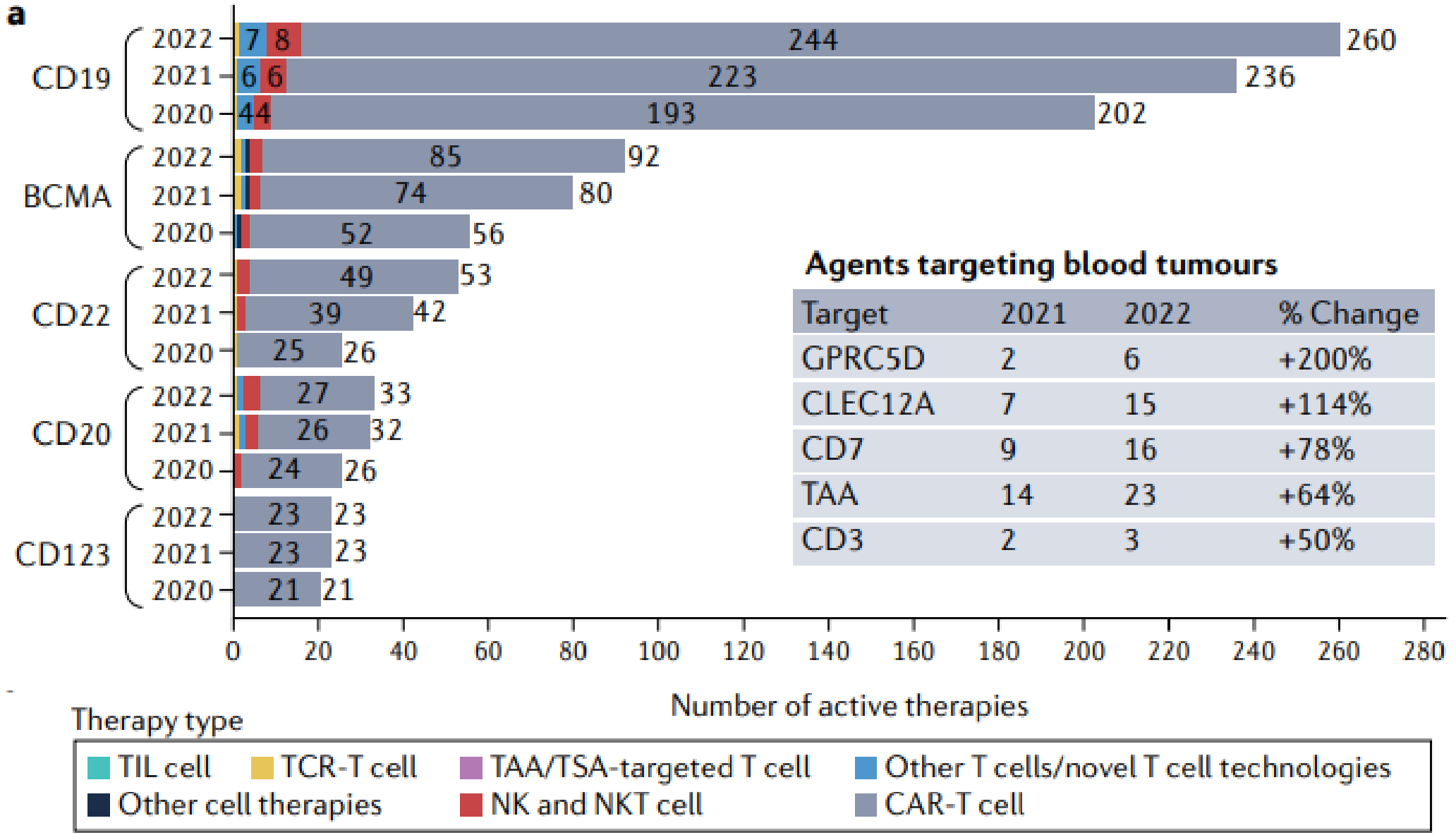
Evolving Cancer Cell Therapy Landscape



Comparison of cell therapy agent development pipeline across various therapy types from 2020 to 2022. APC, antigenpresenting cell; CIK, cytokine-induced killer; NK, natural killer; RBC, red blood cell; TAA, tumourassociated antigen; TCR, T cell receptor; TIL, tumour-infiltrating lymphocyte; TSA, tumour-specific antigen. The pie chart shows the composition of the 'other cell therapies' category in 2022.

Saez-Ibañez et al. *Nature Reviews Drug Discovery* 2022

Heme Tumor Targets of CAR Therapies

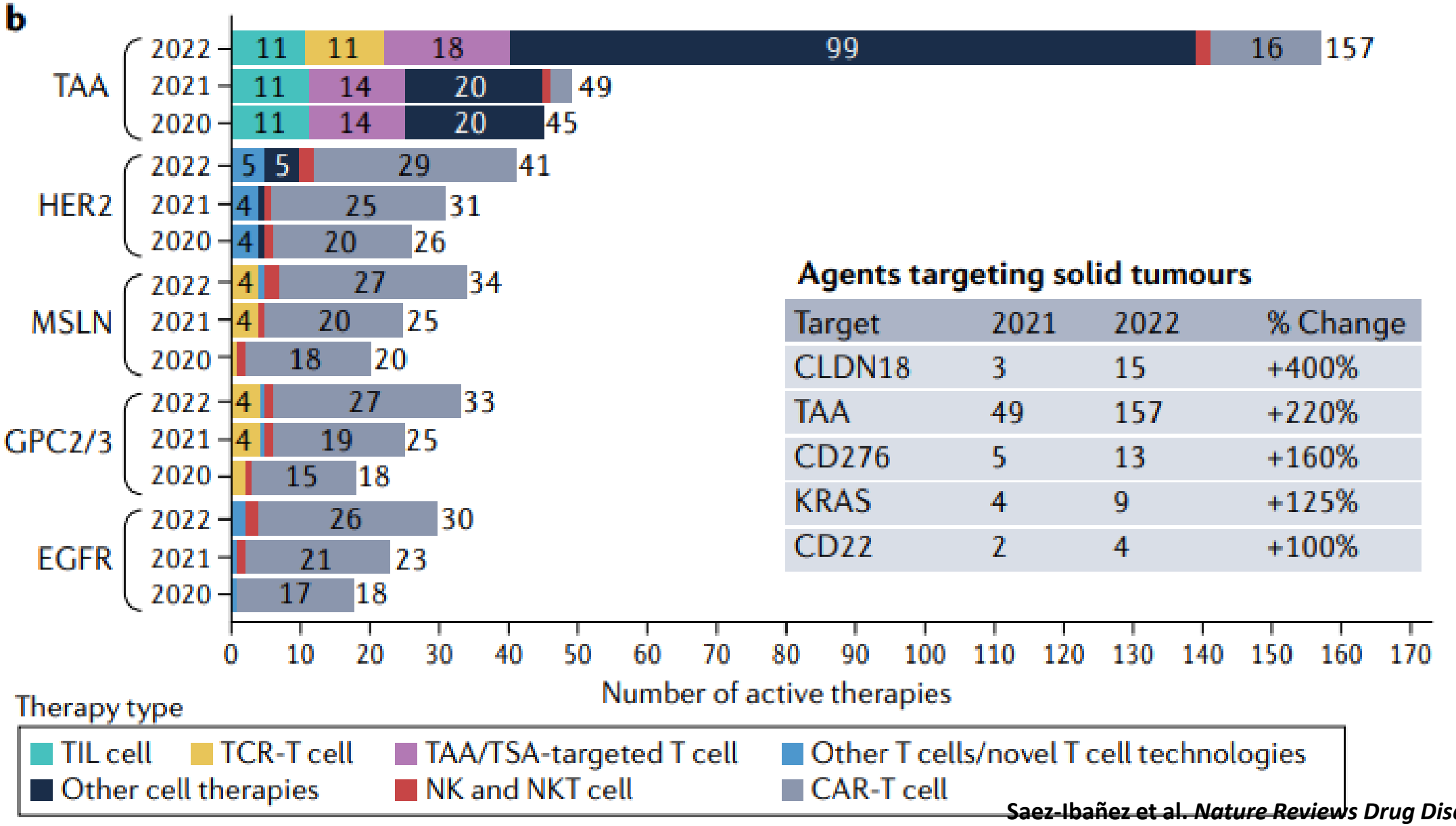


Agents targeting blood tumours

Target	2021	2022	% Change
GPRC5D	2	6	+200%
CLEC12A	7	15	+114%
CD7	9	16	+78%
TAA	14	23	+64%
CD3	2	3	+50%

Saez-Ibañez et al. *Nature Reviews Drug Discovery* 2022

Solid Tumor Targets of CAR Therapies

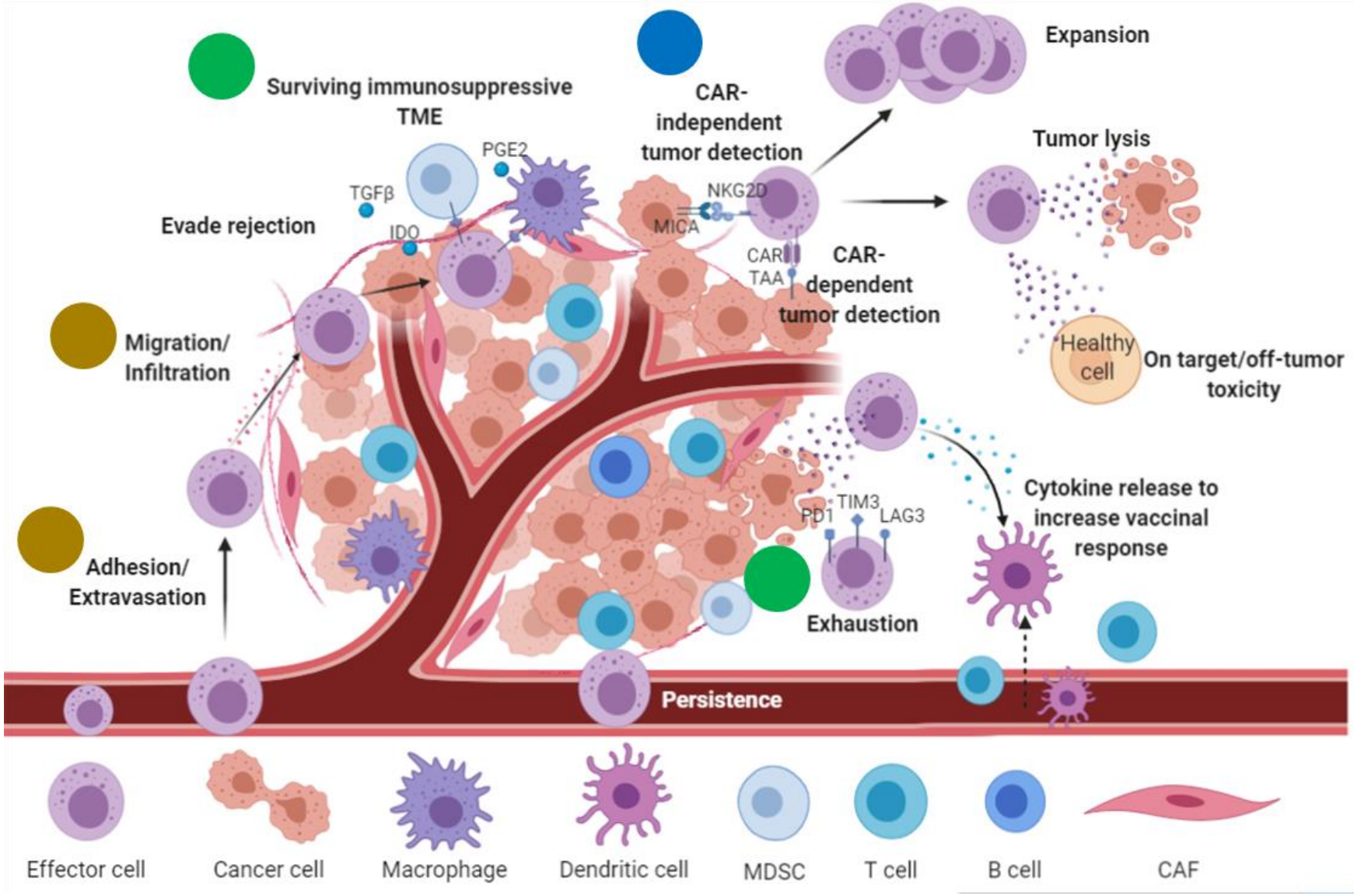


Agents targeting solid tumours

Target	2021	2022	% Change
CLDN18	3	15	+400%
TAA	49	157	+220%
CD276	5	13	+160%
KRAS	4	9	+125%
CD22	2	4	+100%

Saez-Ibañez et al. *Nature Reviews Drug Discovery* 2022

Challenges in the Solid Tumor Space with CAR Therapies



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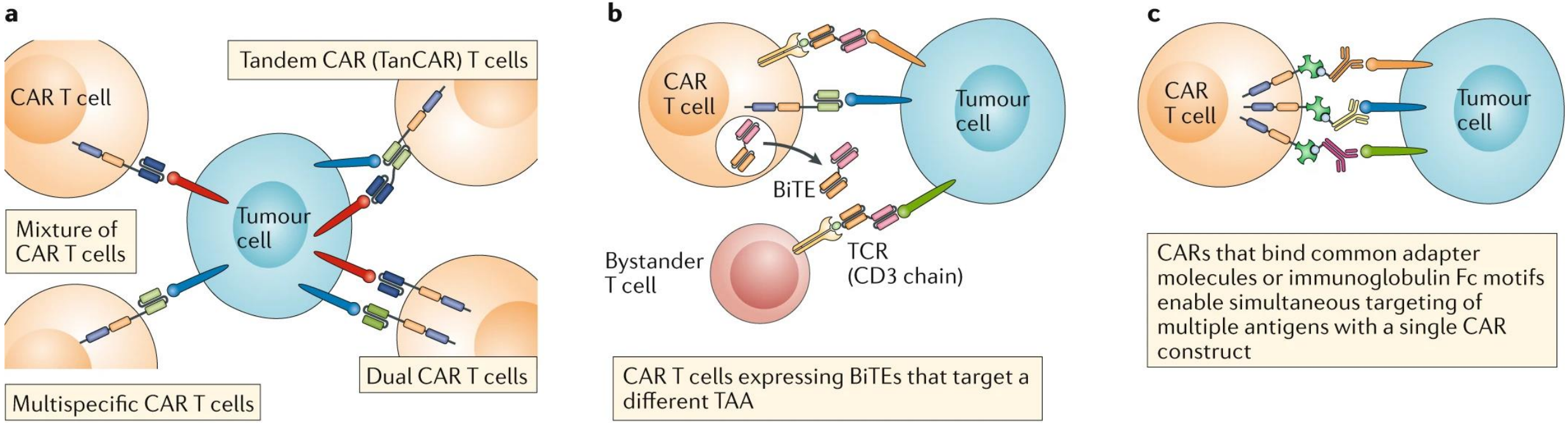
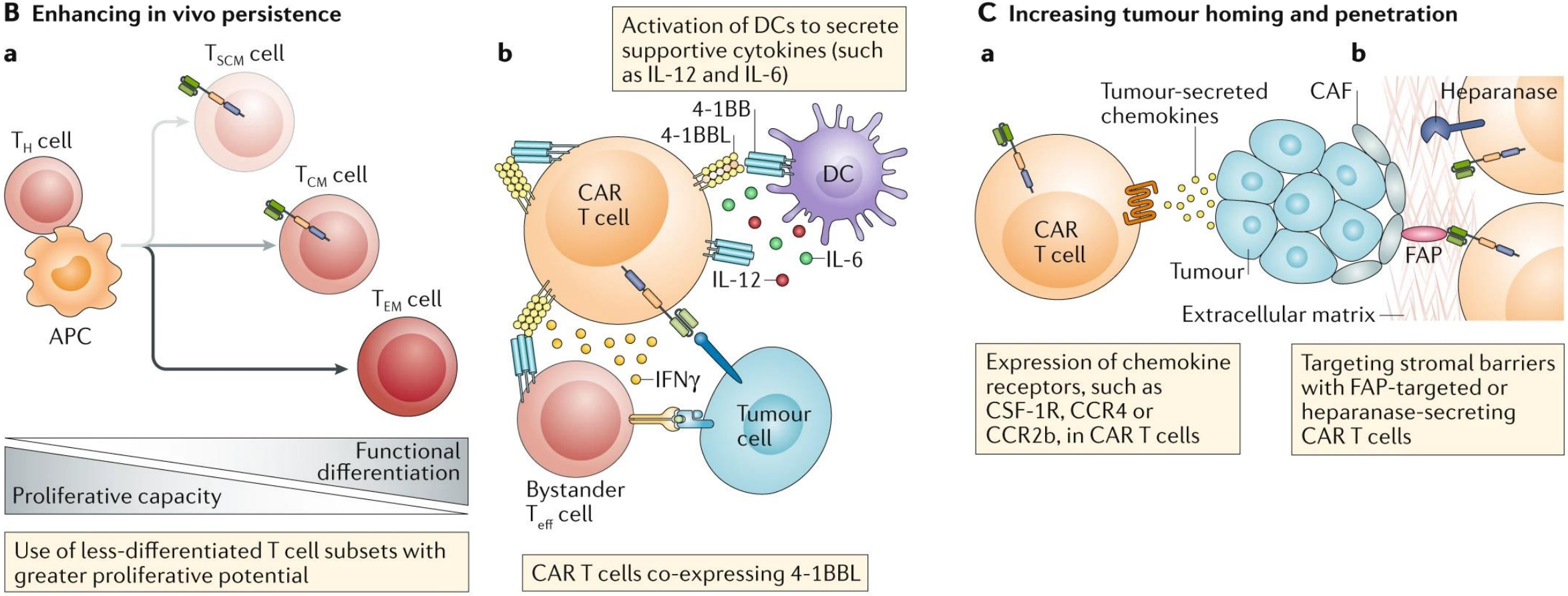
Overcoming antigen escape or heterogeneity



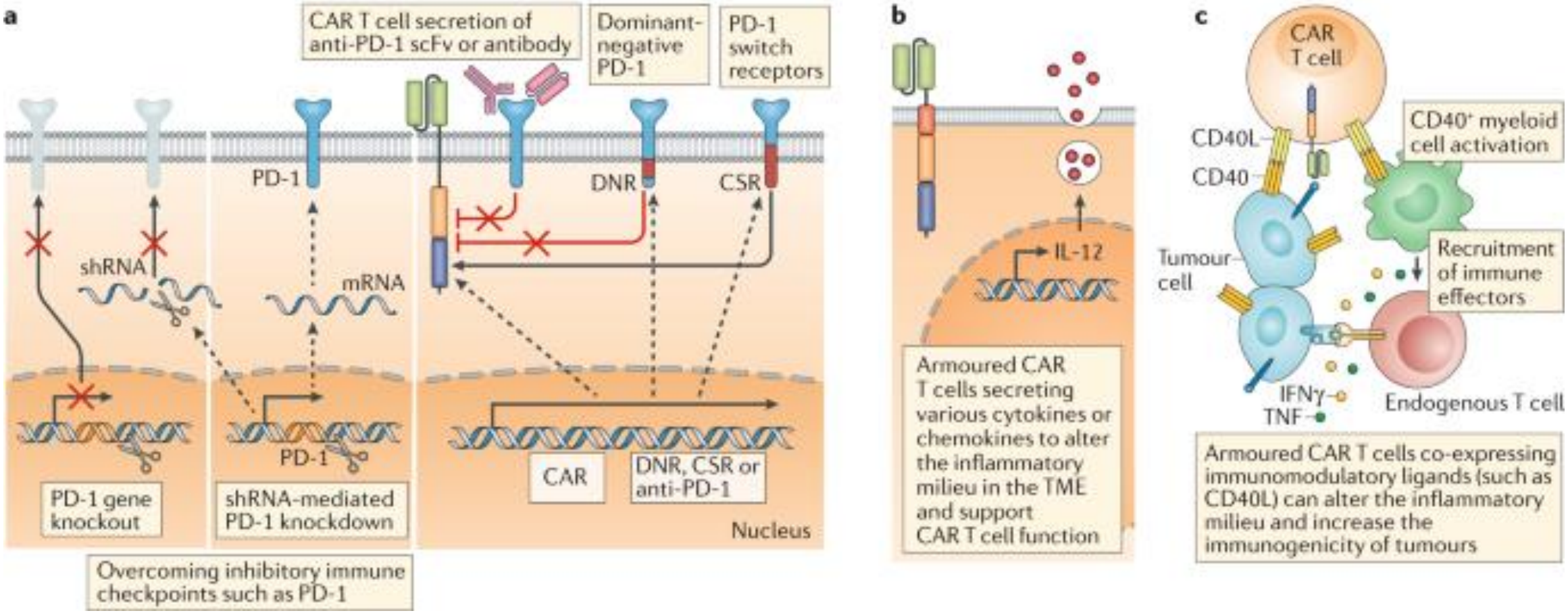
Rafiq, S et al. *Nat Rev Clin Oncol* 2020 https://doi.org/10.1038/s41571-019-0297-y

Enhancing in vivo persistence and tumor homing/penetration



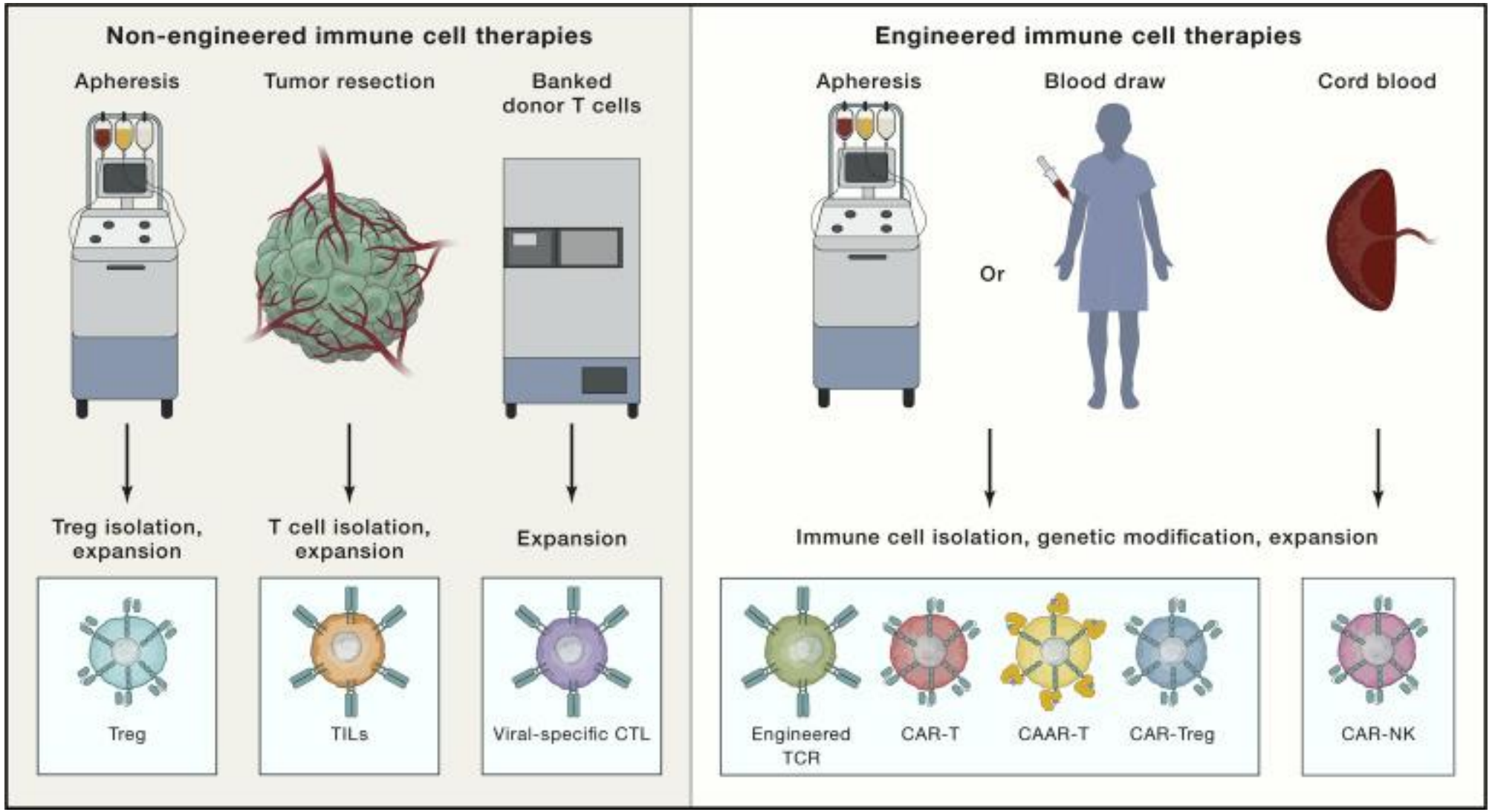
Rafiq, S et al. *Nat Rev Clin Oncol* 2020 https://doi.org/10.1038/s41571-019-0297-y

Overcoming Immunosuppression in the TME



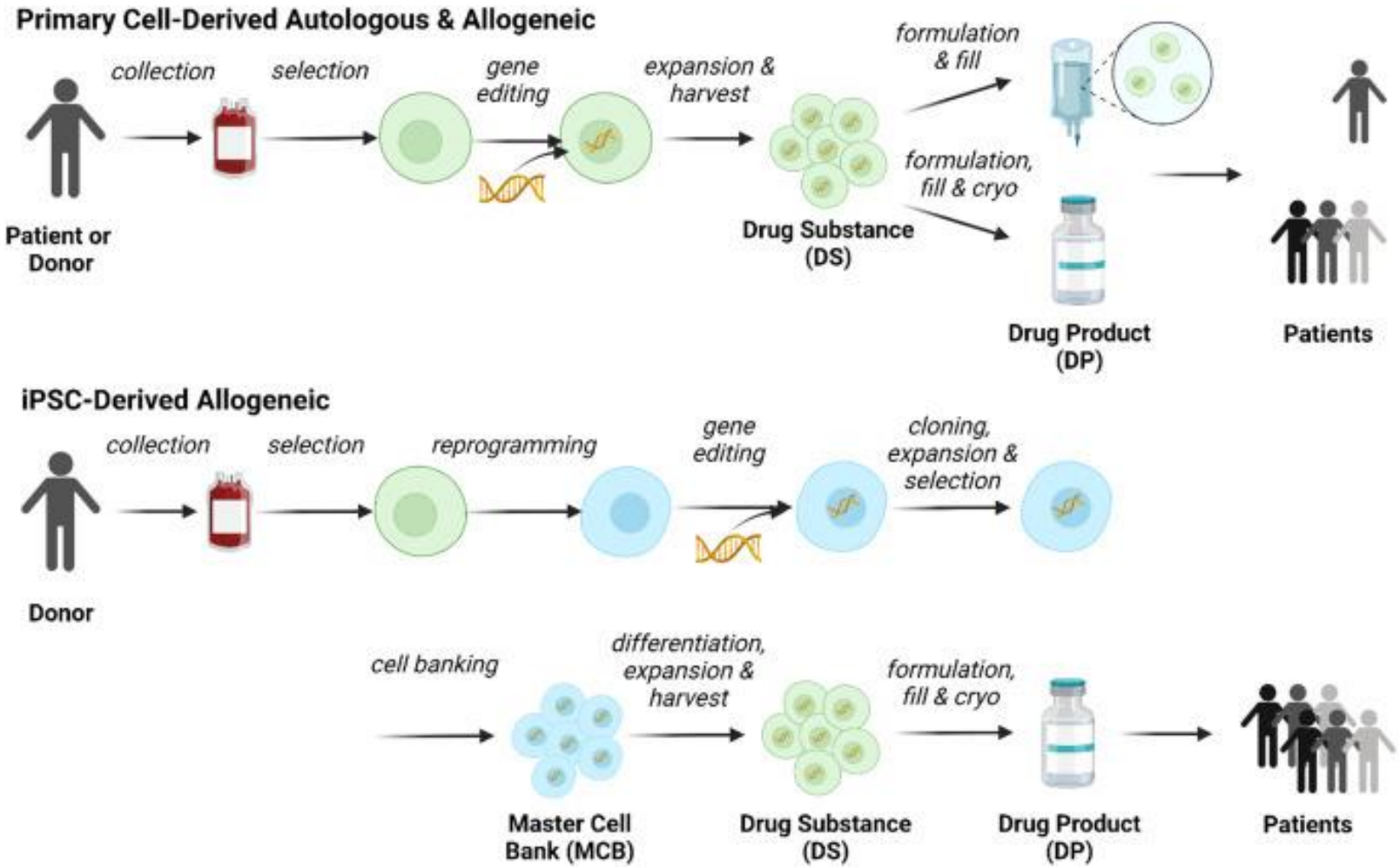
Rafiq, S et al. *Nat Rev Clin Oncol* 2020 https://doi.org/10.1038/s41571-019-0297-y

The Continuum of Immune Cell Therapies



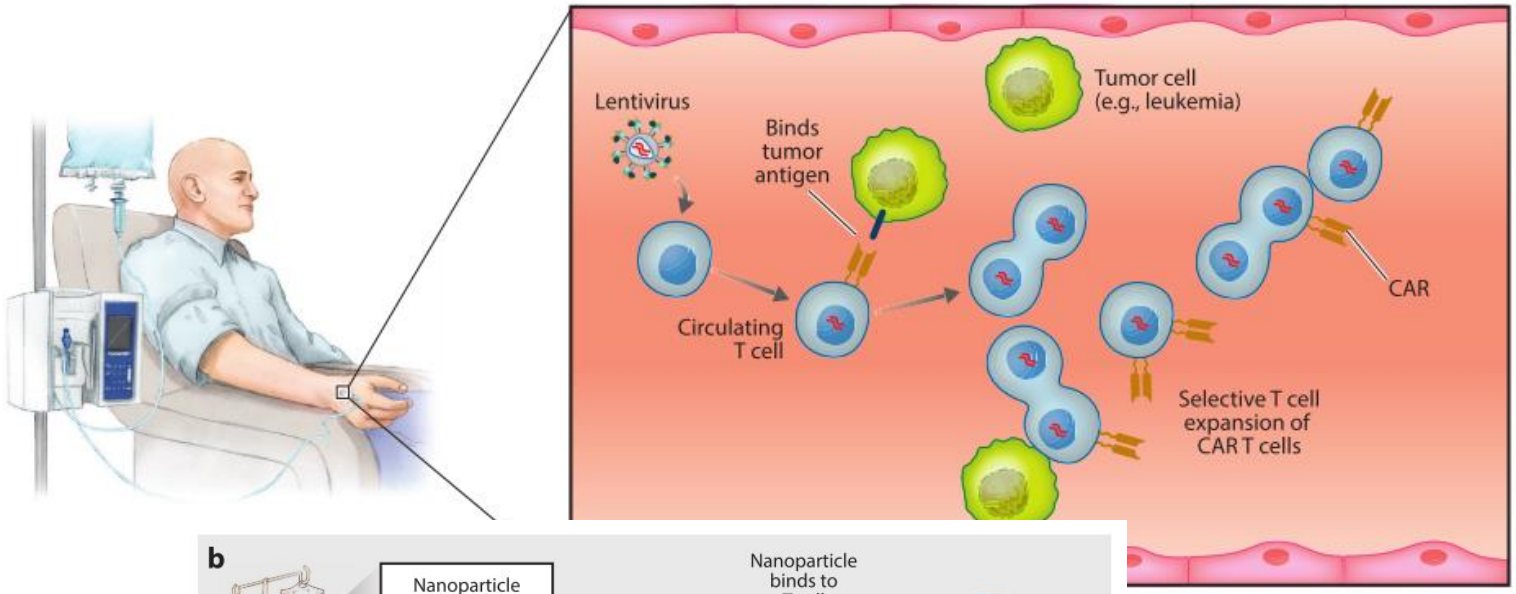
Weber et al. Cell, 2020, https://doi.org/10.1016/j.cell.2020.03.001.

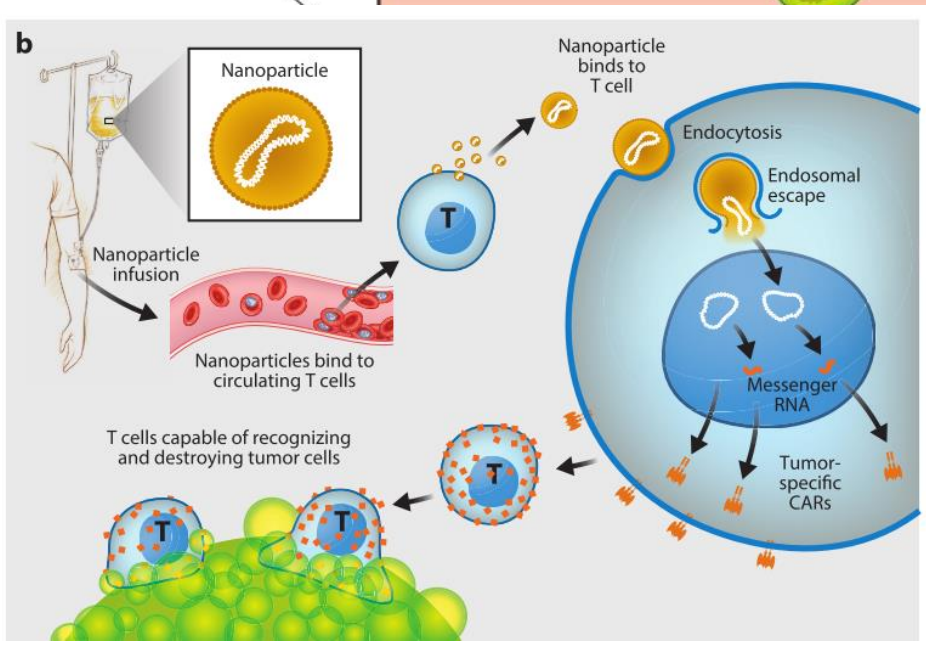
Additional Enhancements in CAR therapy: induced pluripotent stem cell therapies (iPSCs)



Daschnau et al. Cytotherapy, 2023
https://doi.org/10.1016/j.jcyt.2022.08.001

Additional Enhancements in CAR therapy: In vivo CAR reprogramming





- If reduction of the cost for adoptive immune cell transfer could be reduced, the treatment could be more common treatment method
- In vivo CAR reprograming is the technology to induce CAR expression in the body of patients
  - Can be viral or non-viral delivery
- Cost of goods could be reduced compared with autologous and allogeneic ex vivo manufacturing

(Zhang, Parayath et al. 2019)

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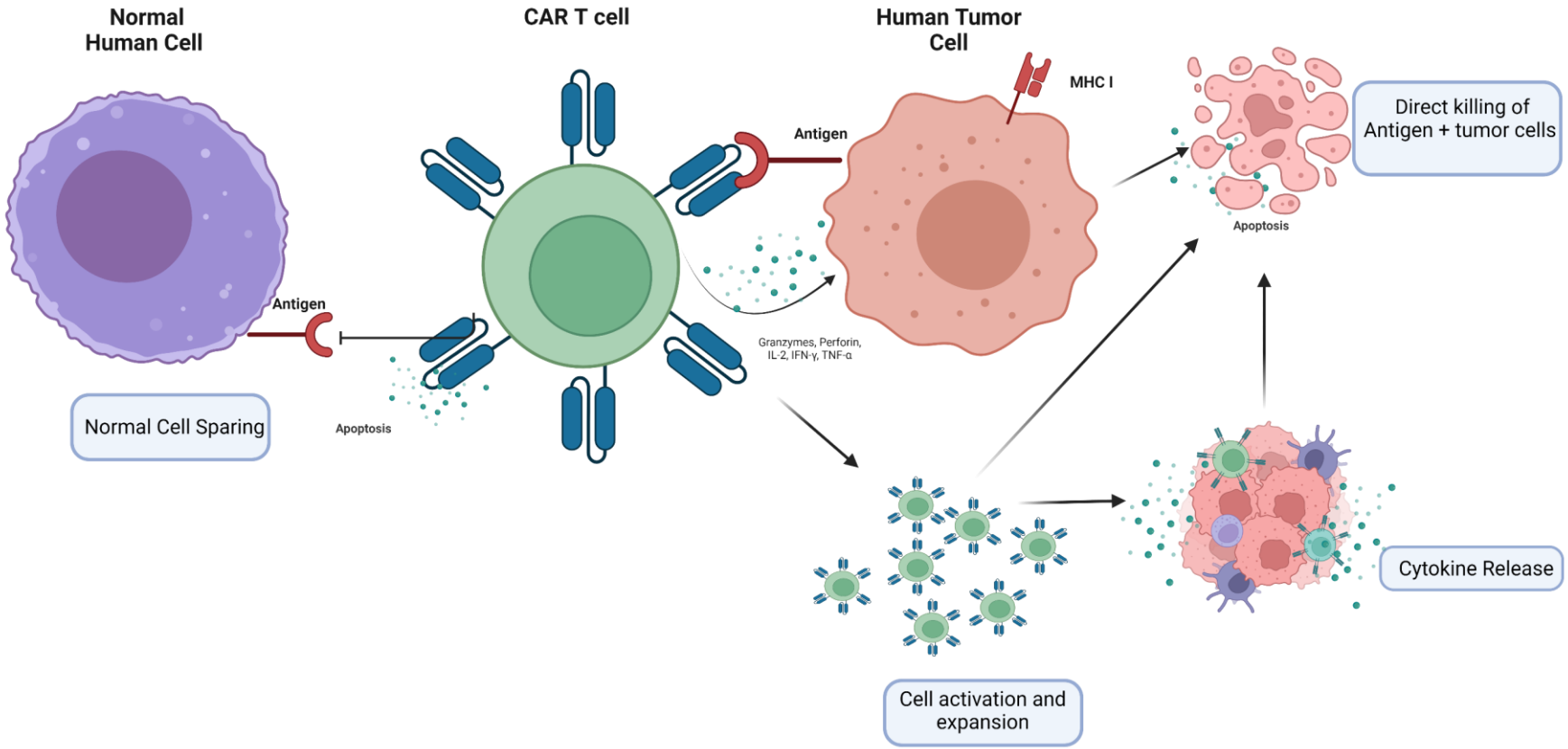
Considerations for Nonclinical Development of CAR Therapies (example autologous αβ CAR T)

- Pharmacology
- Pharmaco-(cellular) kinetics and biodistribution
- Safety (Toxicology)



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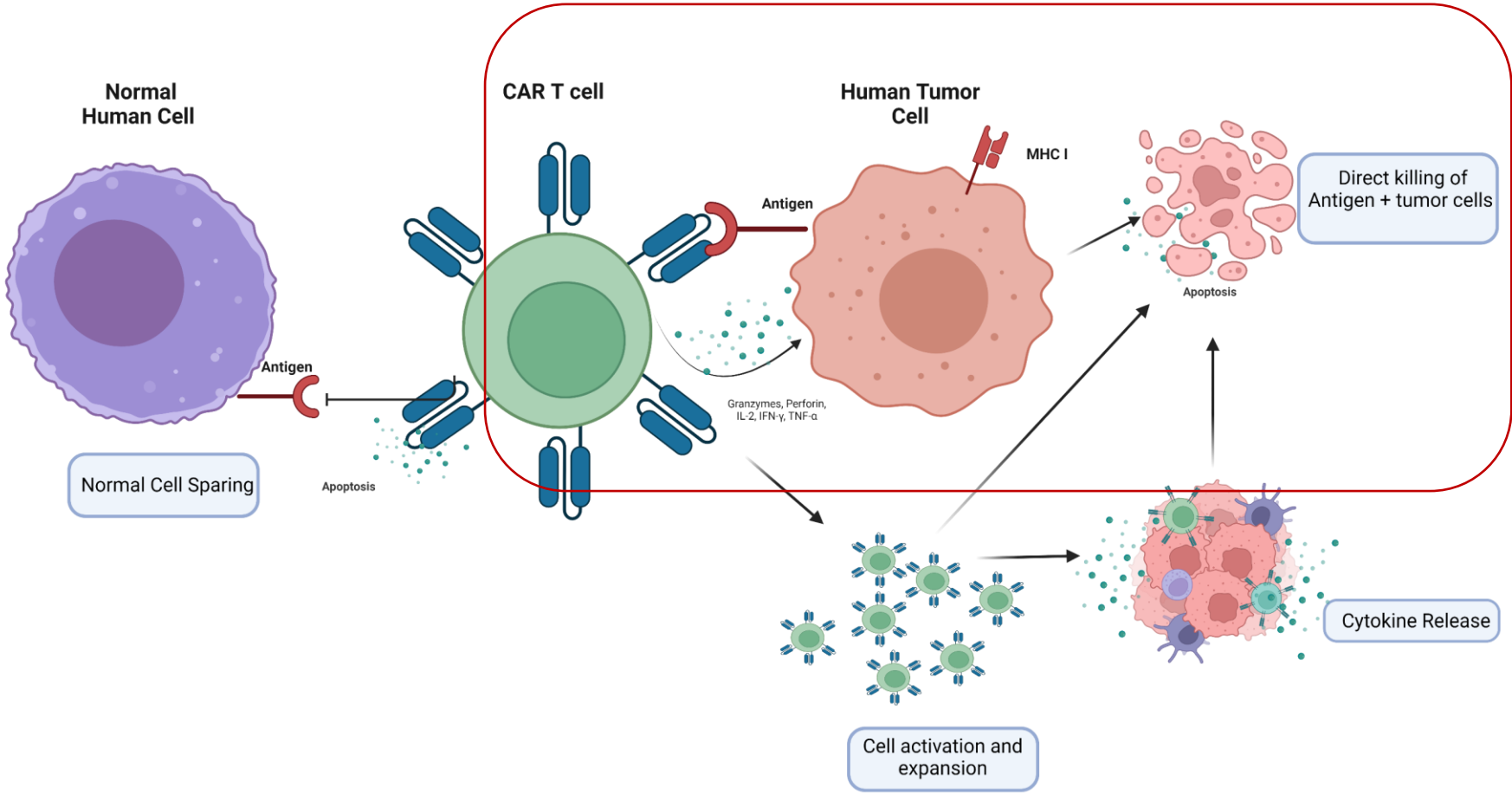
Considerations for Nonclinical Development of CAR Therapies (example autologous αβ CAR T)

- **Pharmacology**
- Pharmaco-(cellular) kinetics and biodistribution
- Safety (Toxicology)



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Nonclinical Pharmacology (example αβ CAR T)

In vitro considerations

- T cell functionality
  - Where does the ScFv bind the target (epitope binding)?
  - Does the CAR bind to human target?
    - Relative or absolute binding affinity (scFv-fusion construct/cell)
    - Does target binding activate the T cell?
      - Proliferation, cytokine secretion, cell killing
      - Flow cytometric assessment of activation markers (CD25, CD69, CD137)

Nonclinical Pharmacology (example αβ CAR T)

In vitro considerations continued…

- Species cross-reactivity*
  - Does the CAR specifically bind to natural target in animal models (rodent or monkey)?
    - DNA sequence alignment (total, extracellular membrane, epitope binding domain)
    - Demonstrated binding and activity against cells expressing human, rodent, non-human primate
      - Binding: flow cytometry, competition assay
      - Activity: T cell activation with native or recombinant protein from each species (isolated cells or tissues, engineered cells)
        - Proliferation
        - Cytokine secretion
        - Cell killing

*limitations in nonclinical species assessment of efficacy and safety due to human-origin of product, and disease state driving activity/toxicity

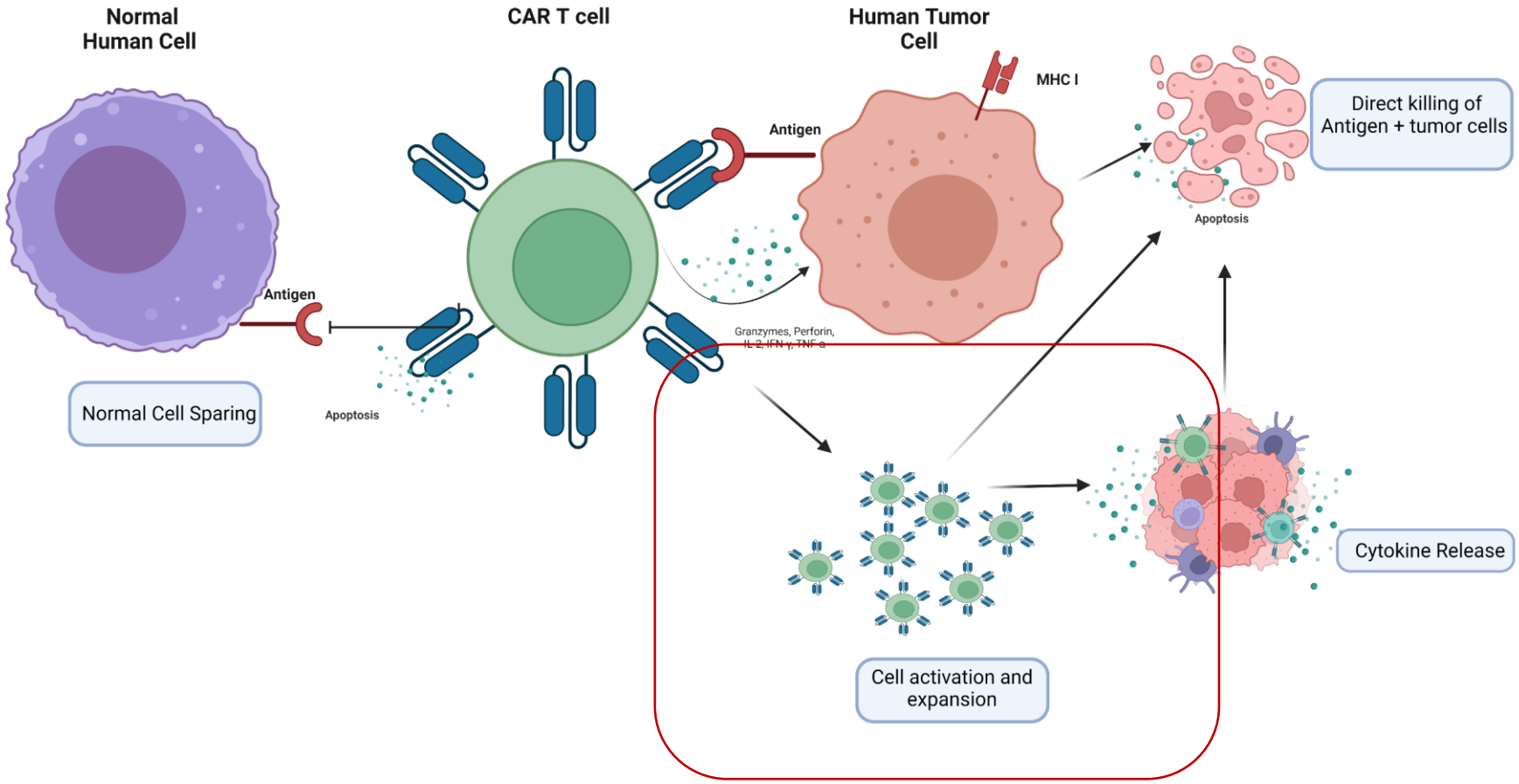
Nonclinical Pharmacology (example αβ CAR T)

In vitro and *in vivo*

- Functional activity of the CAR
  - Can the human CAR T cell kill tumor cells expressing target?
    - *In vitro*
      - T cell dependent cellular cytotoxicity (TDCC) assay
        - Assess cell killing of CAR Therapy in varying effector:target (E:T)
          - CAR-expressing and untransduced (UTD) cells
          - Tumor-associated Antigen (TAA) expressing cells and TAA negative cells
    - *In vivo*
      - Xenograft tumor models: Use immunodeficient mouse bearing a human tumor, treat with human CAR T cells
        - Allows testing of the human cell product in a relevant animal model to demonstrate product potency and activity (Endpoints: Survival, tumor size, T cell expansion, cytokine expression, tolerability)
        - Can be used to compare different CAR constructs (lead selection)
        - Can be used to refine CAR T cell design (co-stimulatory domain, other design enhancements)
        - Can be used to compare CAR T cells produced by different manufacturing strategies
      - Syngeneic models (may require surrogate target binder)
        - Allows evaluation of pharmacology and safety where an intact immune system is required
        - Allows testing of the drug combinations where immune modulation is a factor

Considerations for Nonclinical Development of CAR Therapies (example autologous αβ CAR T)

- Pharmacology
- **Pharmaco-(cellular) kinetics and biodistribution**
- Safety (Toxicology)



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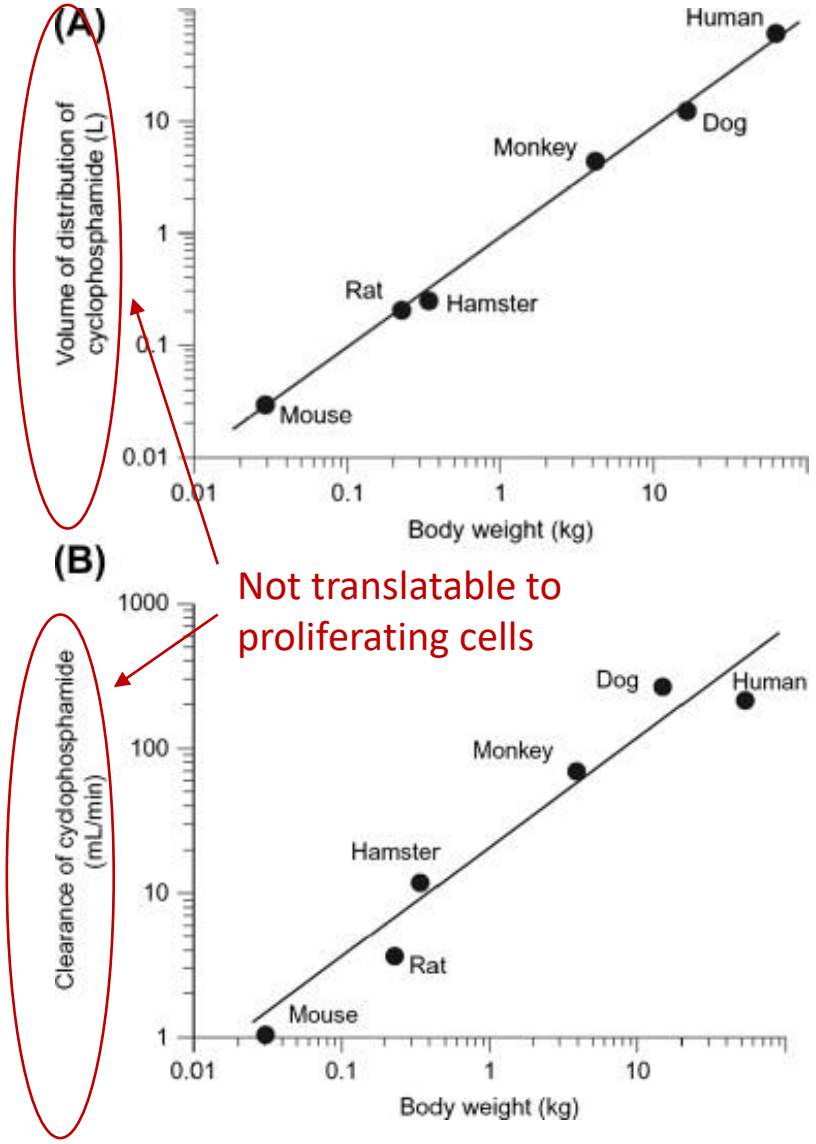
Reminder: Dose-Response Relationships do not Apply to Proliferating Cell Therapies

- For small molecules/biologics, the administered dose *is* the final dose, and so exposure of these modalities is predictable from non-clinical data

- Dose selection based on allometric scaling should not be used for proliferating therapeutics (ab CAR T, iCART, CAR Treg)
  - Can not predict final cell "exposure" from initial dose: tumor burden, underlying immune status of the patient, concurrent medications all affect proliferation in unpredictable ways
  - Body weight scaling is sometimes used, although there is no apparent scientific basis for its use
  - Toxicity at any dose level in non-clinical studies must be considered a concern
    - Understanding the MOA of the toxicity can be important in crafting a weight of evidence approach for translatability and impact on FIH dose selection



Prothero J. Growth 1979;43:139–50 [2] (heart weight) and Adolf EF. Science 1949;109:579–85 [1]

Nonclinical Pharmacokinetics and Biodistribution (example αβ CAR T)

In vitro and *in vivo*

- Cellular Kinetics
  - CAR T cell expansion and resolution in xenograft tumor model
    - Can be used to inform potency, activity of CAR
    - Set timepoints to fully characterize expansion and retraction/clearance

- Biodistribution
  - If there is homology/cross-reactivity of tumor target in study animals, biodistribution data may inform tolerability/safety
  - If no homology/cross-reactivity, utilize for interpretation of efficacy/safety data within the study

- Bioanalysis
  - If further engineered for cytokine/chemokine secretion or expression, analysis of those analytes should be captured in vitro and in vivo at different kinetic timepoints

Considerations for Nonclinical Development of CAR Therapies (example autologous αβ CAR T)

- Pharmacology
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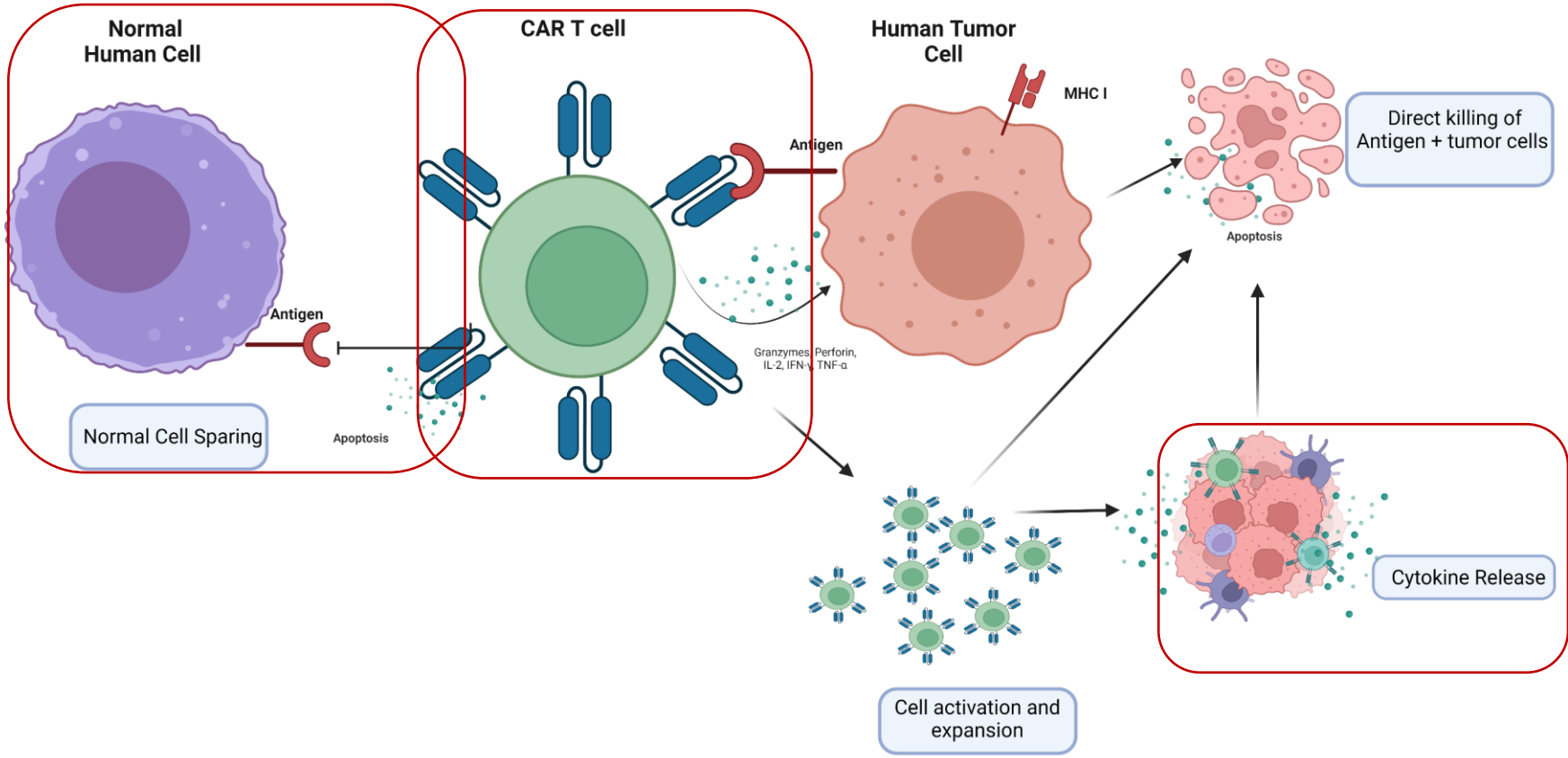
- **Safety (Toxicology)**



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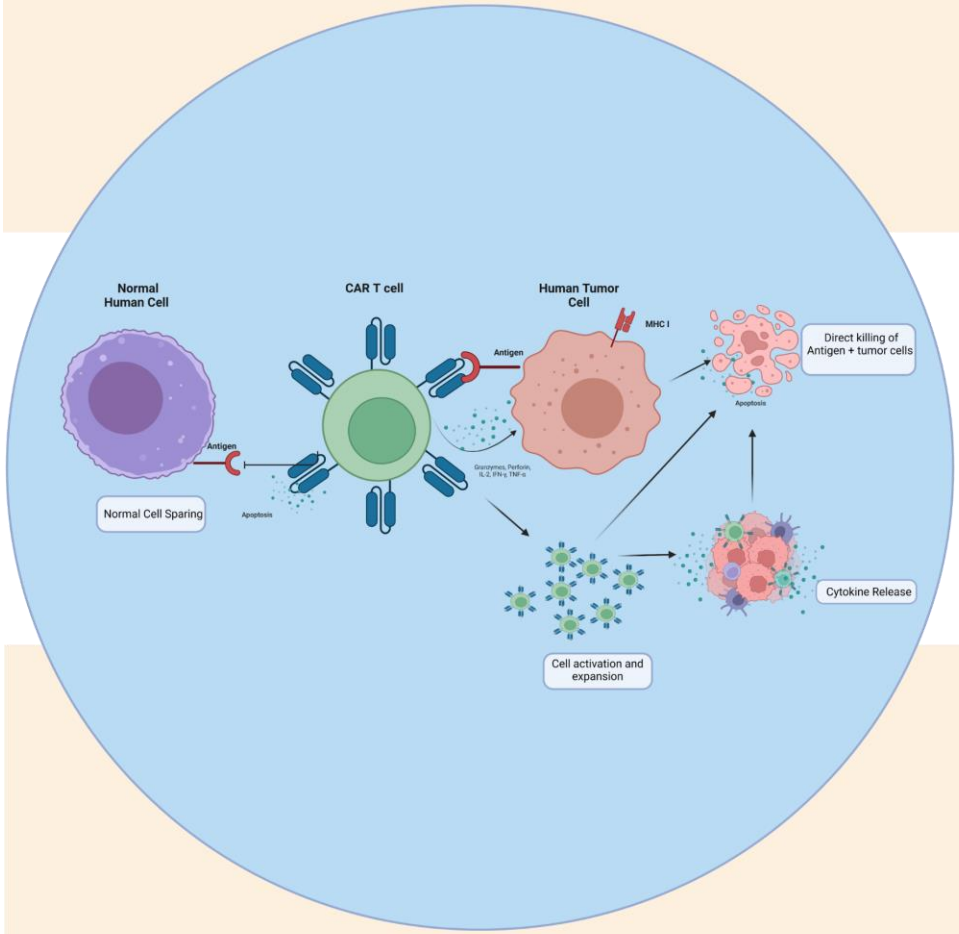
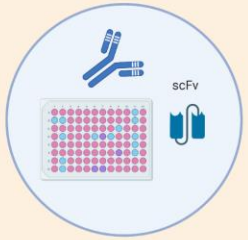
Nonclinical Toxicology (example autologous αβ CAR T)

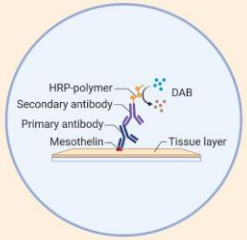


Off-tumor cytotoxicity

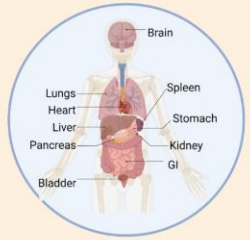
Retrogenix off-target binding screen



Target Distribution in Human Tissue by IHC

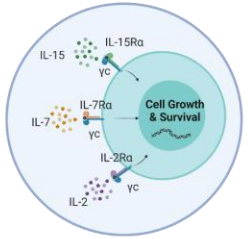


Normal Cell Killing *in vitro*



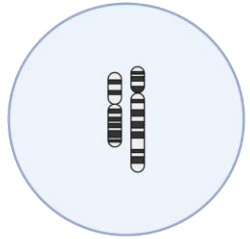
Tumorigenicity

Cytokine-independent Growth Assay

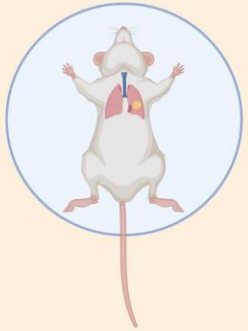


Integration Site Analysis

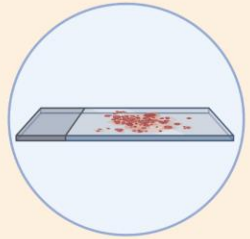
Karyotyping



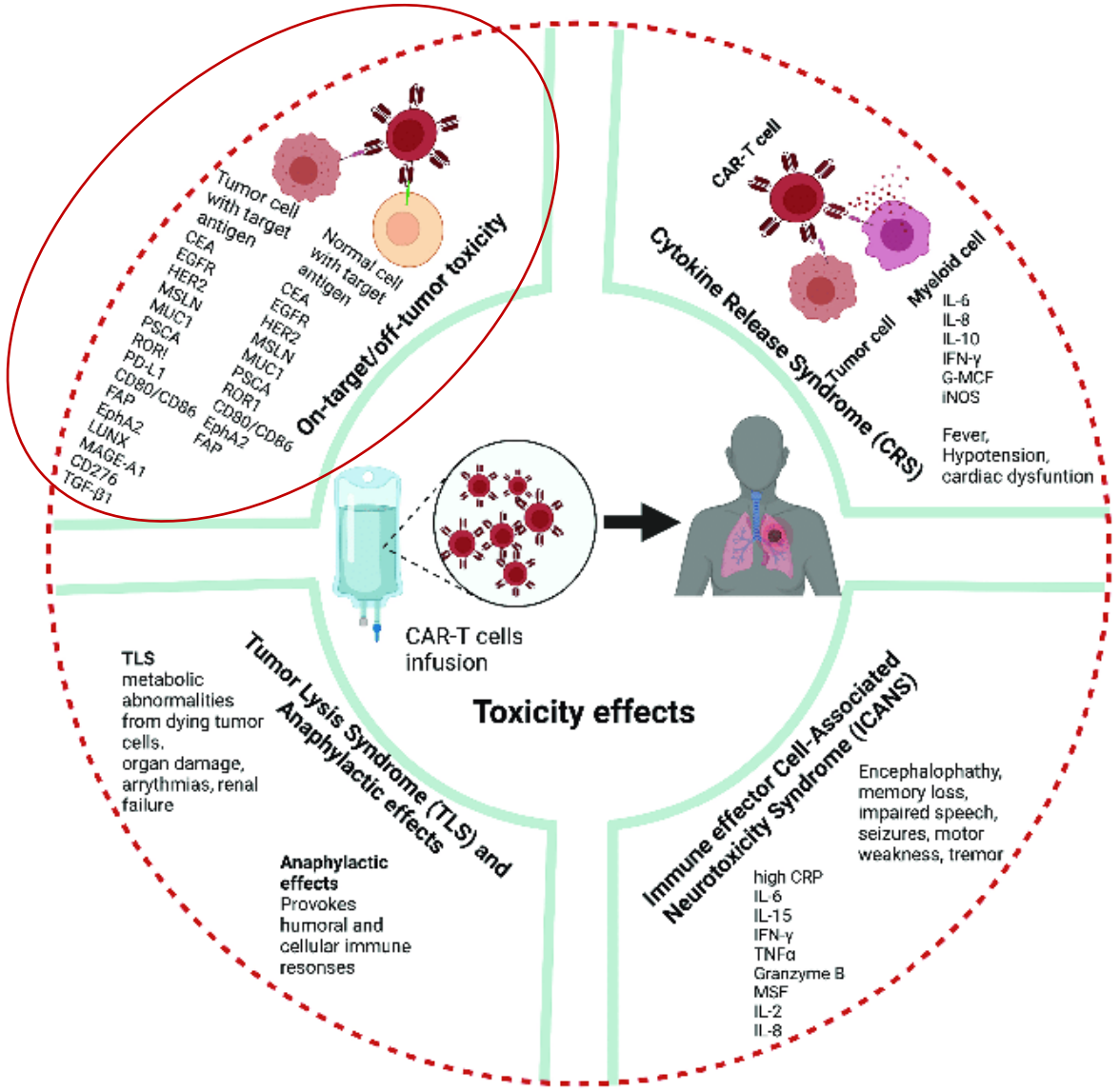
General Toxicity



Combined Pharmacology & Toxicology Study

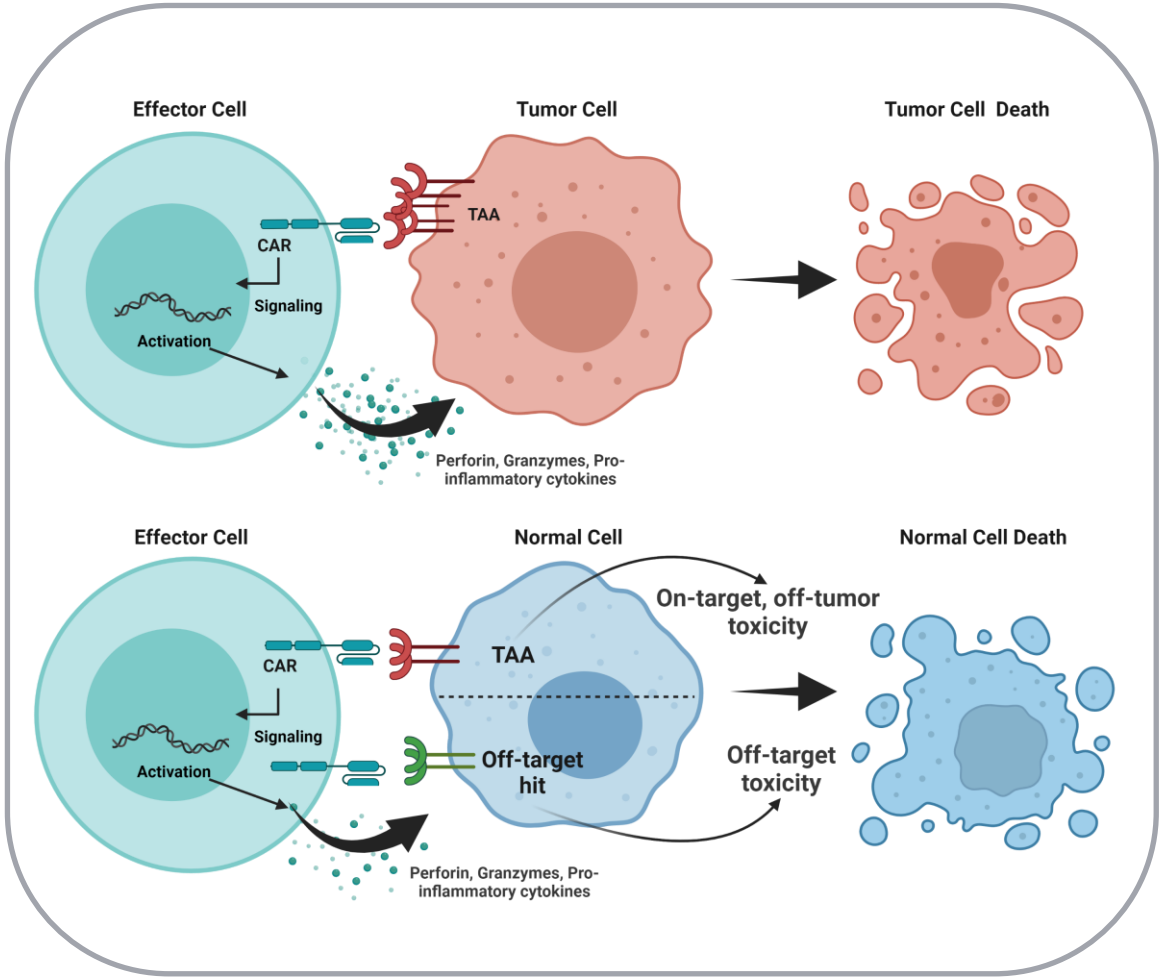


Common side-effects of CAR-T cell therapies



Kandra et al. *Frontiers in Immunology* 2022

Off-tumor cytotoxicity is a key safety concern for CAR-T cell therapies



On target, off-tumor toxicity in CAR T cell Therapy Clinical Trials

Phase	Target	Indication	Efficacy	Toxicity On-target, Off-tumor
I/II	CAIX[1]	metastatic renal cell carcinoma	PD: 100% (3/3 pts)	G2-G4 liver toxicity (100% 3/3 pts), CAIX expression bile duct epithelium
I/II	HER2[2]	Metastatic colon cancer	NA (1/1 patient death)	G5 (100%, 1/1 pts), HER2 expression healthy lung tissue
I	CEA[3]	Advanced CEA+ malignancy	OR: 0% (0/14 pts)	G3 lung toxicity (100% 14/14 pts), CEA expression healthy lung tissue
I	Claudin 18.2[4]	GI cancers	OR: 49% (18/37 pts) DC: 73% (27/37 pts)	Mucosal erosion G3 (3%, 1/37 pts) G1/G2 (14% 5/37 pts) Claudin 18.2 expression healthy GI

[1]Lamers CH et al. *J Clin Oncol*. 2006
[2]Morgan RA et al. *Mol Ther*. 2010
[3]Thistlethwaite FC et al. *Cancer Immunol Immunother*. 2017
[4]Qi C et al. *Nat Med*. 2022

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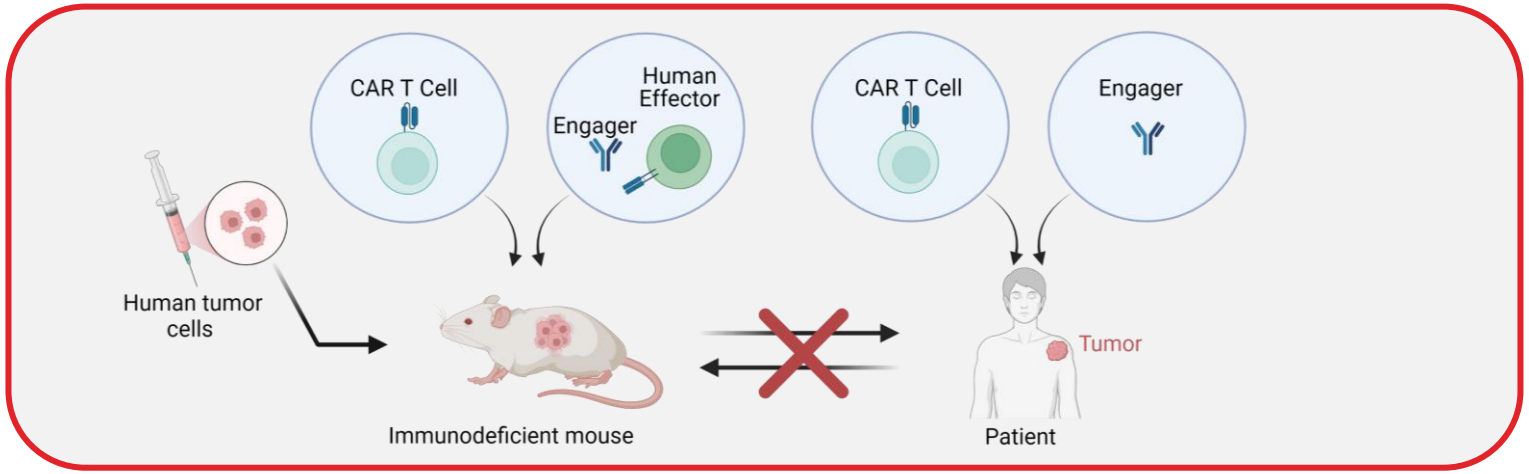
Challenges with preclinical assessment of off-tumor cytotoxicity



In an ideal world

- ✓ *Conserved target expression & biology*
- ✓ *Binder cross-reactivity between human & preclinical species*
- ✓ *Ability to assess efficacy (anti-tumor activity) & safety (off-tumor cytoxicity) within an animal model.*

In reality

- ✓ *Species specific differences in target expression or biology*
- ✓ *Lack of binder cross-reactivity.*
- ✓ *Safety cannot be assessed in animal model.*

Nonclinical Toxicology (example αβ CAR T)

Off-tumor Toxicity

- Selectivity
  - Does the CAR bind <u>specifically</u> to the target?
    - Screen extracellular proteome for non-specific binding
      - Retrogenix membrane proteome array
      - Primary cells, cell lines, tissue sections…

Nonclinical Toxicology (example αβ CAR T)

Off-tumor Toxicity

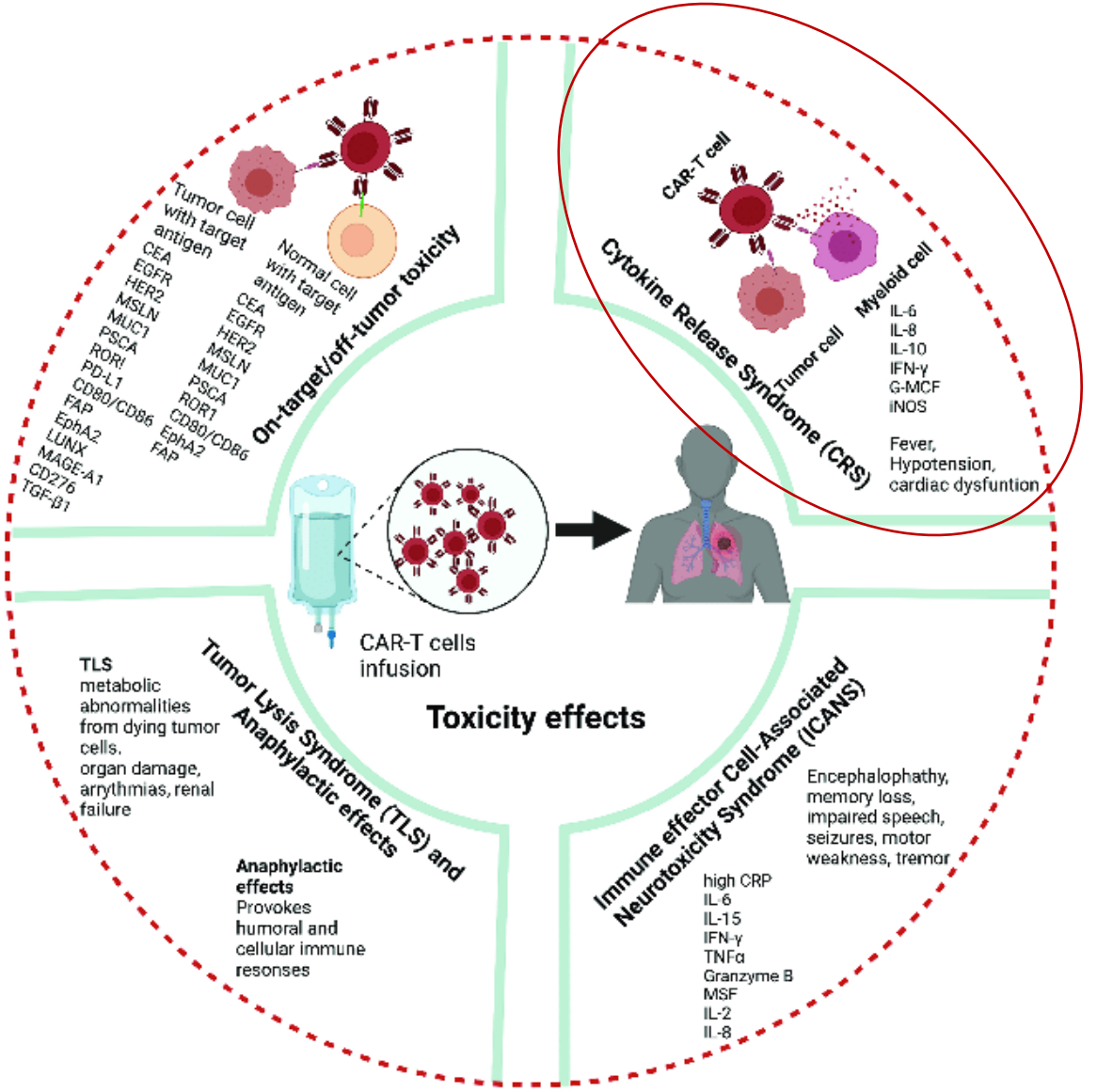
- Target expression
  - Where is the target expressed (normal cells, tissues)?
    - In silico/bioinformatic assessment
      - Evaluation of genomic and proteomic expression libraries
    - In vitro/ex vivo assessment of normal tissues
      - Tissue microarrays: In situ hybridization, immunohistochemistry
      - Test expression by mass spectroscopic assessment of membrane preparations (evaluate the extracellular membrane proteome)
  - Can expression be recognized by the CAR?***
    - Tissue cross-reactivity (TCR) study with the CAR (or the targeting element, ScFv) on a panel of normal tissues (immunohistochemistry)
    - Activation of CAR on targeted normal cells or tissues
      - Flow cytometry
      - In vitro TDCC or tumor vs normal TAA-expressing cell killing assay

*** TCR not required in Oncology indications based on ICH S9 Q+A
https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s9-guideline-nonclinical-evaluation-anticancer-pharmaceuticals-questions-answers-step-5_en.pdf

Nonclinical Toxicology (example αβ CAR T)

Cytokine Release Syndrome (CRS)

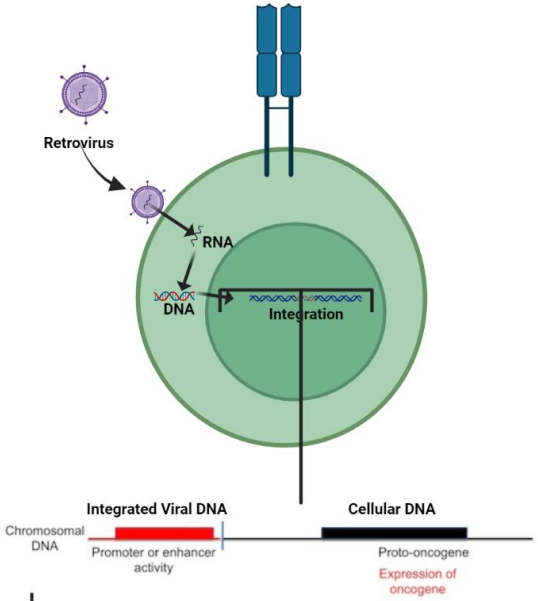
- Identified as a cell therapy concern
  - Recommendation for measuring cytokine secretion both in vitro and in vivo
  - Nonclinical assessment considered as “Hazard ID”
    - Added to risk management plan

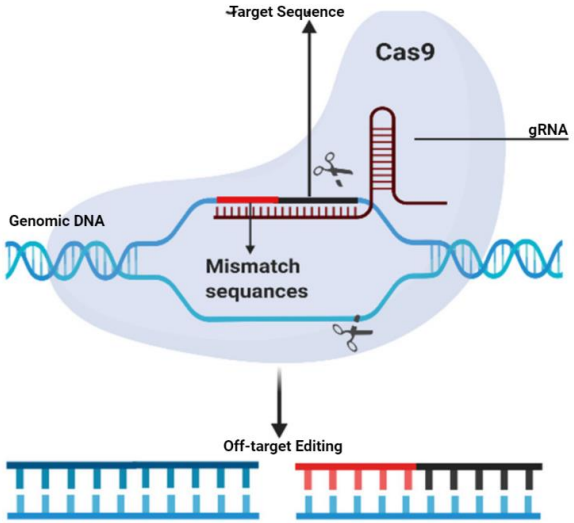


Kandra et al. *Frontiers in Immunology* 2022

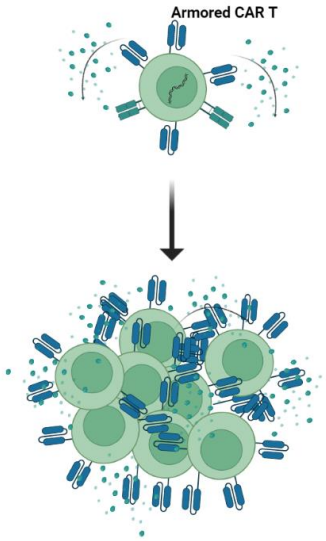
Multiple mechanisms can drive transformation or tumorigenic growth of cell therapy products

Process-related

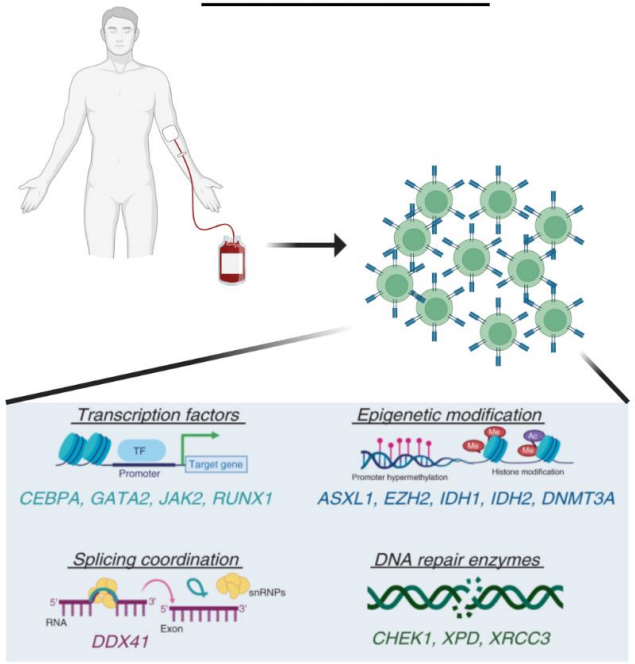


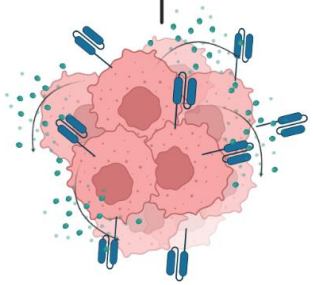


Product-related



Donor-related





What tools can we use to adequately characterize or predict this transformation potential with cell therapy products?

Created in Biorender.com and presented by CAR T Consortia

Nonclinical Toxicology (example αβ CAR T)

Tumorigenicity*

- Assessment of tumorigenicity is required, but long-term *in vivo* assessment of αβ CAR T cells is limited due to GvHD

- Cytokine-independent Growth Assay
  - IL-2 Independent growth assays have become a "standard surrogate/alternative" to *in vivo* tumorigenicity assessment of CAR Ts
  - Limitations in true positive controls, standardization of the assay, interpretation of results

- Insertion Site Analysis
  - Ensuring that there is no insertional bias into proto-oncorich regions of the genome that could lead to transformation

- Karyotyping
  - Monitor damage to whole chromosomes based on editing of the cells (or of donor cell product in general) or process-related excipients that could lead to transformational changes

**These some examples, general recommendation to use weight-of-evidence-based approach depending on the product*

Nonclinical Toxicology (example αβ CAR T)

General Toxicity

- Although stand-alone toxicity studies are not recommended due to the human-origin of cell therapy product and the need for the disease model to drive activity, safety assessments can be added to in vivo pharmacology studies
- On efficacy studies in human tumor-bearing immunocompromised mice, safety evaluations can include comprehensive gross pathology, clinical pathology, histopathology endpoints as well as other assessments (as appropriate), and integration of data with in-life parameters
- Similar endpoints may be added to any animal study to get an “early look” at safety or to aid in determining the potential cause of any unscheduled deaths.
  - Regulatory expectation to report and explain any safety-related findings, independent of the type of study (efficacy, CK, etc)
  - These results will typically be integrated into the pharmacology sections of regulatory findings

Overview

PART I: Summary

- Background on CAR Therapy
  - **Evolving field with potential in being transformative for several diseases**
- Considerations for Nonclinical Development of CAR Therapies (example of $\alpha\beta$ CAR T)
  - **Many limitations in the translatability of nonclinical assessments to clinic, but important to characterize the activity, kinetic, and safety profile of these products**

PART II:

- Considerations for Clinical Study Design
- Future of Cell Therapy as a Treatment Modality for Oncology

Overview

PART I:

- Background on CAR T Therapy
- Considerations for Nonclinical Development of CAR T Therapies

PART II:

- **Considerations for Clinical Study Design**
- **Future of Cell Therapy as a Treatment Modality for Oncology**

Early Stage CAR T Cell Trials: Understanding the Regulatory Environment

- Regulatory agencies are becoming very informed with regard to cell and gene therapies
- To design clinical trials one must be aware of the general disease area guidance as well as the cell and gene therapy and in some cases CAR T cell specific guidance documents.
- Specific guidance documents to be aware of include:
  - Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products Guidance for Industry: FDA—2015
  - Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products Draft Guidance for Industry: FDA—2022
  - Long Term Follow-Up After Administration of Human Gene Therapy Products Guidance for Industry: FDA—2020
  - Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells: EMA—2021
  - Optimizing the Dosage of Human Prescription Drugs and Biological Products for the Treatment of Oncologic Diseases Draft Guidance for Industry: FDA—2023

Regulatory Guidance Early Phase Trials Dose Selection

- One of the main purposes of an early phase study is dose finding
- Regulatory agencies acknowledge this is more complicated in cell therapies.
- Justification of selected doses is complicated by available models
  - Regulatory authorities acknowledge that comparison between animal and human doses may not be sufficient
  - Justification using other clinical trials such as similar cell types, similar targets or similar exposures in other modalities is highly recommended
- Manufacturing considerations in Dose selection
  - Unlike other products doses may be in a range. Justification for the range is needed as well as assurance that range allows for adequate separation of dose cohorts
  - FDA specifically recommends that weight should be considered in the dose for safety reasons, but this has significant impact on allogeneic product manufacturing
- Standard approach of maximum tolerated dose (MTD) may not apply to cell therapies
  - Some products are expected to have limited toxicity
  - In vivo behavior may be vastly different making this hard to characterize in a small study
  - Manufacturing constraints may limit dose before toxicity does

Regulatory Guidance Other Dosing Considerations

- Repeat Dosing:
  - Regulators consider the persistence of cell therapies when providing guidance on repeat dosing
  - Therapies designed for long term persistence may not benefit from repeat dosing and may amplify toxicity
  - Consideration should also be given to repeat conditioning regimens which may add toxicity
  - Justification including biology, safety, regimen and previous experience should be provided in repeat dosing regimens
- Staggering:
  - Late complications are considered a significant risk in cell therapies so dosing of multiple patients close together is not recommended
  - In dose escalation long stagger periods between patients dosed should consider the potential duration of action of the cell therapy and allow observation of acute and subacute toxicity.
- Manufacturing Failure:
  - The risk of manufacturing failure is especially prominent for autologous products
  - If conditioning therapy is given this can cause significant harm to patients so should not be given until a dose is assured
  - Protocols should state if remanufacture is possible

Regulatory Guidance and Patient Selection

- Regulatory agencies consider CAR T Cell therapies to have significant clinical risks.
- Early phase studies are therefore expected to target patients where risk/benefit ratio is positive despite high toxicity potential
- In general, this means targeting patients who can demonstrate failure of other options and limited available therapies
  - In some rare diseases where there are no other therapeutic options this may only require demonstration of seriousness of the course of the disease.
- Physical condition of patients must be balanced with exhaustion of options. The population must be healthy enough to tolerate and benefit from the therapy despite potential side effects.
  - This also impact interpretability of the data
- If the product is specifically designed for pediatric patients additional safeguards must be in place.

Regulatory Guidance Clinical Monitoring

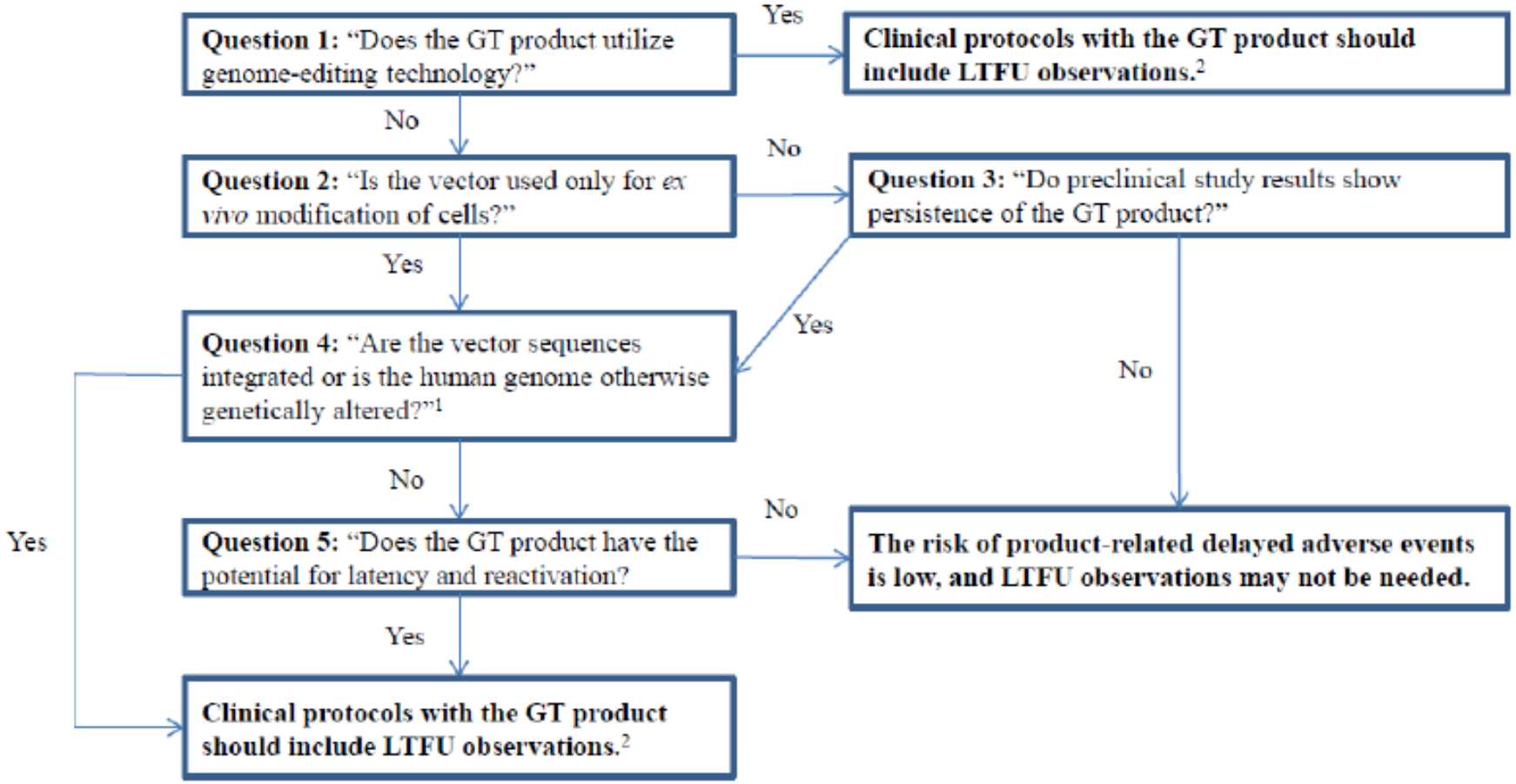
- In early phase studies of cell therapies, regulators consider 2 different aspects of patient safety monitoring.

1. Dose Limiting Toxicities (DLTs):
   - These are serious and acute toxicities that occur during an early safety window.
   - The FDA has given specific suggestions about language for DLTs surrounding cytokine release, autoimmunity and infusion reactions
   - Definition of DLTs should also consider prior experience with the target, overlapping tissue expression or experience in animal studies
   - Number of patients treated in a cohort should be sufficient to see distinctions based on DLTs
2. Stopping Rules:
   - Not all toxicities in CAR T studies are acute
   - Issue such as secondary malignancy, replication competent virus or graft vs. host related reactions may not occur in a typical DLT window
   - Events or toxicities of this type that would stop the study at any time for evaluation should be documented in the clinical protocol

Regulatory Guidance Long Term Safety Monitoring

- Regulators have acknowledged some complications that are specific to cell therapies that require long term evaluation even in early phase trials
- Immunogenicity: may persist as an issue as long as the cell product persists and may increase as patient immune recovery improves
- Persistence: This includes clinical pharmacology measures of persistence and tissue distribution as well as persistence of activity if possible, using biomarkers (e.g. B Cell aplasia with CD 19 targeting agents)
- Complications of Vectors: evaluation for clonality or replication competent vectors are recommended

Do You Need a Long term Follow Up Study?



Long Term Follow Up Studies and Evaluations

- Most CAR T clinical trials have 5 years of follow up built into the original study.
- However, patients who do not respond to therapy or relapse will need to pursue other treatments long before 5 years
- At the time that the initial study is opened an option for long term follow for these patients up needs to be available.
- Eventually, all studies will need a study that can cover the 15 year follow up required for long term safety evaluation, so the ideal scenario is to have both studies open simultaneously.
- If this is not an option and alternative schedule of events can be integrated into the primary study to allow limited follow up for patients who go on to other therapies.
- If this option is tried then the clinical protocol must allow patients to be enrolled on other clinical trials and receive other disease targeting therapies while still enrolled under certain conditions

Long Term Follow-Up After Administration of Human Gene Therapy Products Guidance for Industry: FDA—2020

First In Human Indications and Study Design Considerations

1. Is your target aimed at a single indication or multiple?
   - With single indication targets a simple phase 1 design including a dose escalation and expansion is usually favored.
2. If your target can be aimed at multiple indications, is the safety profile expected to be different in different indications?
   - If there isn't a reason to believe that safety may be different, then a single dose escalation in which all disease types are enrolled together may be considered to establish a uniform dose.
   - However, to fully characterize safety and understand potential for efficacy separate expansion by indication are likely going to be required.
   - If there is potential to need different doses in different indications, then separate studies are a better option.
3. If your target can be aimed at multiple targets is efficacy measured similarly?
   - The caveat to the all indications in one dose escalation study is if your indications are too disparate for the efficacy data to be meaningful.
   - Multiple solid tumors all looking at RECIST criteria for response are easier to place in a basket trial than multiple autoimmune conditions

First In Human Indications and Study Design Continued

4. Who is your first in human population compared to your goal population?
   - Regulators expect that FIH populations will be patients with few options and serious conditions who can take on the risk of untested CAR T studies
   - If this is the population intended for your label, then a continuous phase 1/2 study can be considered.
   - In this case your pivotal phase 2 study may still be single arm. However, a concurrent real-world evidence of outcomes in this population may be a helpful comparator.
5. If you want to treat earlier line patients, what outcomes will be different?
   - Phase 1 studies usually rely on response as primary endpoints, but in early lines survival may be prioritized by regulators
   - To get a read on this do you need a longer study follow up? Is there a potential biomarker of durability you can use?

Safety Monitoring Standardization

- In early CAR T studies CRS and ICANS were graded and treated according to algorithms defined in the individual clinical trials by the sponsor.
- While this can still be done, most sites are now comfortable with a set of algorithms and definitions that have been published. This also allows for more readily comparable data.
- All CAR T studies should consider using grading systems such as:
  - ASTCT Consensus CRS Grading
  - ASTCT ICANS Consensus Grading for Adults
- These grading systems do not line up specifically with standard data capturing approaches for other AE's so databases an statistical analyses must consider these differences in order to not double count or under characterize adverse events.
- Published treatment algorithms such as CARTOX are also commonly used
- If there is a safety switch or other modality in your constructs algorithms and directions for use should be included in your study

Lee et al 2019, University of Texas MDACC 2019

Safety Monitoring and Treatment Beyond the Basics

- Allogeneic Cell Therapies should also consider standard approach to grading of graft vs. host disease (GvHD) utilizing grading systems used in stem cell transplant.
  - Mount Sinai Consortium Acute GvHD Criteria
  - NIH Consensus grading criteria for Chronic GvHD
- Consideration should be given to your target, delivery mechanism and known clinical or animal experience.
- If there is reason to believe in a serious or common complication due to your target or cellular modality, then recommendations for addressing this toxicity may be considered if a potential mechanism and treatment modality are known.
  - If approaches used in other therapies can be leveraged these can be used for guidance.

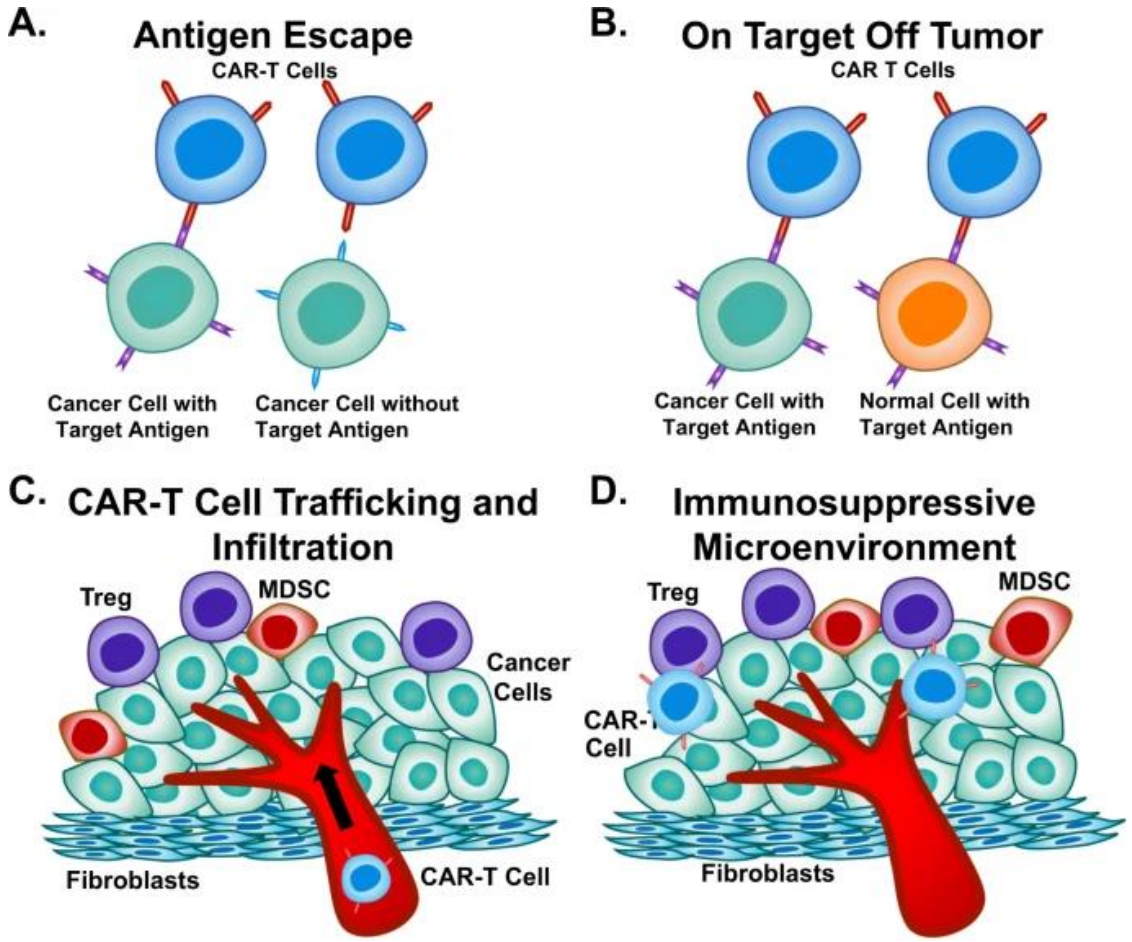
Donor Type Impact on Patient Selection and Trial Design

- Autologous Donors:
  - Collection of source material from the patient means that patients are enrolled with active disease and must have cells collected and manufactured.
    - Protocols must include inclusion criteria/exclusion criteria that assure safety for collection procedure.
    - If the disease is aggressive then consider allowing bridging therapy. This may be defined in the protocol or physician choice but must be captured in your database.
    - For slow developing diseases or rapid manufacturing bridging may be excluded
    - If bridging is allowed, then repeat disease evaluation is needed before lymphodepletion
  - Collection of source material from the patient may impact cell quality
    - If there are know characteristics of donors which impact ability to manufacture these should be considered I the patient population
- Allogeneic Donors:
  - Risks Related to donor matching should be considered
    - Can patients with prior transplant be included? What about prior GvHD
    - Are cells more likely to be rejected and does this impact dosing plan? Multi-dosing?
  - If editing has been used to address allogeneic risks
    - Potential toxicities from editing must be incorporated into short and long term follow up

Considerations for Non-Oncology CAR T Patients

1. Has the target/product been utilized in Oncology first?
   - If the product has already been used in an Oncology indication, then under certain circumstances no dose finding or a short run-in may be all that are required
   - If the target has been used, then it may be helpful to utilize clinical data to support dosing plans
2. Can the established oncology dose be used in non-malignant conditions?
   - Is there likely to be significant difference in starting material characteristics?
   - Is there likely to be differences in safety or overall patient health?
3. Is the conditioning regimen appropriate for non-malignant indications?
   - Most oncology clinical trials utilize chemotherapy agents to generate an environment to support cell expansion and persistence
   - These regimens may not be appropriate to expose patients without malignant conditions
   - Other mechanisms of armoring or immune suppression may be considered
4. Does the modality have the same safety impact?
   - Different immune cell types have different risks
   - Innate immune cells have demonstrated less risk of CRS and ICANS
   - Tregs have shown potential to be more immune suppressive
   - Inclusion criteria, DLT’s and monitoring most reflect this biology

Expanding Beyond Hematologic Malignancies



- The future of CAR therapy as a modality is dependent on more generalizable usage.
- In Oncology this means efficacy in solid tumors
- Solid tumor present a number of obstacles not seen in B and Plasma Cell malignancies
- Targets are less consistent expressed and more easily down regulated or internalized
- Targets are also expressed on tissues that cannot be damaged in the way that B Cells can be eliminated.
- Tumors are harder to reach from the blood stream
- And immune tissue once penetrated is harder to impact due to local immune suppression

Sterner 2021

Strategies to Unlock Solid Tumors

1. Antigen Escape
   - Strategies utilizing multiple overlapping targets may limit the impact of low target expression
   - Innate immune cells provide an additional mechanism of action through stress ligands which may be able to target tissues that are not targeted by the CAR
2. On Target Off Tumor Toxicity
   - Continued work is being done to identify antigens with limited toxicity and tumor specificity
   - Neoantigen based approaches and those which can impact internal targets without requiring HLA matching
3. Trafficking and Infiltration
   - Local administration approaches may bypass this need but can add complexity and may not be effective for metastatic disease
   - Conditioning regimens aimed at breaking down tissue stroma are being tried
   - Armoring with cytokine receptors may support trafficking
4. Immunosuppressive microenvironment
   - Cytokine armoring strategies may improve survival and efficacy in tissues
   - Combination regimens may be impactful especially the use of check point blockade

Solid Tumor CAR Therapy Combinations

- Single agent therapy is rarely used in malignant indications
- Even approved CAR T therapies would not be as effective without the combined effect of preceding lymphodepleting chemotherapy.
- Clinical trial designs to unlock solid tumors may consider:
  - Combinations with agents which impact tumor trafficking: tumor stroma may be hard for immune cells to penetrate, agents that break down tumor stroma such as nab-paclitaxel have been tried.
  - Timing with standard agents: lower tumor bulk has been shown to correlate positively with outcome in hematologic malignancies. Cell therapies given as adjuvant to standard chemo regimens might provide favorable tumor bulk and limit additional chemo exposure. Similar timing could be considered with surgery.
  - Combination with agents that improve the activity of CAR-T cells. Some agents have been shown to increase target expression or improve expansion or persistence (e.g. checkpoint inhibitors). These combinations offer the opportunity for synergy.
- Combinations will not all be seen as akin to lymphodepleting chemotherapy by regulators so stepwise approaches may slow clinical development

Limitations to Understanding Clinical Behavior in Solid Tumors

- Solid tumors represent a more complicated clinical environment as well.
- In hematologic malignancies blood is representative of the location for pharmacokinetic and dynamic behavior of cell therapies.
  - Blood samples are relatively non-invasive and easy to capture repeatedly
  - Clinical studies rely heavily on blood samples to understand the expansion, persistence and activation of CAR T cells
  - Blood samples allow CAR T Cell behavior to be chronicled over time and compared to response and tumor biology.
- Blood is not representative of solid tumors so blood samples are not able to identify any of the obstacles to success in solid tumors
- Further it is not known if pharmacokinetic and dynamic properties defined in the blood will reflect what is actually occurring in the tumor even if none of these obstacles is encountered
- Lack of adequate understanding of CAR T Cell behavior will hinder patient selection and future improvements

Biopsies and the Need for New Clinical Tools

- Biopsies remain the mainstay of understanding solid tumors
  - Biopsy tissue is needed to assess target expression for patient enrollment onto study
  - Biopsies are needed to understand off tumor effects when the occur
  - Biopsies are the only way to understand trafficking into tumors and intra-tumoral behavior
- Biopsies are invasive and cannot be done repeatedly
  - Unlike blood samples tumor tissue is difficult to get and can cause pain and injury to patients
  - It is unlikely that more than one biopsy can be done on study so unlike hematologic diseases, behavior over time cannot be established on biopsies
  - This significantly limits understanding of pharmacokinetic behavior
- Correct timing is crucial and yet unknown
  - I mid-cycle biopsy may be the most important test done on a solid tumor study, but only if timed so that CAR T cells are expected to have reached the tumor and been activated
  - However, it is not known when after infusion this will be
  - Clinical trials often infer timing from hematologic malignancies or animal models but this timing is flawed

Developing Better Clinical Tools for Solid Tumors

- Imaging has been the mainstay of evaluation for solid tumors and represents an area of interest for clinical evaluation.
- Broadly available techniques such as metabolic imaging (e.g. PET Scan) may show intra-tumoral inflammation but do not immediately distinguish from tumor growth and do not aid in understanding kinetics.
- Techniques are beginning to be developed to track CAR T cells by imaging.
  - Adding a tracible element to the vector would allow expansion and trafficking to be understood but adds complexity and takes up valuable space
  - Labeling of infused cells with radio-opaque substances can identify trafficking but is diluted by dividing cells so cannot quantify kinetics.
  - Infusion of a radio tag specific to the CAR can show trafficking over time and repeat infusions may help understand kinetics but requires multiple exposures and washout time and the modality itself would require safety studies
- Despite these limitations new imaging modalities will likely be essential for understanding CAR therapy in solid tumor and for guiding clinical biopsies

Barriers to Generalized Use of CAR Therapy

- **Cost**
  - Approved cell therapies have enormous overhead and time so they are priced at a third to half a million dollars
  - While some of this cost is offset by the potential for a single use curative agent the market is not likely to absorb this in multiple high volume disease states
  - Allogeneic approaches may relieve some of this stress
- **Manufacturing**
  - Despite multiple CAR T cells with the same target access remains limited by manufacturing capacity
  - Resource limitation such as skilled technicians, reagents and vector supply will only worsen this as cell therapies become more prevalent
  - Allogeneic approaches and better equipment and supply chain management may improve these issues
  - Innovations such as local manufacturing may also be seen in the future
- **Patient Concerns**
  - Patient material may have limited quality and lead to manufacturing failures
  - Time to manufacture may limit access for patients who have rapidly progressing diseases may not have access.
  - Safety also limits access to patients who have good general health and access to specialized centers
  - Allogeneic approaches, new manufacturing innovations, different cell types and better safety prophylaxis may alleviate this

Summary of CAR Therapy Lecture

- **CAR Therapy is an evolving field with unique nonclinical and clinical challenges**

- **From this course, the following learning objectives should be achieved**

  - Describe the fundamental concepts of how a CAR T cell works
  - Understand the basic nonclinical assessments of CAR T therapy in support of clinical development
  - Understand considerations applied for clinical trial design of cell therapies
  - Recognize the barriers for future CAR T development in the field of Oncology

HESI Distance Learning Course: CAR Therapy

Post-Lecture Quiz Questions

Question 1: Which of the following of are functions of an αβ CAR T cell product (select all that apply):

a) Recognize a tumor cell via the antigen recognition domain

b) Secrete granzyme and perforin to kill a tumor cell

c) Secretes cytokines and chemokines to cause an immunosuppressive effect on T cells

d) Secretes cytokines and chemokines to expand and activate other T cells

Question 2: Stand-alone in vivo toxicity studies are required to characterize the safety and support clinical development of αβ CAR T products for oncology indications. True or False?

a) True

b) False

Question 3: Which of the below are unique regulatory complexities of CAR T cell studies:

a) Manufacturing considerations and clinical experience with other agents may be more important than scaling from animal models or identifying the MTD in dose justification

b) Due to the potential for cure regulators recommend first in human studies in early line patients

c) FIH studies may have short follow up periods due to rapid primary endpoints like response regardless of safety risks

d) Side effects in CAR T studies are usually rapid and predictable so dosing multiple patients in a short time is acceptable to regulators

Question 4: Broad use of CAR Therapies is limited by:

a) Safety concerns

b) Cost

c) Applicability outside of heme malignancies

d) Manufacturing constraints

e) All of the above

HESI Distance Learning Course: CAR Therapy

Post-Lecture Quiz Answers

Post-Lecture Quiz Answers

- Question 1: Answer A, B, and D
- Question 2: Answer B
- Question 3: Answer A
- Question 4: Answer E