คณะเภสัชศาสตร์
FACULTY OF PHARMACEUTICAL SCIENCES
Chulalongkorn University

เภสัชจุฬา 111 ปี 2024
111 YEARS ANNIVERSARY, PHARM CHULA

การควบคุมคุณภาพของผลิตภัณฑ์เซลล์แสดงผลทางภูมิคุ้มกัน

23 December 2024
9.00 – 15.00 hr

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- This presentation is intended solely for academic purposes and should not be used as a reference for regulatory, clinical, or manufacturing decisions.
- Any information provided here must be verified against official standard references or guidelines for accuracy and authority.
- Some of the content, including parts of the presentation and speech, may reflect the speaker's personal opinions or views, which are based on current scientific knowledge.
- It is important to note that the accuracy of the information is valid only at the time of the presentation. Users are encouraged to consult credible sources for updated and reliable information.

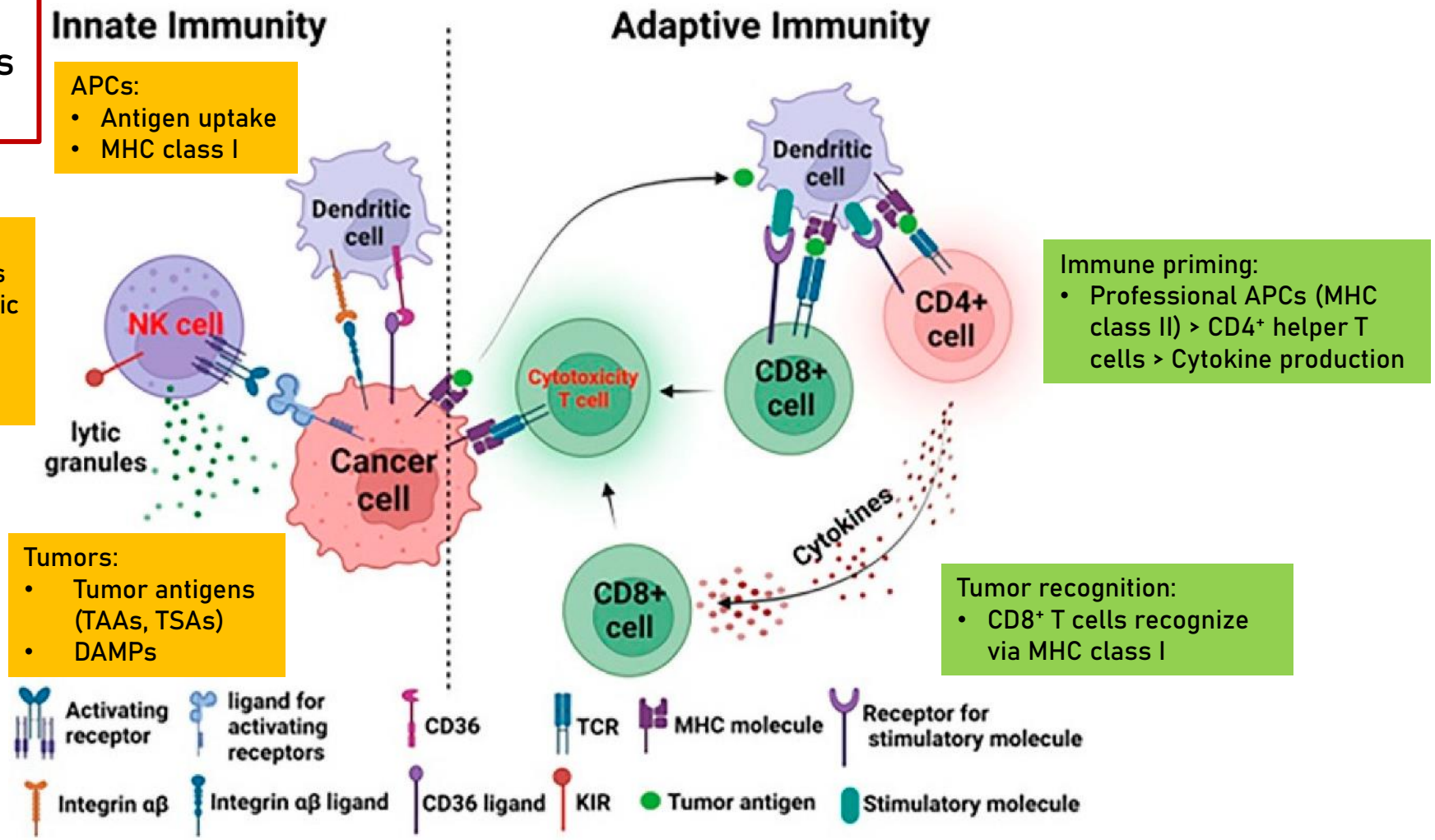
Key topics

1. Introduction to IECs
2. Control Strategy: Monographs and Specifications
3. Quality control
4. Future prospects of IEC treatment

Tumor Immunology: Introduction

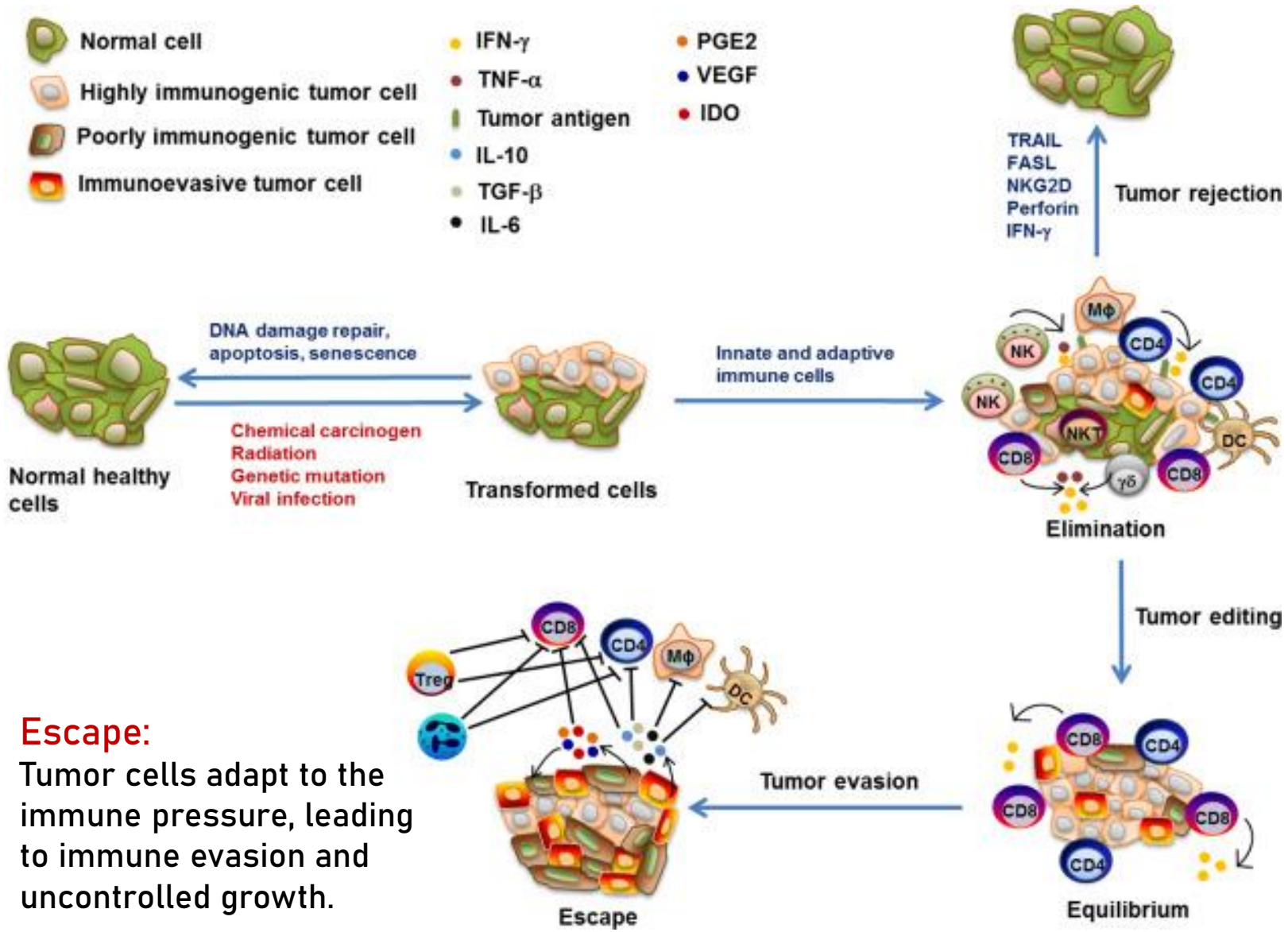
Innate immunity is nonspecific and rapidly generates defense reactions within foreign antigen encounters.

Adaptive immunity is based on the clonal selection of T cells and B cells with receptors recognizing specific 'non-self' antigens.



Yu Y. The Function of NK Cells in Tumor Metastasis and NK Cell-Based Immunotherapy. Cancers (Basel). 2023 Apr 16;15(8):2323.

Tumor Immunology: Immunosurveillance



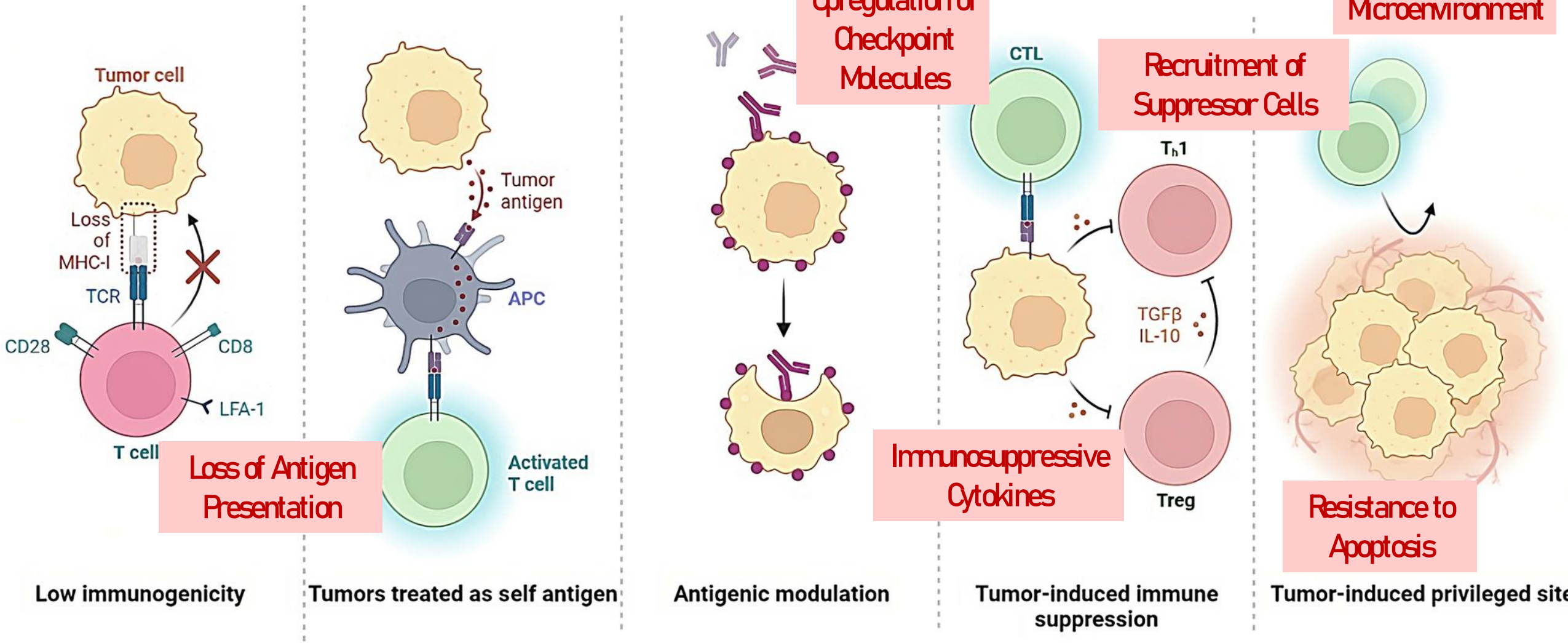
Elimination:
Immune cells are successfully detected and destroyed by immune cells.

Escape:
Tumor cells adapt to the immune pressure, leading to immune evasion and uncontrolled growth.

Equilibrium:
Tumor cells can persist to immune responses, so they remain dormant.

Sourav Paul, Girdhari Lal, Chapter 9 - Development and Function of Natural Killer Cells and Its Importance in Cancer Immunotherapy, Editor(s): MA Hayat, Immunology, Academic Press, 2018, Pages 117-140

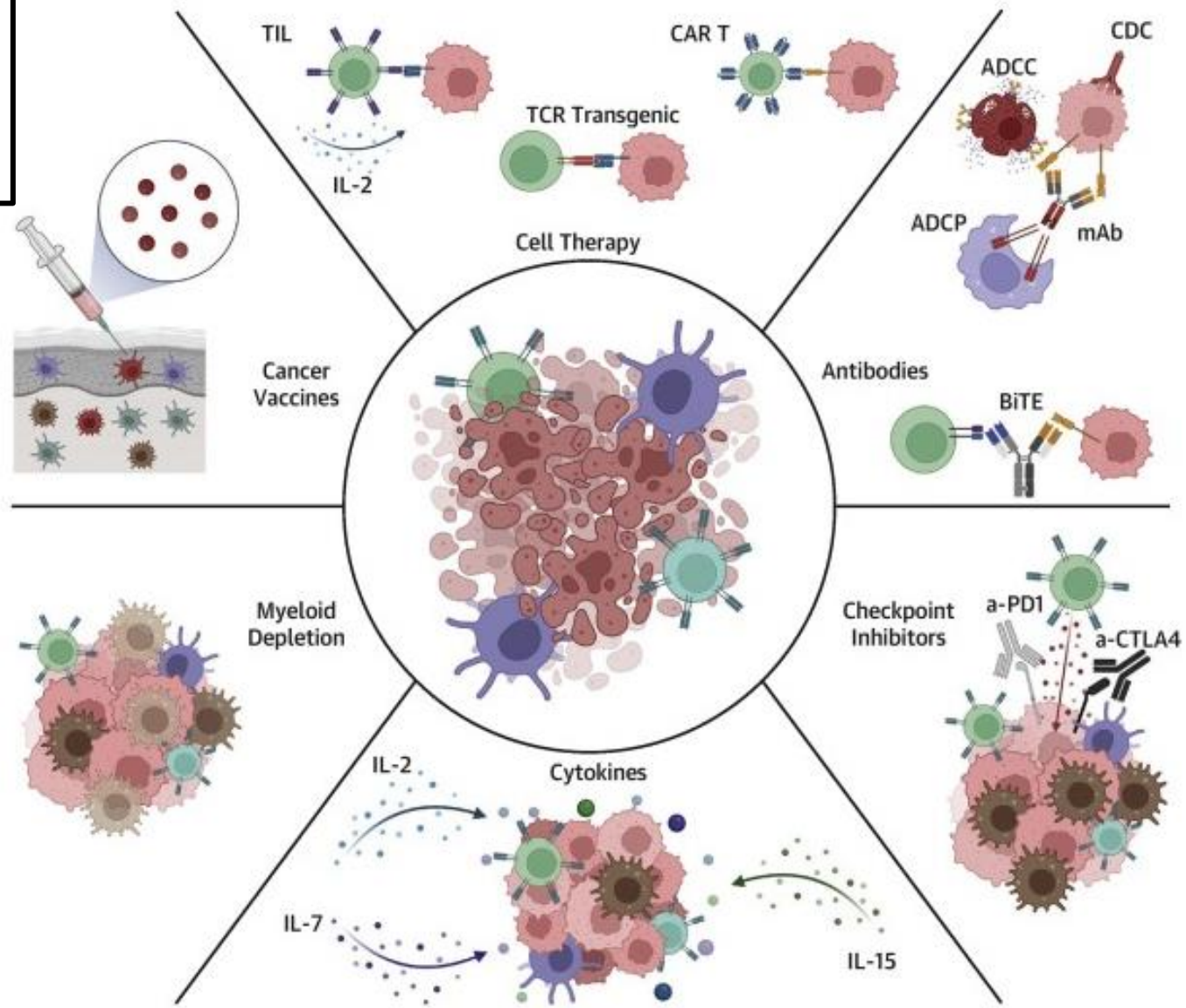
Tumor Immunology: Immune Escape



Kallingal, A, Olszewski, M, Maciejewska, N et al. Cancer immune escape: the role of antigen presentation machinery. J Cancer Res Clin Oncol 149, 8131–8141 (2023).

Tumor Immunology: Immunotherapy

Cell therapy:
- TILs
- CAR therapy
- CIK cells



Biologics:
- mAbs
- ADCs
- BITEs
- Cytokine therapy

Small molecules:
- Targeted therapy

Welty NE, et al. J Am Coll Cardiol CardioOnc. 2022;4(5):563-578.

Recap: Tumor Immunology and Immunotherapy

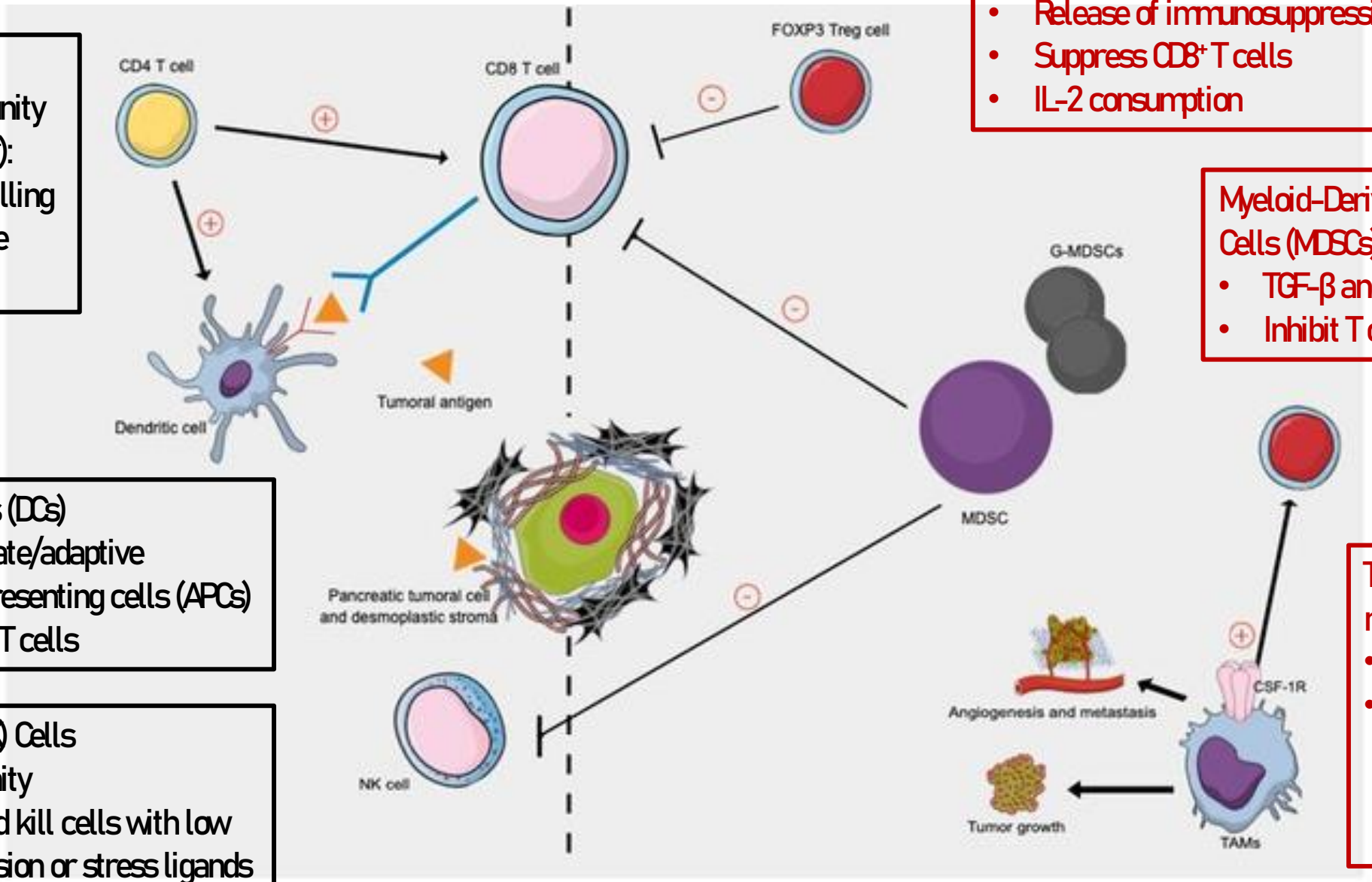
- Tumor Immunology: The immune system's ability to recognize and respond to abnormal cells, such as tumors.
- Immunosurveillance and Immune Escape: The dynamic balance between tumor growth and immune control, occurring in three phases: elimination, equilibrium, and escape.
- Immunotherapy: Cancer treatments based on tumor immunology principles, including targeted therapies, immune checkpoint inhibitors, immune effector cells, and cytokine-based therapies.

Immune Effector Cells: Overview

T Cells ($CD8^+$, $CD4^+$)
- Adaptive immunity
- Cytotoxic ($CD8^+$): Direct tumor killing
- ($CD4^+$): Cytokine secretion

Dendritic Cells (DCs)
- Bridge innate/adaptive
- Antigen-presenting cells (APCs)
- Activating T cells

Natural Killer (NK) Cells
- Innate immunity
- Recognize and kill cells with low MHC-I expression or stress ligands



Regulatory T Cells (Tregs)
- $FoxP3^+$, $CD25^+$
- Release of immunosuppressive cytokines
- Suppress $CD8^+$ T cells
- IL-2 consumption

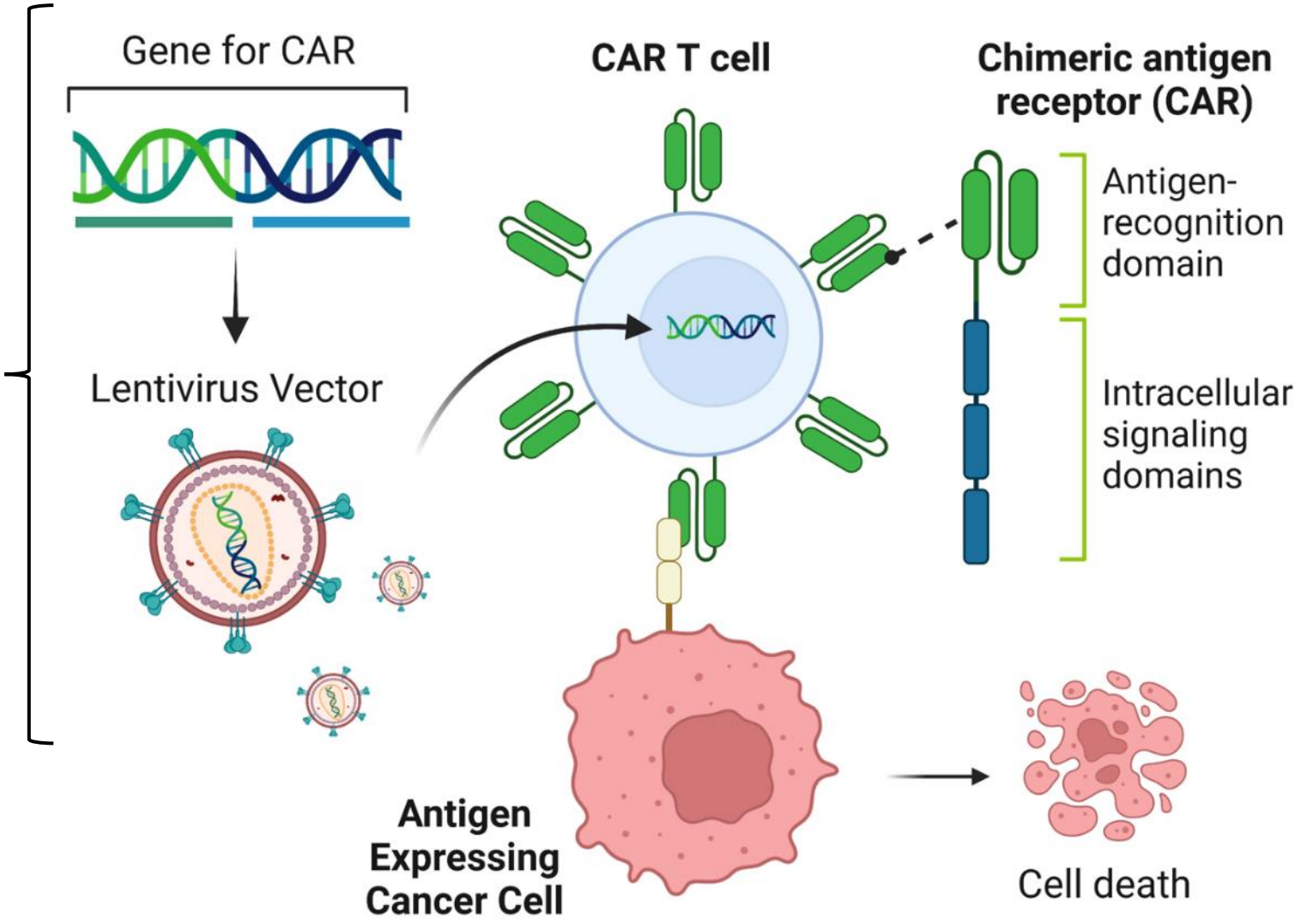
Myeloid-Derived Suppressor Cells (MDSCs)
- TGF-β and IL-10 Secretion
- Inhibit T cell activation

Tumor-associated macrophages (TAMs)
- M2 polarization
- Promote angiogenesis, suppress T cells, recruit Tregs

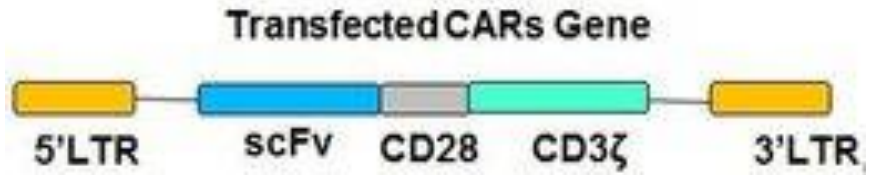
Muller M, Haghnejad V, Schaefer M, Gauchotte G, Caron B, Peyrin-Biroulet L, Bronowicki JP, Neuzillet C, Lopez A. The Immune Landscape of Human Pancreatic Ductal Carcinoma: Key Players, Clinical Implications, and Challenges. Cancers (Basel). 2022 Feb 16;14(4):995.

Immune Effector Cells: CAR Therapy

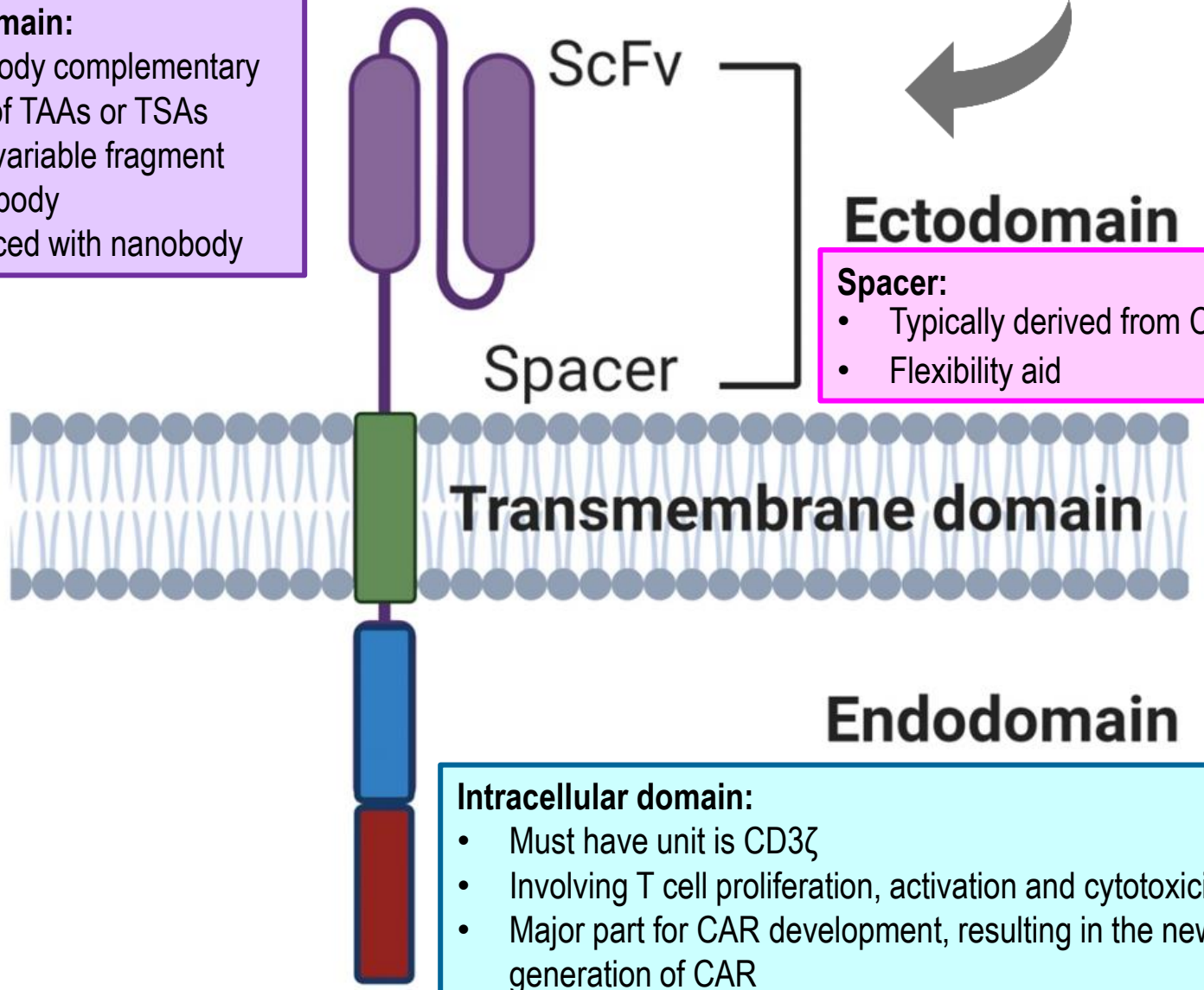
Genetic modification using recombinant DNA technology



Immune Effector Cells: CAR Characteristics



Extracellular domain:
- Antigen-antibody complementary
- Recognition of TAAs or TSAs
- Single chain variable fragment (scFv) of antibody
- Can be replaced with nanobody

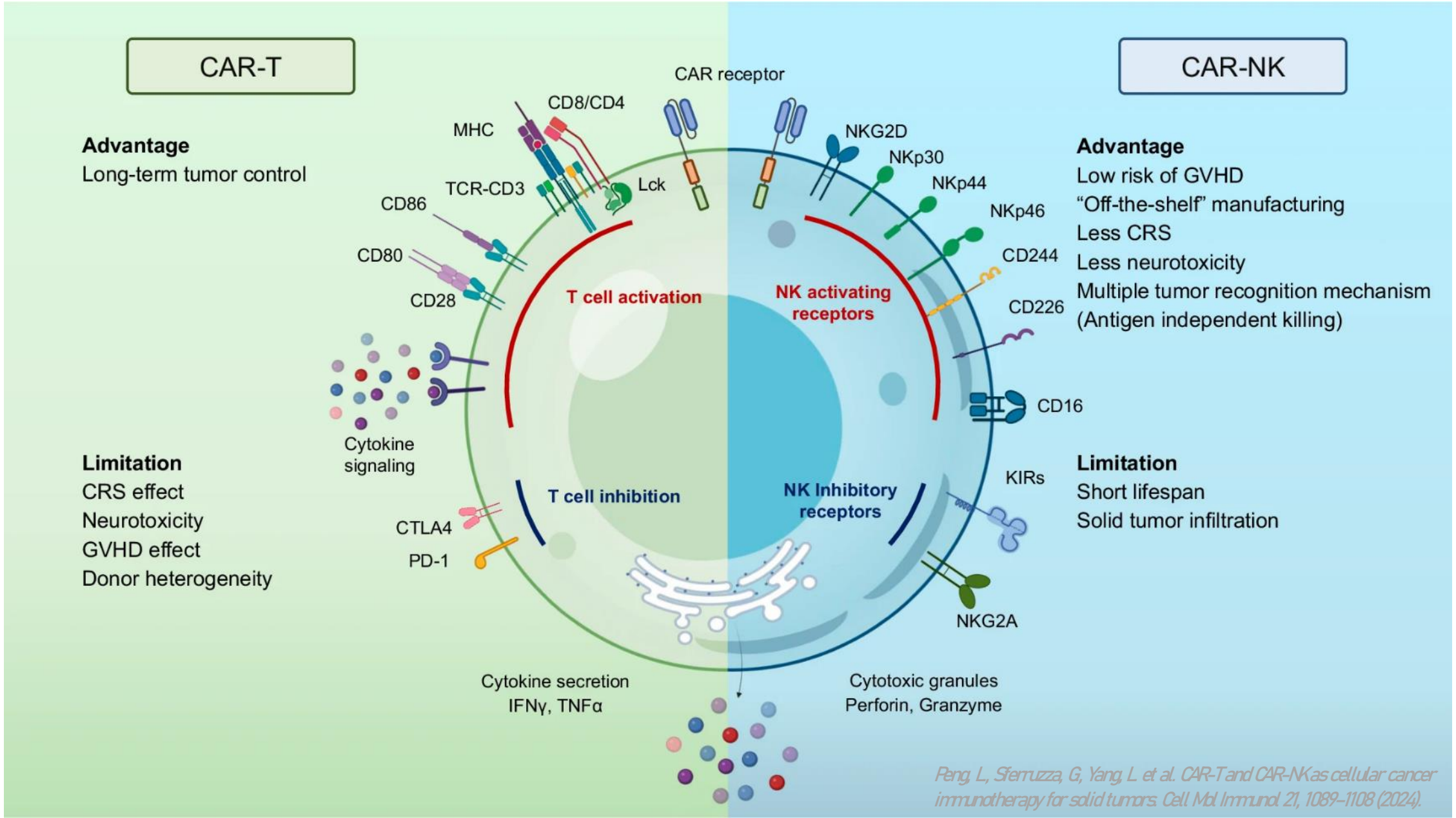


Spacer:
- Typically derived from CD28 or CD8
- Flexibility aid

Transmembrane domain:
- Typically derived from CD28 or CD8
- Linker of extra- and intracellular domain
- Signal transfer after the binding to recognition antigen

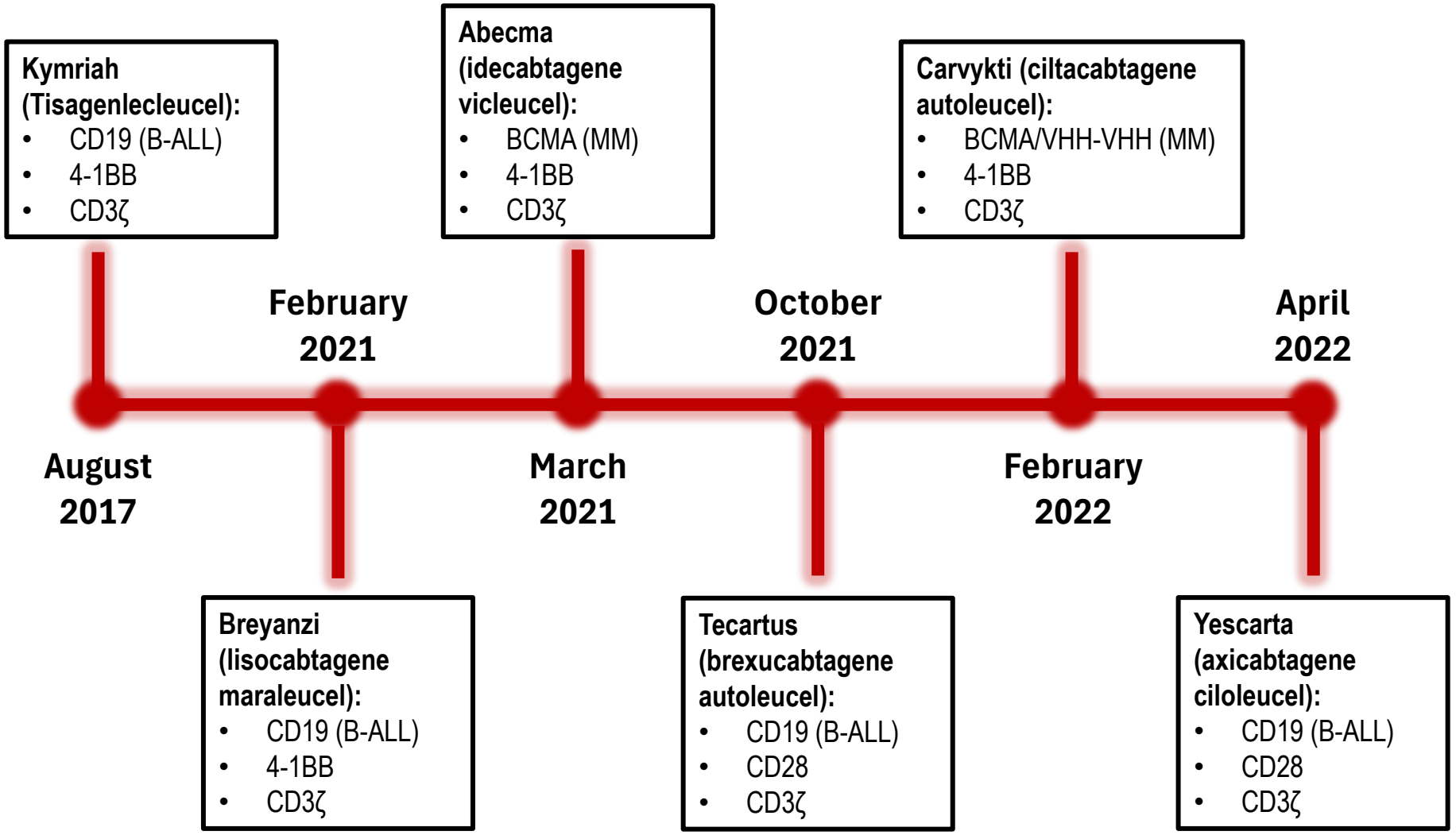
Intracellular domain:
- Must have unit is CD3ζ
- Involving T cell proliferation, activation and cytotoxicity
- Major part for CAR development, resulting in the new generation of CAR

Immune Effector Cells: CAR T vs CAR NK Cells

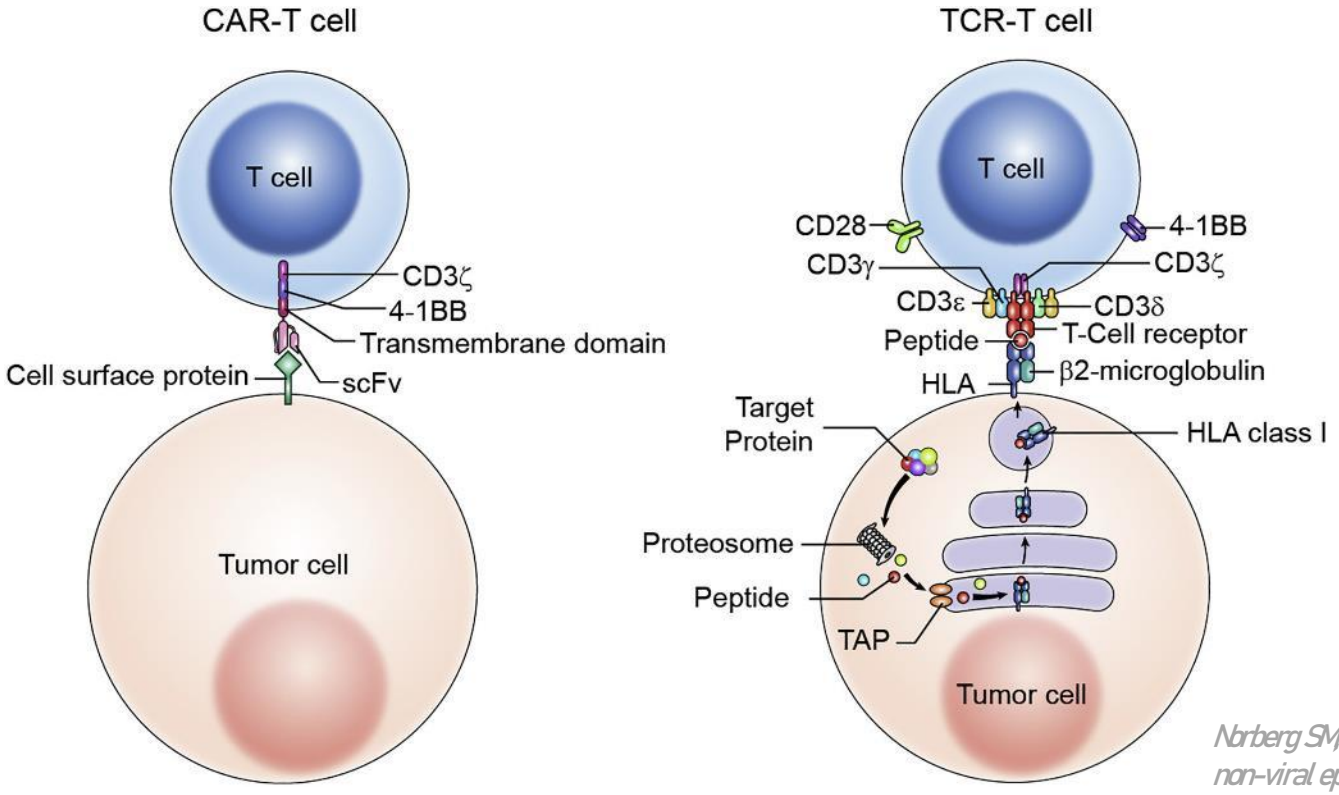


Peng L, Sferruzza G, Yang L, et al. CAR-T and CAR-NK as cellular cancer immunotherapy for solid tumors. Cell Mol Immunol 21, 1089–1108 (2024).

Immune Effector Cells: CAR Products



Immune Effector Cells: TCR Therapy



Norberg SM, Hinrichs CS. Engineered T cell therapy for viral and non-viral epithelial cancers. Cancer Cell. 2023 Jan 9;41(1):58-69.

Features	TCR Therapy	CAR Therapy
Target antigen	Intracellular antigens presented by MHC molecules	Surface antigens only
MHC Dependency	Yes, requires compatibility with specific HLA alleles	No, MHC-independent
Applications	Suitable for solid tumors and hematologic malignancies	Primarily effective in hematologic cancers, limited in solid tumors
Feasibility of Allogeneic Use	Limited due to MHC restriction	Increasing feasibility with TCR-knockout strategies
Development Stage	Experimental, under clinical investigation	Established, with FDA-approved products

Immune Effector Cells: TCR T Cell Products

Tecelra®
afamitresgene autoleucel
suspension for IV infusion

Tecelra

- Afamitresgene autoleucel (afami-cel)
- First FDA-approved T-cell receptor (TCR) therapy
- For metastatic synovial sarcoma

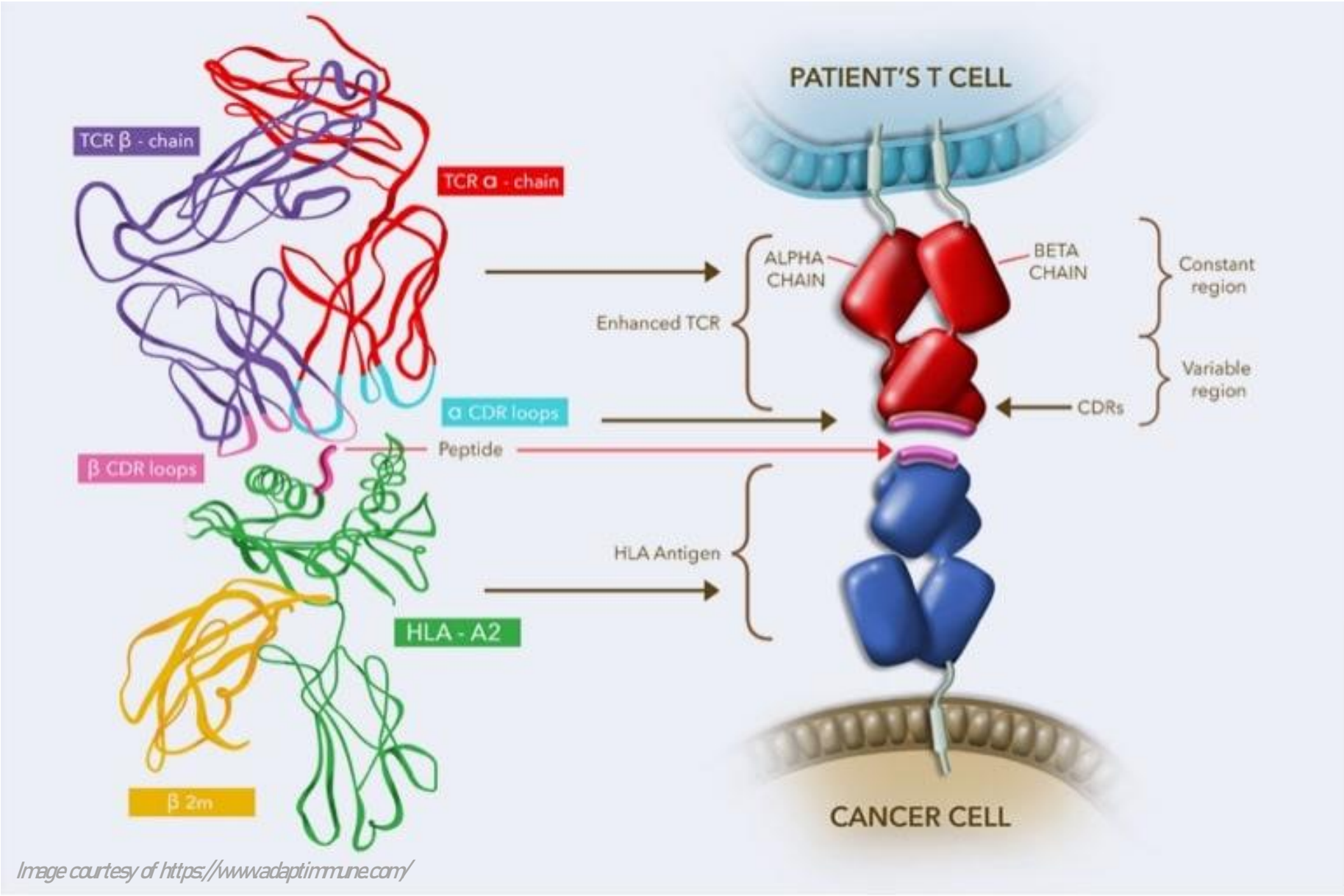


Image courtesy of https://www.adaptimmune.com/

CAR & TCR Products: Manufacturing Process

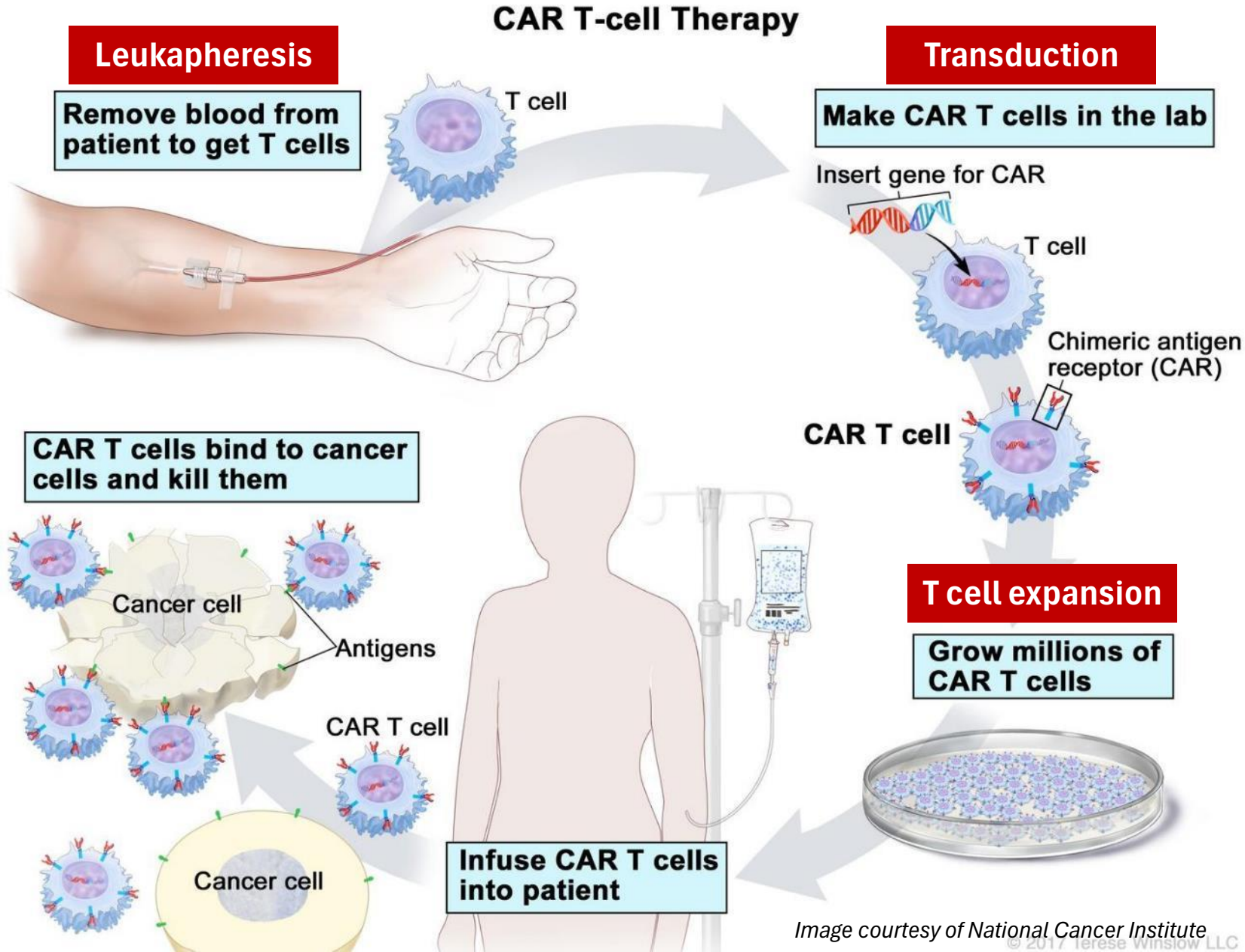


Image courtesy of National Cancer Institute

Immune Effector Cells: TILs

Tumor-Infiltrating Lymphocytes (TILs)

- Immune effector cells found within the tumor microenvironment (TME)
- TIL population: $CD8^+$ T cells, $CD4^+$ T cells, γδT cells, B cells, NK cells
- TILs can recognize multiple antigens, including neoantigens, which are unique to individual tumors
- TIL therapy is particularly promising for solid tumors, such as melanoma, where TILs naturally infiltrate

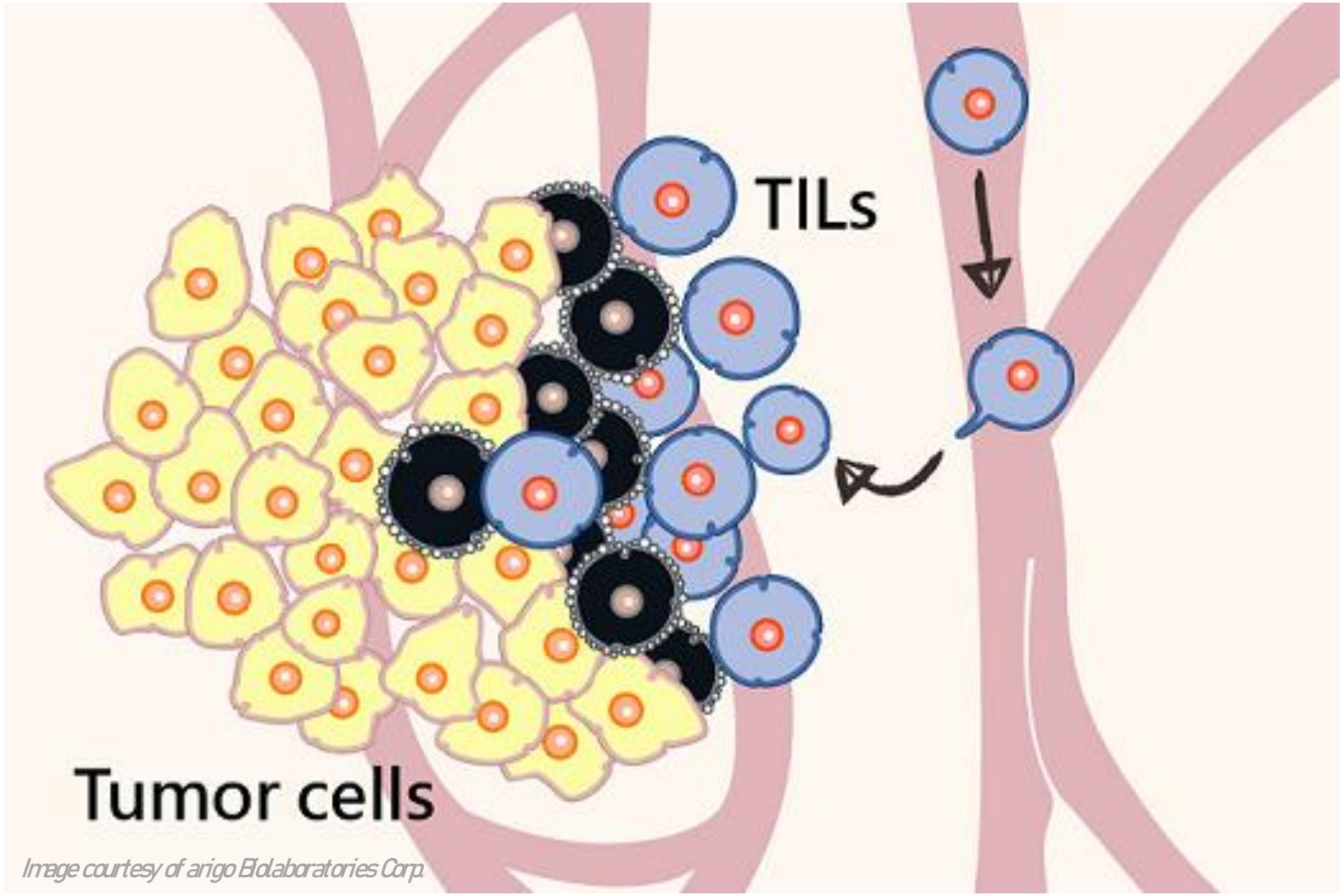


Image courtesy of arigo Biolaboratories Corp.

Immune Effector Cells: TIL Product and Manufacturing

AMTAGVI™ (lifileucel) Suspension for IV infusion

AMTAGVI (lifileucel), Iovance Biotherapeutics, Inc.

EXCISE: A tumor lesion is surgically resected

EXTRACT: Tumor tissue is fragmented and pooled fragments are cultured with IL-2 for TIL to accumulate in media

EXPAND: Massive expansion of TIL occurs in the presence of OKT3 & feeder cells, yielding a polyclonal mixture of 10^9 – 10^{11} TIL

PREPARE & INFUSE: Patient receives non-myeloablative lymphodepletion and is infused with their (autologous) expanded TIL and IL-2

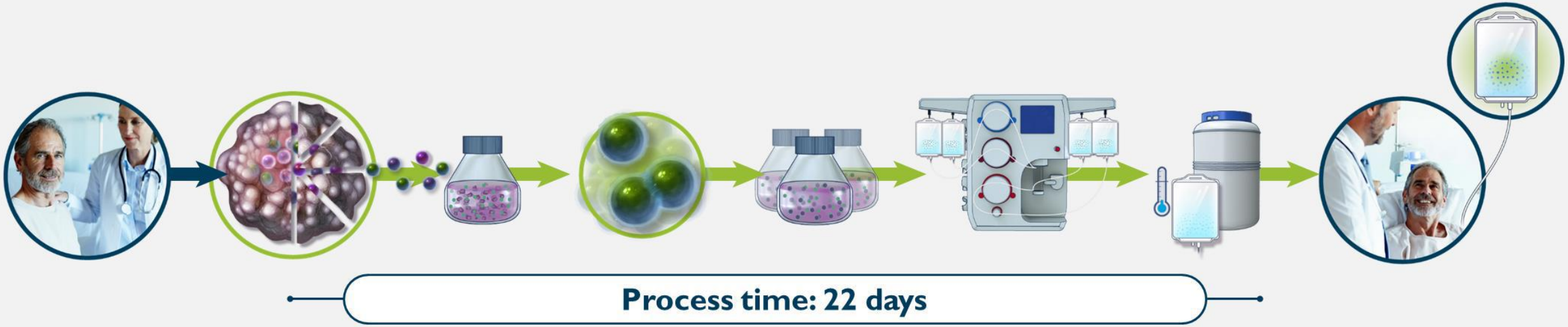
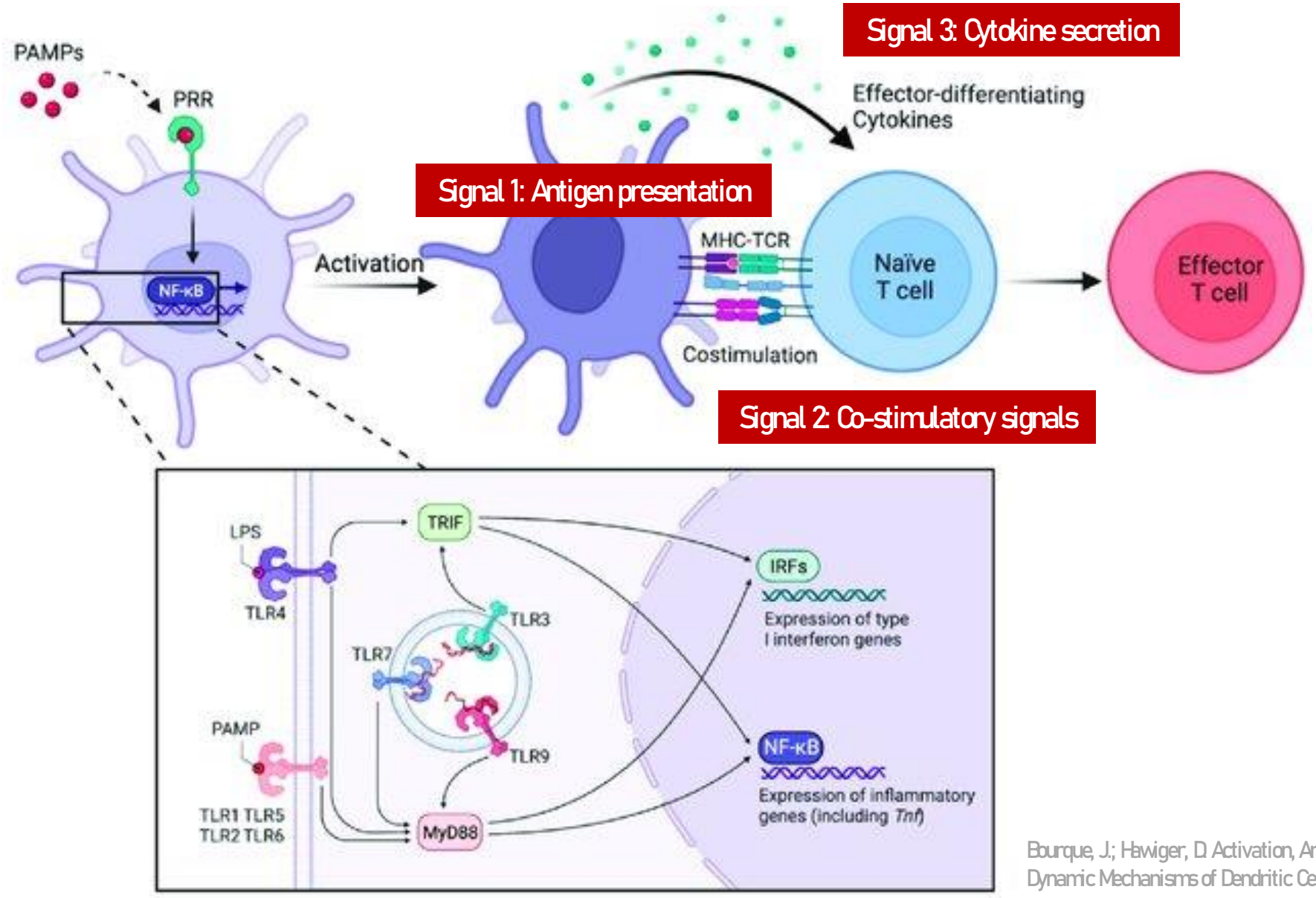


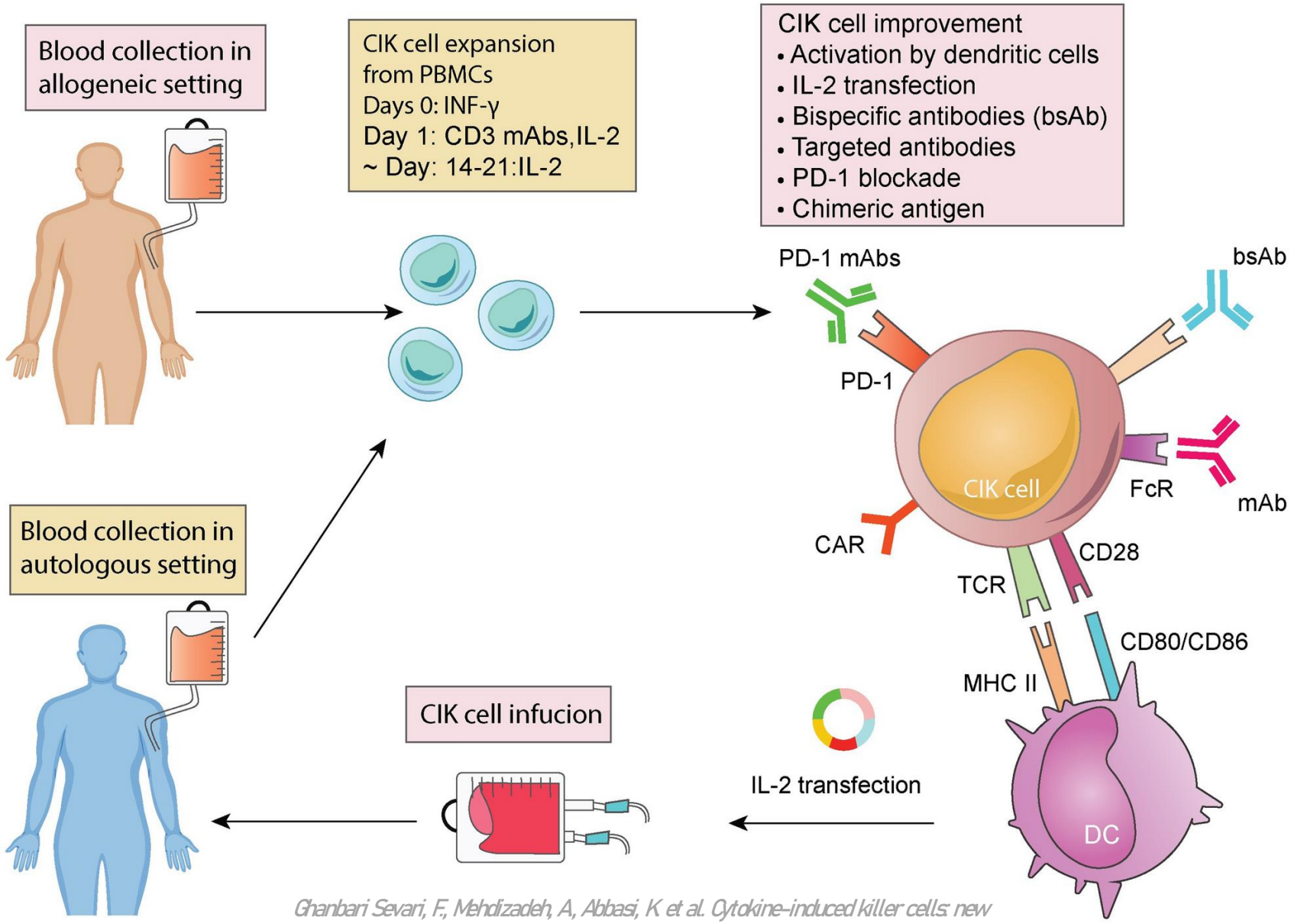
Image courtesy of IOVANCE Biotherapeutics, Inc.

Immune Effector Cells: CIKs



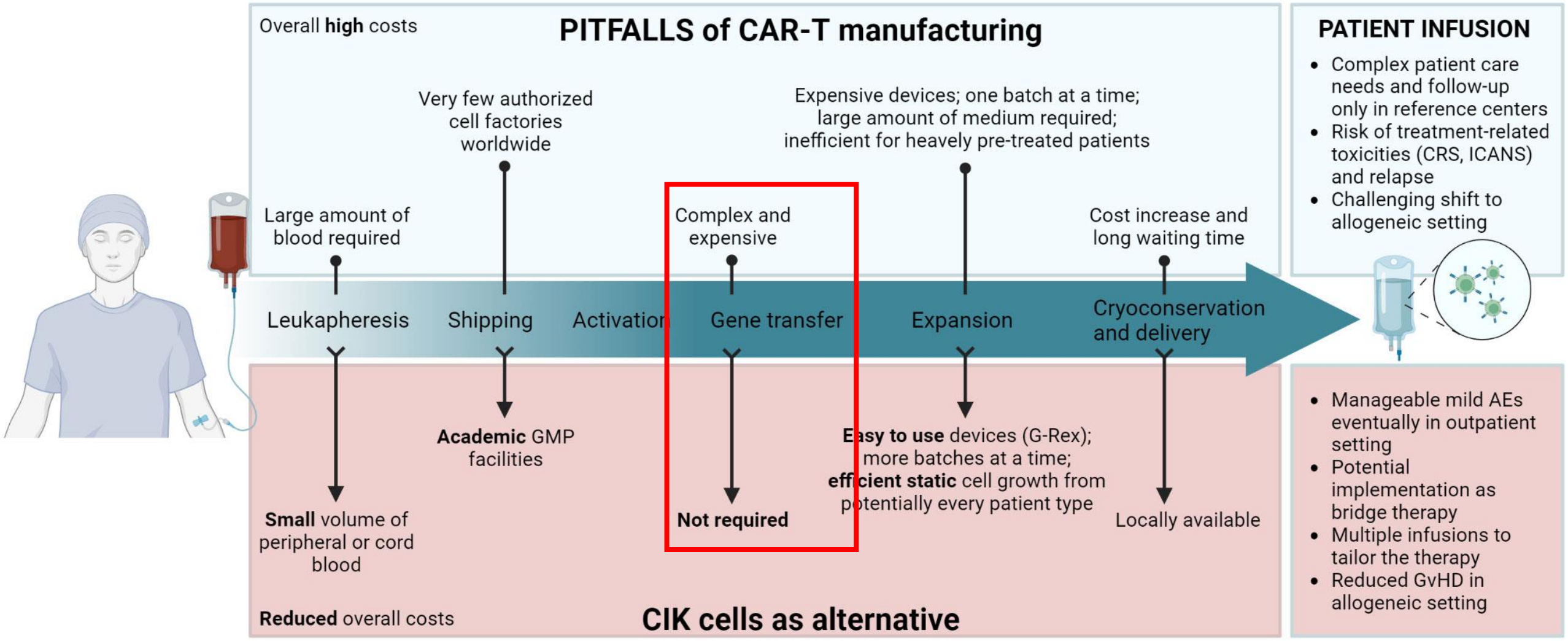
Bourque, J.; Hawiger, D. Activation, Amplification, and Ablation as Dynamic Mechanisms of Dendritic Cell Maturation. *Biology* 2023, *12*, 716.

Immune Effector Cells: Manufacturing of CIK cell products



Ghanbari Sevari, F., Mehdizadeh, A., Abbasi, K. et al. Cytokine-induced killer cells: new insights for therapy of hematologic malignancies. Stem Cell Res Ther 15, 254 (2024).

Immune Effector Cells: CIK cells vs CAR T cells



Cappuzzello E, Vigolo E, D'Accardio G, Astori G, Rosato A, Sommaggio R. How can Cytokine-induced killer cells overcome CAR-T cell limits. Front Immunol. 2023 Aug 22;14:1229540.

Recap: Immune Effector Cells

- **Immune Effector Cells:** T cells ($CD8^+$ cytotoxic T lymphocytes & $CD4^+$ T helper cells), dendritic cells, and NK cells.
- **Current options:** CAR T/NK cells, TCR-engineered T cells, tumor-infiltrating lymphocytes (TILs), and cytokine-induced killer (CIK) cells.

Biologics & ATMPs: Lot Release

Biologics

- Manufacturers: Production and In-Process Testing, Final Product Testing, Documentation Submission
- Regulatory authorities: Regulatory Review, Lot Certification and Release

Cell and gene therapy products

- Short Shelf Life
- Autologous Products: Limited amount
- Complexity: Hgh variation

To ensure that the products meet the requirements on

- Quality
- Safety
- Efficacy

ประกาศกระทรวงสาธารณสุข
เรื่อง กำหนดชนิดหรือรายการของยาชีววัตถุที่ต้องได้รับ
หนังสือรับรองรุ่นการผลิตก่อนออกจำหน่ายหรือส่งมอบให้ผู้ใช้

เนื่องจากยาชีววัตถุบางชนิดหรือบางรายการ มีกระบวนการผลิตที่มีความซับซ้อนและใช้วัตถุดิบที่มีโอกาสการปนเปื้อนสูง ตลอดจนประกอบด้วยตัวยาสำคัญซึ่งเป็นสารชีวโมเลกุลที่มีโครงสร้างซับซ้อน จึงทำให้เกิดความเสี่ยงต่อประชาชนเนื่องจากเกิดความแปรปรวนหรือความไม่สม่ำเสมอในด้านคุณภาพของผลิตภัณฑ์ ซึ่งอาจส่งผลกระทบต่อประสิทธิภาพของยาหรือกระทบต่อความปลอดภัยจากการใช้ยา จึงจำเป็นต้องมีการควบคุมคุณภาพในทุกรุ่นของการผลิต

ดังนั้น อาศัยความในข้อ ๔ ของกฎกระทรวงว่าด้วยการรับรองรุ่นการผลิตยาแผนปัจจุบันที่เป็นยาชีววัตถุ พ.ศ. ๒๕๕๓ รัฐมนตรีว่าการกระทรวงสาธารณสุข จึงออกประกาศไว้ ดังต่อไปนี้

ข้อ ๑ ยาชีววัตถุสำหรับมนุษย์ (Biologics for human use) ชนิดหรือรายการที่ต้องได้รับหนังสือรับรองรุ่นการผลิตก่อนออกจำหน่ายหรือส่งมอบให้ผู้ใช้ ได้แก่ ยาชีววัตถุดังต่อไปนี้

(๑) ผลิตภัณฑ์กลุ่มวัคซีน (vaccines) รวมถึงกลุ่มสารก่อภูมิแพ้ (allergens) ที่ใช้ในการรักษาหรือป้องกันโรค

(๒) ผลิตภัณฑ์กลุ่มเซรุ่ม (serum)

(๓) ผลิตภัณฑ์กลุ่มที่สกัดหรือแยกได้จากเลือดหรือพลาสมา (blood-derived or plasma-derived products)

(๔) สารที่ใช้ในการพิเคราะห์โรคซึ่งใช้โดยตรงต่อร่างกายมนุษย์ (diagnostic agents) รวมถึงสารก่อภูมิแพ้ (allergens) ที่ใช้ในการพิเคราะห์โรคซึ่งใช้โดยตรงต่อร่างกายมนุษย์ ที่มิใช่เครื่องมือแพทย์ตามกฎหมายว่าด้วยเครื่องมือแพทย์

ข้อ ๒ ยาชีววัตถุสำหรับสัตว์ (Biologics for veterinary use) ชนิดหรือรายการที่ต้องได้รับหนังสือรับรองรุ่นการผลิตก่อนออกจำหน่ายหรือส่งมอบให้ผู้ใช้ ได้แก่ยาชีววัตถุดังต่อไปนี้

(๑) ผลิตภัณฑ์กลุ่มวัคซีน (vaccines)

ข้อ ๓ ประกาศกระทรวงนี้ให้ใช้บังคับเมื่อพ้นกำหนดเก้าสิบวันนับตั้งแต่วันถัดจากวันประกาศในราชกิจจานุเบกษาเป็นต้นไป

ประกาศ ณ วันที่ ๓ กุมภาพันธ์ พ.ศ. ๒๕๕๕
วิทยา บุรณศิริ
รัฐมนตรีว่าการกระทรวงสาธารณสุข

Biologics & ATMPs: References

Official

- BP
- USP/NF
- Ph.Eur.
- JP

Non-official

- ICH guidelines
- Supplier Specifications
- In-house Specifications

ข้อ ๓ ให้ตำรายาต่อไปนี้เป็นตำรายาแผนปัจจุบัน

๓.๑ ตำรายาของประเทศไทย ฉบับที่ ๒ เล่มที่ ๑ ภาค ๑ และฉบับเพิ่มเติม (Thai Pharmacopoeia II, Volume I, Part 1 and Supplements) ซึ่งจัดพิมพ์โดยกระทรวงสาธารณสุข

๓.๒ ตำรามาตรฐานยาสมุนไพรไทย เล่มที่ ๑ เล่มที่ ๒ เล่มที่ ๓ เล่มที่ ๔ และฉบับเพิ่มเติม (Thai Herbal Pharmacopoeia Volume I Volume II Volume III Volume IV and Supplements)

๓.๓ ตำรามาตรฐานยาสมุนไพรไทย พ.ศ. ๒๕๖๐ และฉบับเพิ่มเติม (Thai Herbal Pharmacopoeia 2017 and Supplements)

๓.๔ ตำราอินเตอร์แนชนาลฟาร์มาโคเปีย ฉบับพิมพ์ครั้งที่ ๕ และฉบับเพิ่มเติม (The Fifth Edition of The International Pharmacopoeia and Supplements)

๓.๕ ตำราฟาร์มาโคเปียของสหรัฐอเมริกา ฉบับพิมพ์ครั้งที่ ๓๙ (ค.ศ. ๒๐๑๖) และฉบับเพิ่มเติม (The United States Pharmacopoeia, Thirty-Ninth Revision, and The National Formulary, Thirty-Fourth Edition and Supplements)

๓.๖ ตำราบริติชฟาร์มาโคเปีย ฉบับ ค.ศ. ๒๐๑๖ เล่มที่ ๑ - ๕ (British Pharmacopoeia 2016 Volume 1 - 5)

๓.๗ ตำราบริติชฟาร์มาโคเปีย (สัตวแพทยศาสตร์) ฉบับ ค.ศ. ๒๐๑๖ (British Pharmacopoeia (Veterinary) 2016)

๓.๘ ตำรายูโรเปียนฟาร์มาโคเปีย ฉบับพิมพ์ครั้งที่ ๘ (ค.ศ. ๒๐๑๔) และฉบับเพิ่มเติม (The Eighth Edition of The European Pharmacopoeia and Supplements)

“๓.๙ ตำราฟาร์มาโคเปียของประเทศญี่ปุ่น ฉบับพิมพ์ครั้งที่ ๑๗ และฉบับเพิ่มเติม (The Seventeenth Edition of The Japanese Pharmacopoeia and Supplements)”

Biologics & ATMPs: Monographs

USP has stated that it will not develop a new monograph for a biologic unless there is stakeholder consensus supporting its creation, including the support of FDA. USP's proposed revision is intended to align compendial names with FDA's biologics naming approach and avoid potential issues for manufacturers and other stakeholders.

USP statement on monographs for biologics, April 2, 2018

FDA has already communicated to USP the Agency's detailed concerns regarding biological product monographs.[6] In a 2014 letter to USP, FDA cited significant concern that monographs for biological products may impede or delay innovative technology and present an additional, unnecessary burden on regulated industry. As an alternative, FDA encouraged USP to develop optional standards that are "consistent with the flexible approach FDA uses to properly account for the complex nature of biological products."[7]

FDA Letter to USP, March 28, 2018

Biologics & ATMPs: Specifications

A specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a new drug substance or new drug product should conform to be considered acceptable for its intended use. "Conformance to specifications" means that the drug substance and / or drug product, when tested according to the listed analytical procedures, will meet the listed acceptance criteria. Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval.

Acceptance criteria

A list of tests

References

Quality attribute	Test procedure	Acceptance criteria
Appearance	Visual inspection	Complies with in-house description
Identification		
pI of intact protein	Imaged capillary isoelectric focusing	Conforms to reference material
Tryptic peptide mapping	RPLC-UV	Conforms to reference material
Bioidentity	Activity binding ELISA	Conforms to reference material
Purity and impurities		
Product-related substances and impurities: Charge variants		
Main peak	IEX-UV	NLT 65.0%
Acidic peak	IEX-UV	NMT 15.0%
Basic peak	IEX-UV	NMT 20.0%
Product-related substances and impurities: Size variants		
Major peak	CE-SDS (non-reduced)	NLT 97.0%
Heavy and light chain peaks	CE-SDS (reduced)	NLT 99.0%
Monomer	SEC-UV	NLT 98.5%
High molecular weight species	SEC-UV	NMT 1.0%
Low molecular weight species	SEC-UV	NMT 0.5%
Product-related substances and impurities: Post-translational modification (Glycosylation)		
N-glycan profiling	HILIC–Fluorescence detector	Conforms to the limit of each glycan
Process-related impurities		
Residual host cell protein	ELISA	NMT 20 ppm (ng/mg)
Residual protein A	ELISA	NMT 5 ppm (ng/mg)
Residual DNA	qPCR	NMT 40 ppb (pg/mg)
Additional process-related impurity	…	…
Protein concentration	UV spectroscopy	90.0%–110.0% labeled amount
Potency		
Functional assay	Cell-based bioassay	70.0%–130.0%
Additional tests		
pH	USP ⟨791⟩	5.5–6.5
Bacterial endotoxins	USP ⟨85⟩	NMT 0.20 EU/mg
Total microbial count	USP ⟨61⟩	NMT 1 CFU/mL
Mycoplasma	USP ⟨63⟩	Negative result of its nucleic acid

CE: capillary electrophoresis; ELISA: enzyme-linked immunosorbent assay; HILIC: hydrophilic interaction chromatography; IEX: ion exchange chromatography; NLT: not less than; NMT: not more than; pI: isoelectric point; qPCR: quantitative polymerase chain reaction; RPLC: reversed phase liquid chromatography; SDS: sodium dodecyl sulfate; SEC: size exclusion chromatography; USP: United States Pharmacopoeia; UV: ultraviolet.

Limpikirati PK, Mongkoltipparat S, Denchaipradit T, Siwasophonpong N, Pornnopparat W, Ramanandana P, Pianpaktr P, Tongchusak S, Tian MT, Pisitkun T. Basic regulatory science behind drug substance and drug product specifications of monoclonal antibodies and other protein therapeutics. J Pharm Anal. 2024 Jun;14(6):100916.

Biologics & ATMPs: Specifications

ICH HARMONISED TRIPARTITE GUIDELINE

SPECIFICATIONS: TEST PROCEDURES AND ACCEPTANCE CRITERIA FOR NEW DRUG SUBSTANCES AND NEW DRUG PRODUCTS: CHEMICAL SUBSTANCES

Q6A

Current *Step 4* version

dated 6 October 1999

ICH HARMONISED TRIPARTITE GUIDELINE

SPECIFICATIONS: TEST PROCEDURES AND ACCEPTANCE CRITERIA FOR BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS

Q6B

Current *Step 4* version

dated 10 March 1999

ICH Q6A	
Drug substances	Drug products
Universal tests • Description • Identification • Assay • Impurities	
Specific tests • Physicochemical properties • Particle size • Polymorphic forms • Chirality • Water content • Microbial limits	Specific tests (e.g., parenteral products) • Sterility • pH/Osmolarity • Endotoxin/Pyrogens • Particulate matter • Extractables • Water content/Reconstitution time • Re-dispersibility
ICH Q6B	
• Appearance and Description • Identity • Quantity • Purity and Impurities • Potency	• Appearance and Description • Identity • Quantity • Purity and Impurities • Potency • General tests & Additional testing for unique dosage forms

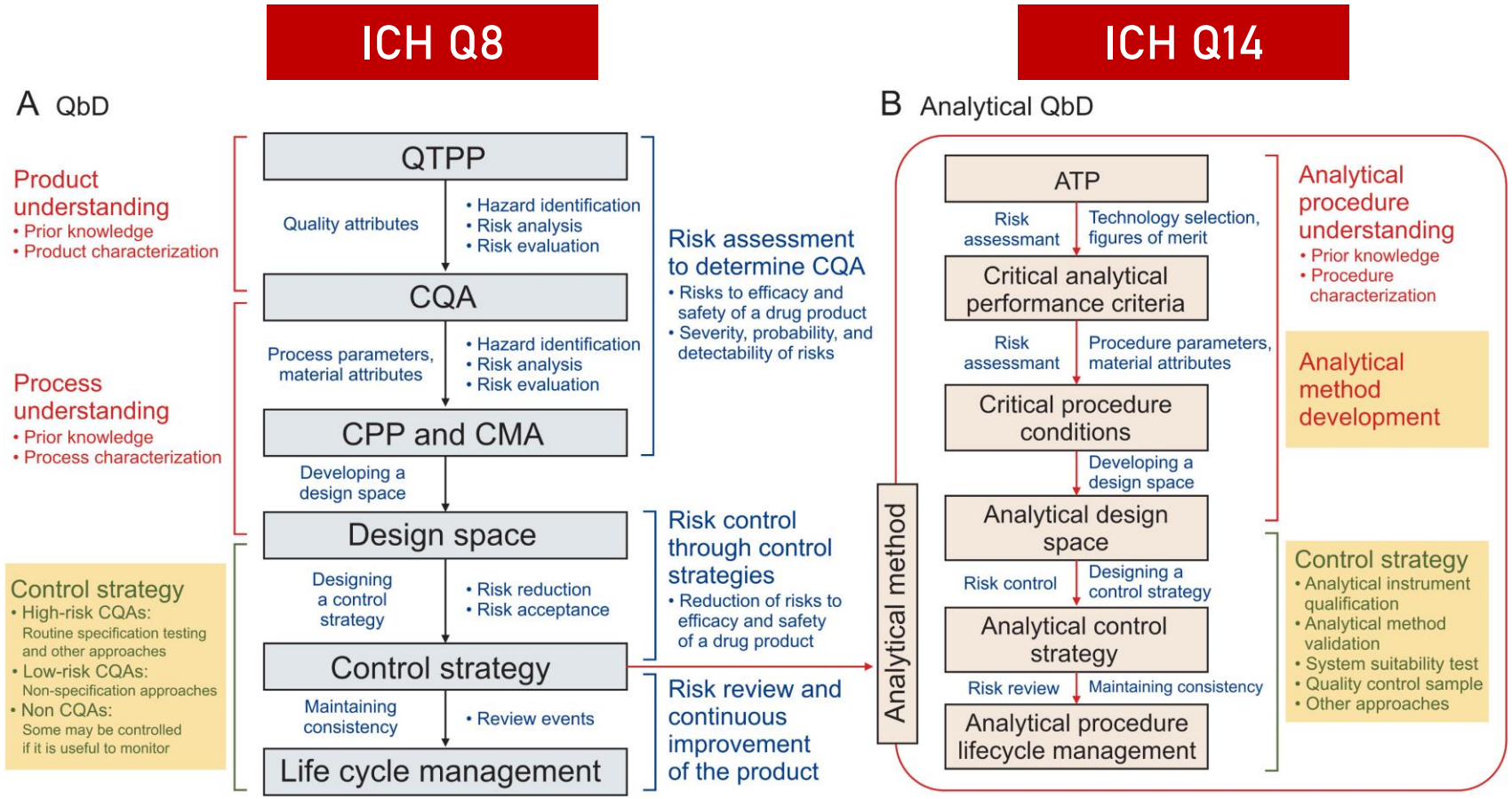
Biologics & ATMPs: Setting up a specification

Quality focusing

- Quality-by-design approaches: ICH Q8, Q9, Q10, Q14
- Integrate scientific understanding in products and processes with quality risk management (QRM)

Safety and Efficacy focusing

- Patient-centric specification: Rely on clinical data and patient impact
- Tighter criteria for high-risk CQAs impacting efficacy and safety

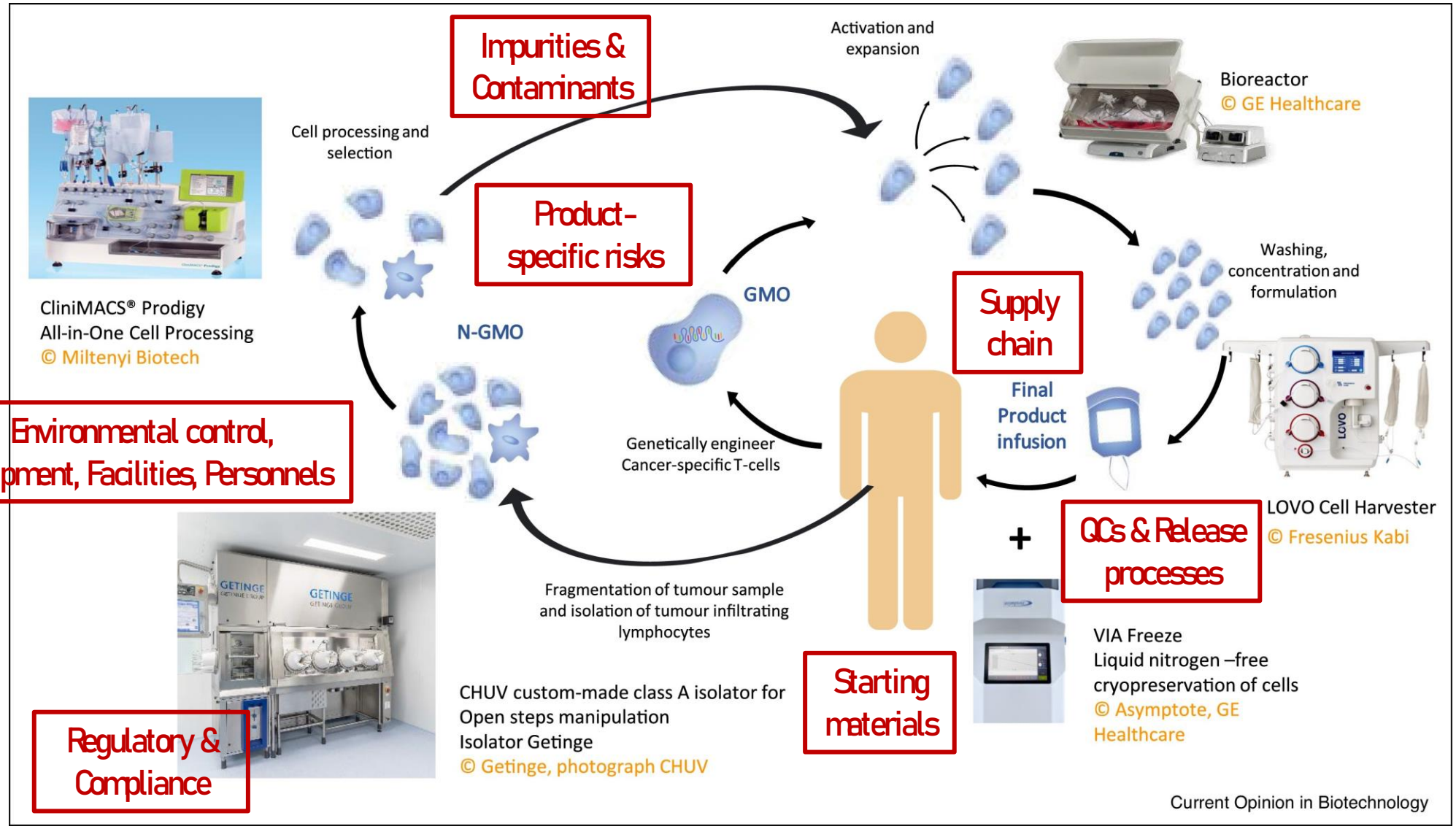


Limpikirati PK, Mongkoltipparat S, Denchaipradit T, Siwasophonpong N, Pornnopparat W, Ramanandana P, Pianpaktr P, Tongchusak S, Tian MT, Pisitkun T. Basic regulatory science behind drug substance and drug product specifications of monoclonal antibodies and other protein therapeutics. J Pharm Anal. 2024 Jun;14(6):100916.

Recap: Control Strategy for Biologics & ATMPs

- **Monographs:** Monographs for biologics and ATMPs are often absent in USP. References should rely on ICH guidelines for general concepts and framework.
- **Specifications:** Compose of a list of tests with references and acceptance criteria. Universal tests are similar to chemical drugs but include potency tests due to biologics' complexity.
- **Setting-up a specification:** Must address the quality, safety, and efficacy of the products.

Risk management: Sources



Iancu EM, Kandalaft LE. Challenges and advantages of cell therapy manufacturing under Good Manufacturing Practices within the hospital setting. Curr Opin Biotechnol. 2020 Oct;65:233-241.

Risk management: Identifications

That is the reason why…

"Cell therapy products are really only defined by their process."

David Courtman
Director, Cell Manufacturing, Biotherapeutics Core Facilities,
Ottawa Hospital Research Institute

"In other words, everything you do in your manufacturing, you need to have evidence that it is safe and that it's producing what you expect. If you have that, you are in a much better space in terms of moving ahead in translation."

Ottawa Hospital Research Institute
Institut de recherche de l'Hôpital d'Ottawa

A short guide to cell therapy manufacturing, part 1
https://medium.com/the-expression/a-short-guide-to-cell-therapy-manufacturing-part-1-ad61e29f94b2

Quality-by-design: Concepts

- **QbD** is a concept based on that the quality should be designed into a product.
- In pharmaceutical sciences, QbD helps to predefine the characteristics of end-products based on desired outcomes, such as clinical performance, patient compliance and satisfaction, or safety.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

PHARMACEUTICAL DEVELOPMENT

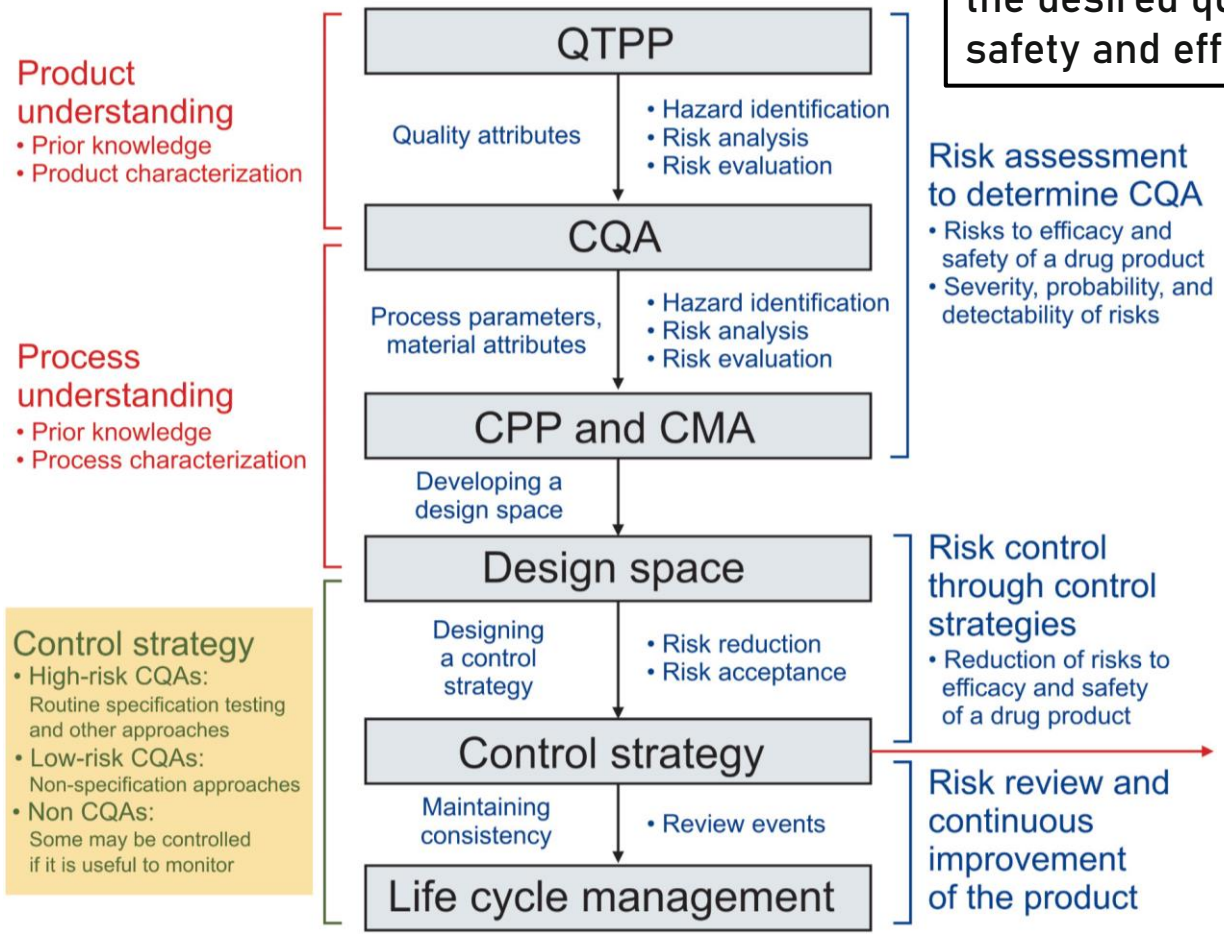
Q8(R2)

Quality-by-design: Elements

QTPP is a prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product.

CQA is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

CMA and CPP are defined as "A material or process whose variability has an impact a critical quality attribute and therefore it should be monitored or controlled to ensure desired drug product quality".

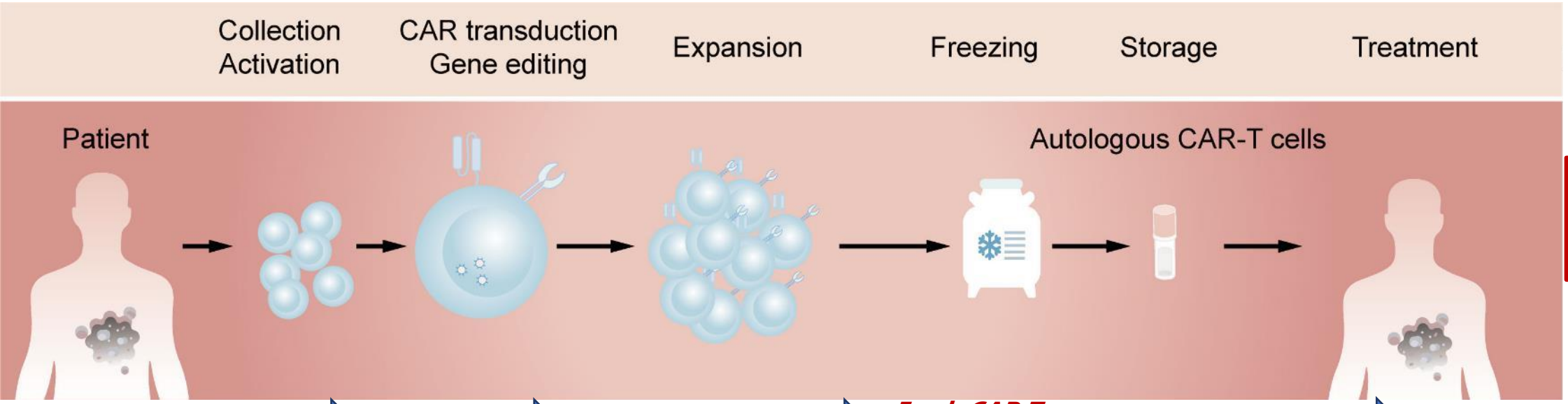


Limpikirati PK, Mongkoltipparat S, Denchaipradit T, Siwasophonpong N, Pornnopparat W, Ramanandana P, Pianpaktr P, Tongchusak S, Tian MT, Pisitkun T. Basic regulatory science behind drug substance and drug product specifications of monoclonal antibodies and other protein therapeutics. J Pharm Anal. 2024 Jun;14(6):100916.

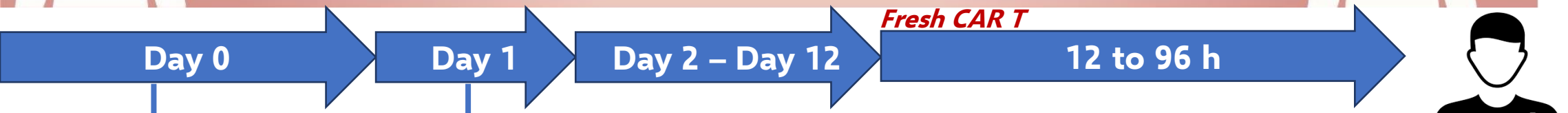
Tools to identify QbD elements:

- Prior knowledge
- Risk assessment
- Mechanistic model, design of experiments, and data analysis
- Process analytical technology

Quality-by-design: Identify QTPPs



Patient with R/R B-ALL



Patient

- T cell identification
- T cell enrichment
- T cell activation

- Transduction using Lentiviral vectors

Ref: Lv Z, Luo F, Chu Y. Strategies for overcoming bottlenecks in allogeneic CAR-T cell therapy. Front Immunol. 2023 Jul 24;14:1199145.

Quality-by-design: QTPPs

Product dosage form	Suspension for IV infusion, Fresh CAR T product
Indications	CD19+ hematological malignancies
Total cells per dose	30 to 1,000 million T cells with at least 10% CD19 CAR T cells
Identity	• Presence of T cells (CD3+) • Absolute number of CD4+, CD8+ T cells
Net volume	30 mL
Process lot size	4 bags of cells (2 for retention, 1 for release testing, 1 for quarantine)
Administration time	IV infusion 30 minutes within 96 h after product release
Transfer conditions	2-8 °C
Container closure system	Identical primary packaging to RLD
Package integrity	No failure
Purity	• Absence of cellular Impurities (NK cells, monocytes, CD19+ B cells) • Absence of magnetic beads

Stability	Shelf life: 96 hours for fresh product
Pre-conditioning Regimen	Cyclophosphamide and fludarabine prior to infusion
Dosing Regimen	Single infusion; split dosing optional
Adverse Event Management	• Recommended protocols for CRS and ICANS management • Hospitalization requirements and follow-up
Potency	• Phenotypic profile confirmation of CD19 CAR T cells • Vector copy number • Cytotoxic assay of CD19+ tumor cells • Cytokine release assay • Cell viability
Safety	• Insertional mutagenesis risk • Replication competent lentivirus • Sterility testing • Endotoxin testing • Mycoplasma contamination testing

Quality-by-design: Identify CQAs

Product dosage form	Suspension for IV infusion, Fresh CAR T product
Indications	CD19+ hematological malignancies
Total cells per dose	30 to 1,000 million T cells with at least 10% CD19 CAR T cells
Identity	• Presence of T cells (CD3+) • Absolute number of CD4+, CD8+ T cells
Net volume	30 mL
Process lot size	4 bags of cells (2 for retention, 1 for release testing, 1 for quarantine)
Administration time	IV infusion 30 minutes within 96 h after product release
Transfer conditions	2-8 °C
Container closure system	Identical primary packaging to RLD
Package integrity	No failure
Purity	• Absence of cellular Impurities (NK cells, monocytes, CD19+ B cells) • Absence of magnetic beads

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Safety	• Insertional mutagenesis risk • Replication competent lentivirus • Sterility testing • Endotoxin testing • Mycoplasma contamination testing

Quality-by-design: Specification Development (1)

Description

- Suspension for IV infusion
- Fresh cell product

Identity

- T cells ($CD3^+$ cells)
- CAR^+ T cells

Quantity

- Number of viable T cells ($CD3^+$ cells)
- Percentage of CAR^+ T cells in total viable T cells

Purity

Organic impurities

- NK cells, Monocytes, B cells
- Residual plasmid DNA
- Product-related impurities

Elemental impurities

- Magnetic beads

Residual solvents

- Culture medium
- DMSO

Mutagenic impurities

- Replicate-competent viruses
- Cells with insertional mutagenesis

Potency

- Cytotoxic assay
- Cytokine release assay
- Vector copy number

Specific tests

- Sterility tests
- Mycoplasma contamination tests
- Bacterial endotoxins
- pH
- Osmolarity
- Particulate matters
- Container closure integrity

Quality-by-design: Specification Development (2)

Specification category	Quality attributes	Test procedures	Acceptance criteria
Appearance and description		Visual inspection	Cell suspension in clear, amber solution; no visible particulates
Identity	CD3+ T cells	Flow cytometry	Meet the requirements in Quantity
	CAR+ cells	Flow cytometry	Meet the requirements in Quantity
Quantity / Assay	Total viable T cells	Flow cytometry	NLT 10×10^8 cells
	Percentage of CAR+ cells	Flow cytometry	NLT 10% of the total viable T cells
Purity	Total cells with CD19+, CD20+, HLA-DR+, CD11c+, CD14+, CD16+, CD56+ or CD123+	Flow cytometry	NMT 0.1% of the total viable cells
	Magnetic beads	ICP-MS	Absence
	DMSO	GC-MS	NMT 0.1%
	Residual DNA	qPCR	NMT 40 ppb
	Replication Competent Lentivirus	RCL assay	NMT 1 RCL/mL
	Oncogene insertion	qPCR	Meet the requirements in Potency (Vector copy number)

Quality-by-design: Specification Development (3)

Specification category	Quality attributes	Test procedures	Acceptance criteria
Potency	Cytotoxic assay	Cell-based assay	NLT 50% cell lysis
	Cytokine release assay	ELISPOT	NLT 100 SFUs per well
	Vector copy number	qPCR	NMT 5 copies per cells
Specific tests	pH	USP <791>	5.5–6.5
	Osmolarity	USP <785>	310–370 mOsm/kg
	Bacterial endotoxins	USP <85>	NMT 0.20 EU/mg
	Sterility	USP <71>	No microbial growth
	Mycoplasma	USP <63>	Negative result of its nucleic acid

Recap: QbD & Specification Development

- Risks: Quality, safety and efficacy of biologics and ATMPs are highly dependent on the processes, Risks must be thoroughly identified and mitigated.
- QbD: QbD a systematic approach that defines the desired characteristics of the final product.
- Setting-up a specification: CQAs, derived from the QbD process, form the foundation for developing product specifications.

Appearance and description

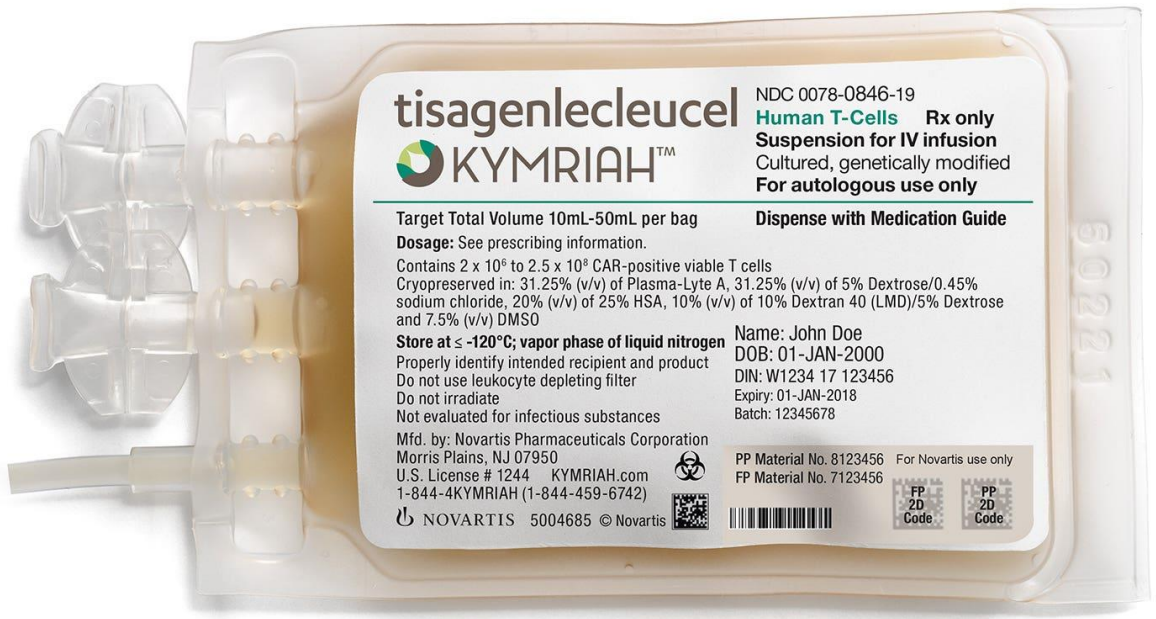
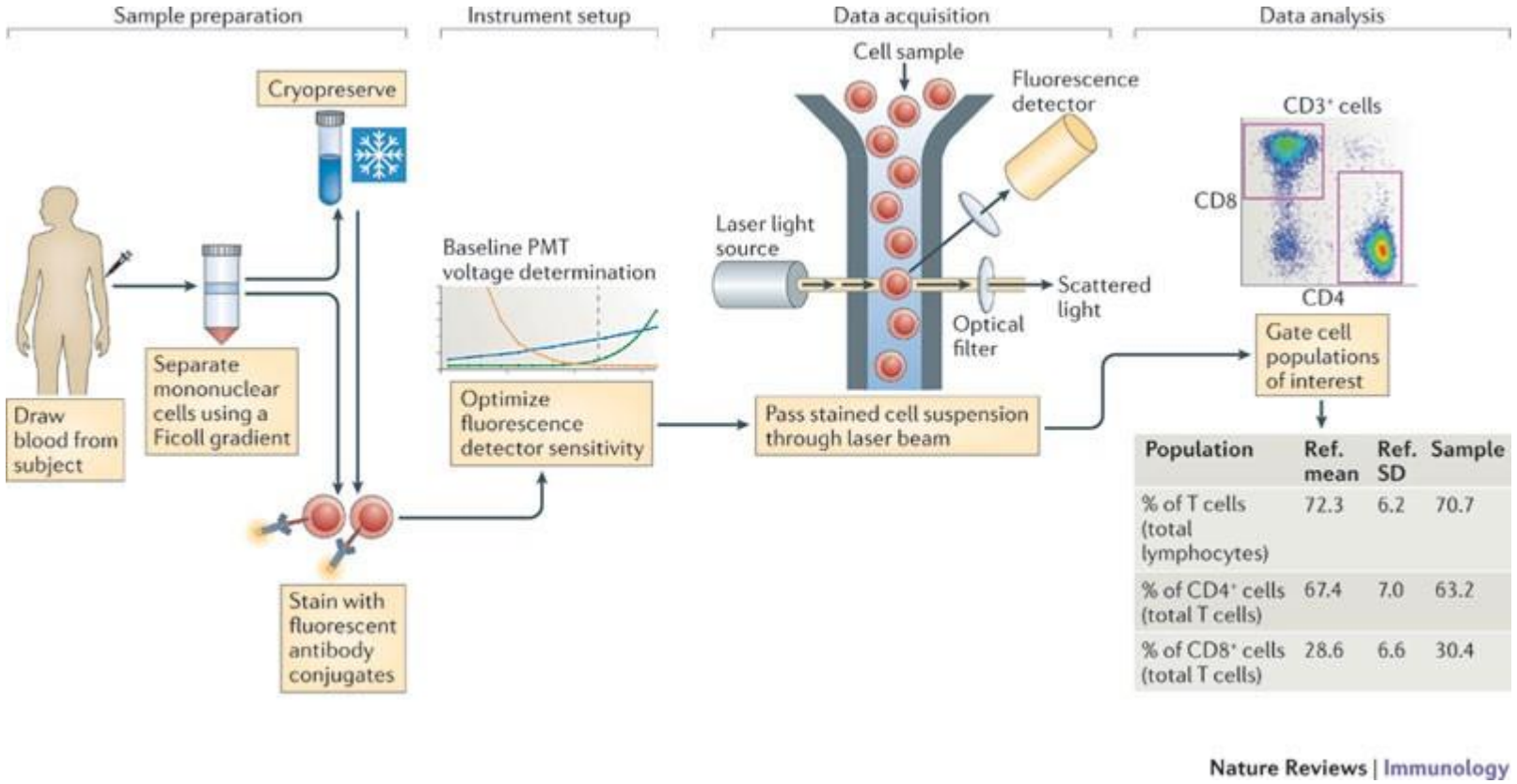


Image courtesy of Novartis Pharmaceuticals Corporation

Image courtesy of Eurofins Viracor, LLC

Identity and Quantity: Flow cytometry

Flow cytometry is a widely used but technically complex tool; the practical application of which is recognised to be diverse and often challenging to standardise. Standardisation is critical in supporting robust data generation, enabling data comparability between users and instruments, and when applied to development of ATMPs, ensuring reproducible product quality, safety, and efficacy throughout the entire product lifecycle.



British Pharmacopoeia: Advanced Therapy Medicinal Products Guidance, Application of Flow Cytometry

Recommended surface markers (special for T cell products):

T cells: CD3 (to define T cells), CD4, CD8, CD45RA and CCR7

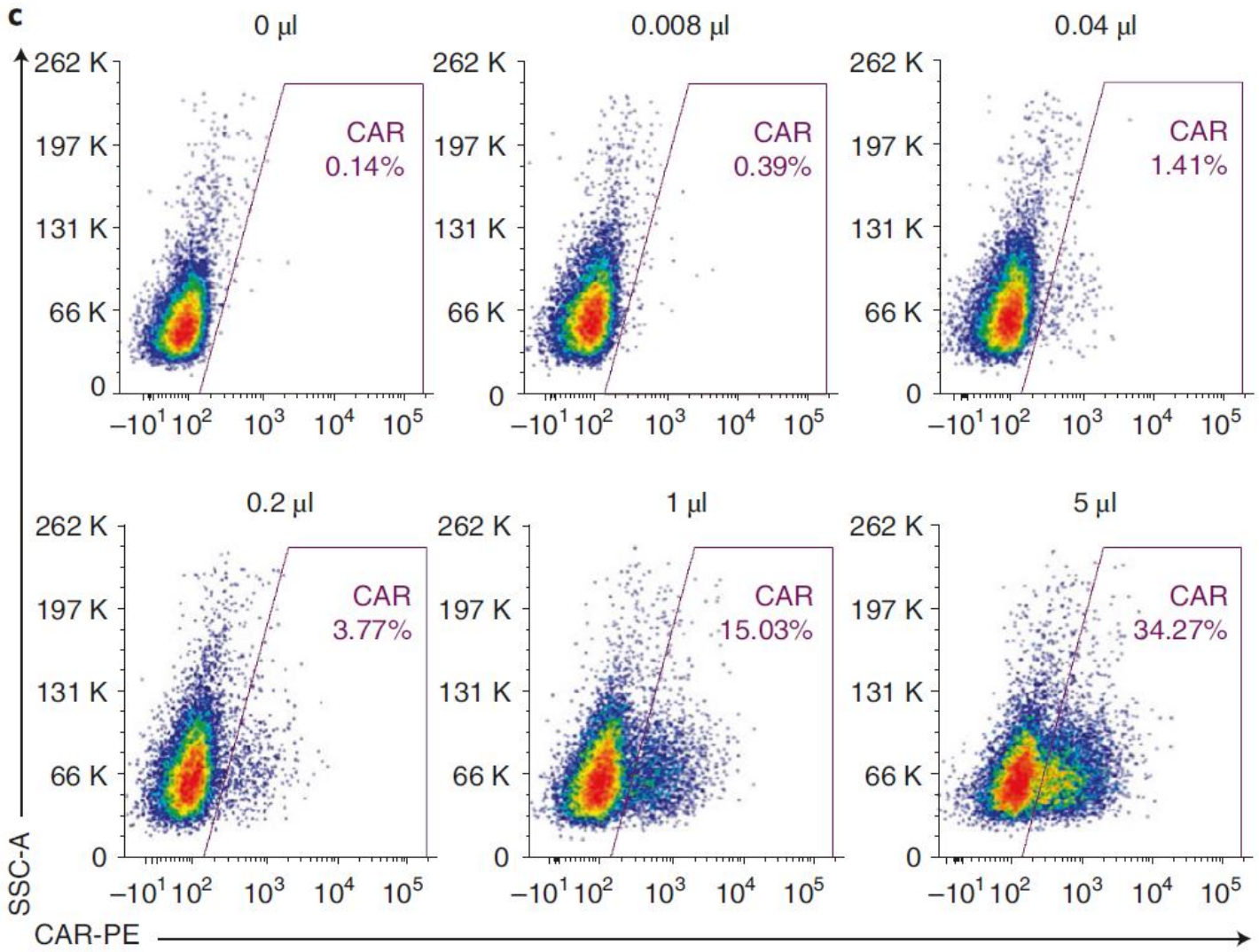
B cells: CD19 and CD20 (to define B cells), CD38 (plasmablasts and transitional B cells), CD24 (transitional B cells), and IgD and CD27 (naive and memory B cell)

NK cells, Dendritic cells, and Monocytes: HLA-DR, CD11c, CD14, CD16, CD56 and CD123

Recommended articles for T cell characterization by Flow cytometry

- Maecker, H., McCoy, J. & Nussenblatt, R. Standardizing immunophenotyping for the Human Immunology Project. *Nat Rev Immuno*l **12**, 191–200 (2012). https://doi.org/10.1038/nri3158

Quantity: Flow cytometry



*Weidner, T., Agarwal, S., Perian, S. et al. Genetic in vivo engineering of human T lymphocytes in mouse models. Nat Protoc **16**, 3210–3240 (2021).*

Gene transfer activity of a CD8-LV stock encoding the CD19-CAR. Serial dilutions of the vector stock were incubated with Molt4.8 cells. CAR expression was measured by the expression of the myc-tag after 4 d via flow cytometry. Side scatter area (SSC-A) is displayed on a linear scale, whereas the axis scale for the CAR (PE) is logarithmic.

Purity: Flow cytometry

nature medicine

BRIEF COMMUNICATION

https://doi.org/10.1038/s41591-018-0201-9

Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell

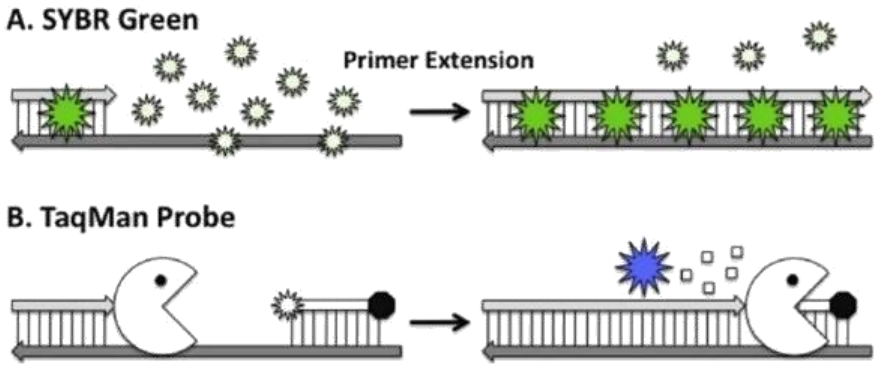
Marco Ruella[1,2,3,4,5,11], Jun Xu[1,2,3,11], David M. Barrett[6,11], Joseph A. Fraietta[1,2,3,4], Tyler J. Reich[1], David E. Ambrose[1], Michael Klichinsky[1,7], Olga Shestova[1], Prachi R. Patel[1], Irina Kulikovskaya[1], Farzana Nazimuddin[1], Vijay G. Bhoj[1,2,3], Elena J. Orlando[8], Terry J. Fry[9], Hans Bitter[8], Shannon L. Maude[6], Bruce L. Levine[1,2,3], Christopher L. Nobles[10], Frederic D. Bushman[10], Regina M. Young[1], John Scholler[1], Saar I. Gill[1,3,5], Carl H. June[1,2,3,4*], Stephan A. Grupp[6], Simon F. Lacey[1,2,3,12] and J. Joseph Melenhorst[1,2,3,12*]

CD19 CAR-transduced B cells (CARB)

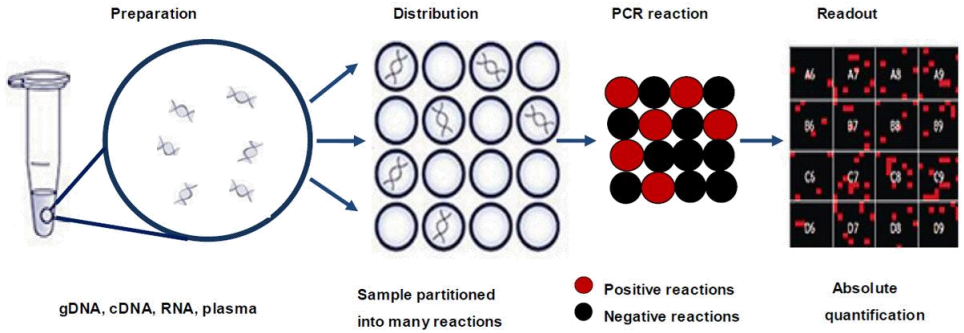
We report a patient relapsing 9 months after CD19-targeted CAR T cell (CTL019) infusion with CD19$^-$ leukemia that aberrantly expressed the anti-CD19 CAR. The CAR gene was unintentionally introduced into a single leukemic B cell during T cell manufacturing, and its product bound in cis to the CD19 epitope on the surface of leukemic cells, masking it from recognition by and conferring resistance to CTL019.

Purity: Quantitative polymerase chain reaction (PCR)

Real-time quantitative PCR (RT-qPCR)



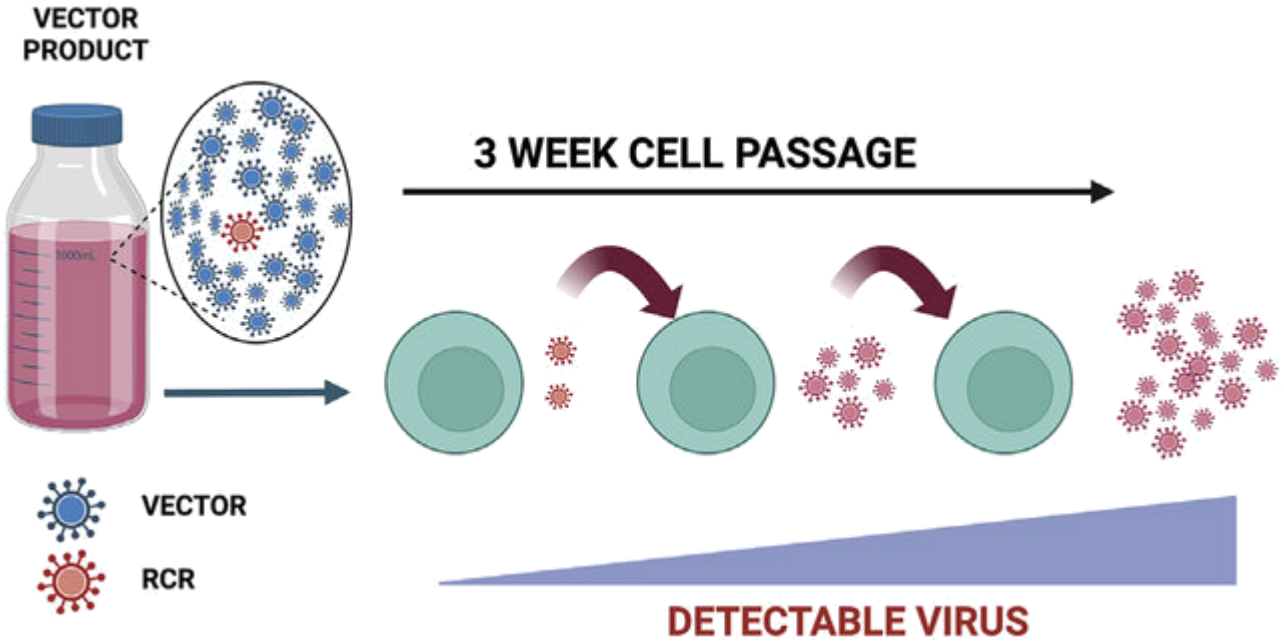
Droplet Digital PCR (ddPCR)



Comparative Strengths of qPCR and dPCR	
Strengths of qPCR	Strengths of dPCR
Established technology	Emerging technology
Relative measurement, ideally suited for gene expression analysis	Absolute measurement eliminates need for standard curve
Wide choice in detection chemistry and reaction volume equates to flexible running costs	High precision for better reproducibility for low input target concentrations
Large dynamic range	Greater sensitivity for rare mutation detection
Higher throughput, automation compatibility	Improved precision for higher copy number variation analysis

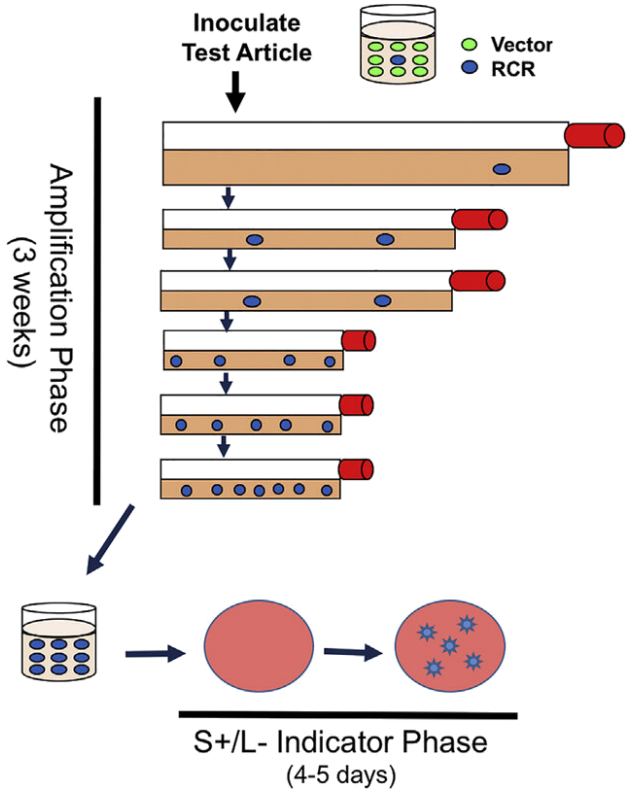
Image courtesy of Drug Discovery World

Purity: RCL assay



Cornetta K, Lin TY, Pellin D, Kohn DB. Meeting FDA Guidance recommendations for replication-competent virus and insertional oncogenesis testing. Mol Ther Methods Clin Dev. 2022 Dec 2;28:28-39.

USFDA, Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up: Guidance for Industry



Ref: Cornetta K, Duffy L, Feldman SA, Mackall CL, Davila ML, Curran KJ, Junghans RP, Tang JY, Kochenderfer JN, O'Cearbhaill R, Archer G, Kiem HP, Shah NN, Delbrook C, Kaplan R, Brentjens RJ, Rivière I, Sadelain M, Rosenberg SA. Screening Clinical Cell Products for Replication Competent Retrovirus: The National Gene Vector Biorepository Experience. Mol Ther Methods Clin Dev. 2018 Aug 17;10:371-378.

Potency: Functional assay

Functional assay: Cytotoxic assay

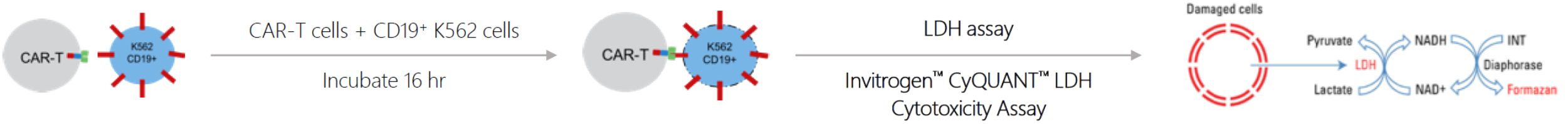


Image courtesy of Invitrogen, Thermo Fisher Scientific

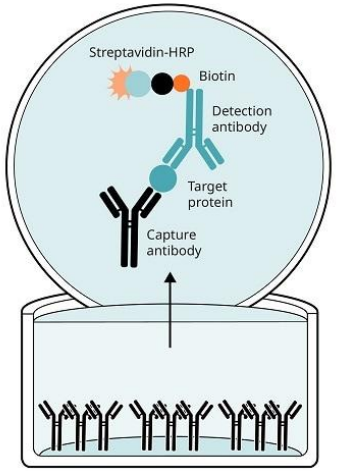
Viability measurement could be conducted using:

- MTT uptake,
- Chromium-51 release (although there is an industry-wide move away from radioactivity-based assays),
- Lactate dehydrogenase activity,
- and luciferase activity (which requires a luciferase expressing tumour target cell line).

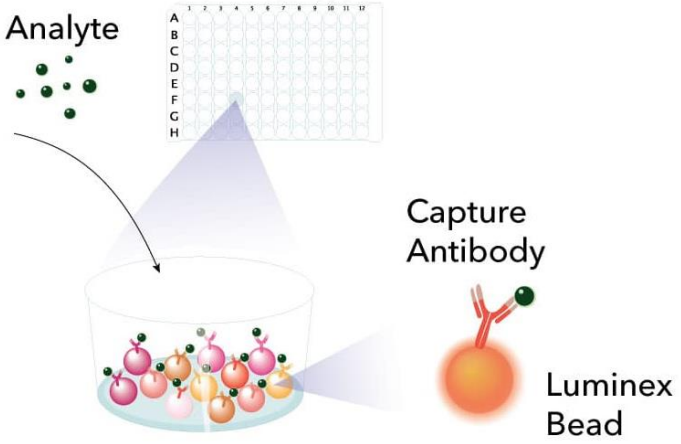
Functional assay: Release of key cytokines

E.g., IFN-γ, IL-2, and Granzyme B

ELISA



ELISPOT or Multiplex assay

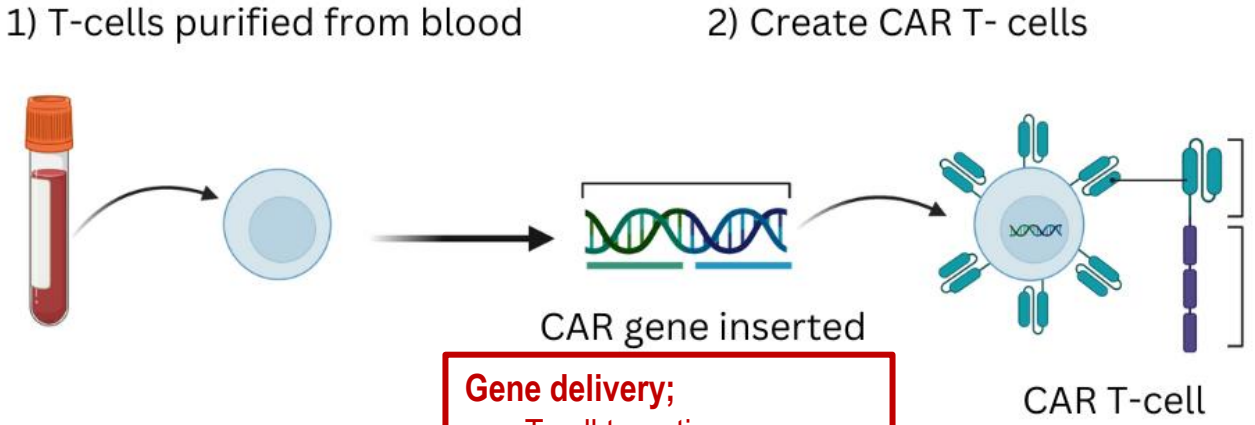


Recap: Quality control

- Tests: Quality control tests should encompass the categories outlined in ICH Q6B, including appearance, identity, quantity, purity and potency.
- Flow cytometry and qPCR: These are essential techniques in cell and gene therapy quality control.
- Functional assay: Cell-Based Assays are required to confirm the potency of the product by evaluating its efficacy in killing target cells or eliciting the desired immune response.

Future of IECs: Overview

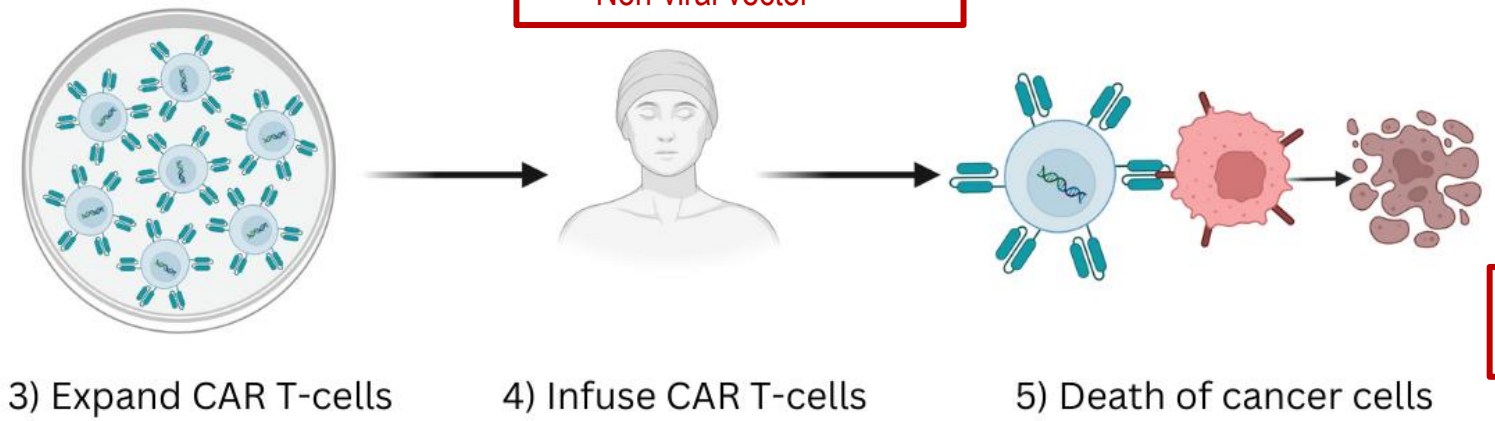
Source of cells;
- Allogeneic cells (off-the-shelf)
- In vivo engineering or reprogramming



Development of new receptors;
- Head-to-head comparison of existing CAR or TCR
- New generation CAR

Gene delivery;
- T cell targeting
- Precision gene insertion
- Non-viral vector

Manufacturing process;
- T cell processing
- Processing time and cost

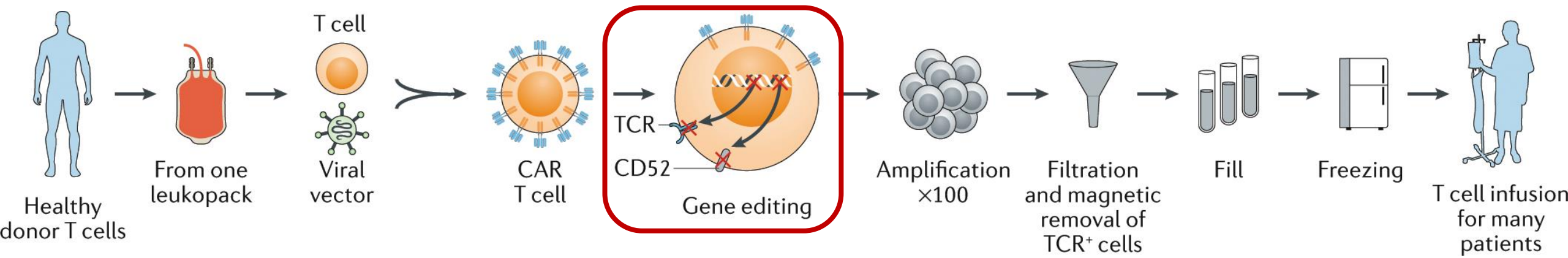


Extended application;
- Solid tumor

Treatment regimen;
- Combination therapy
- ADR management

Off-the-shelf: Allogeneic cell transfer

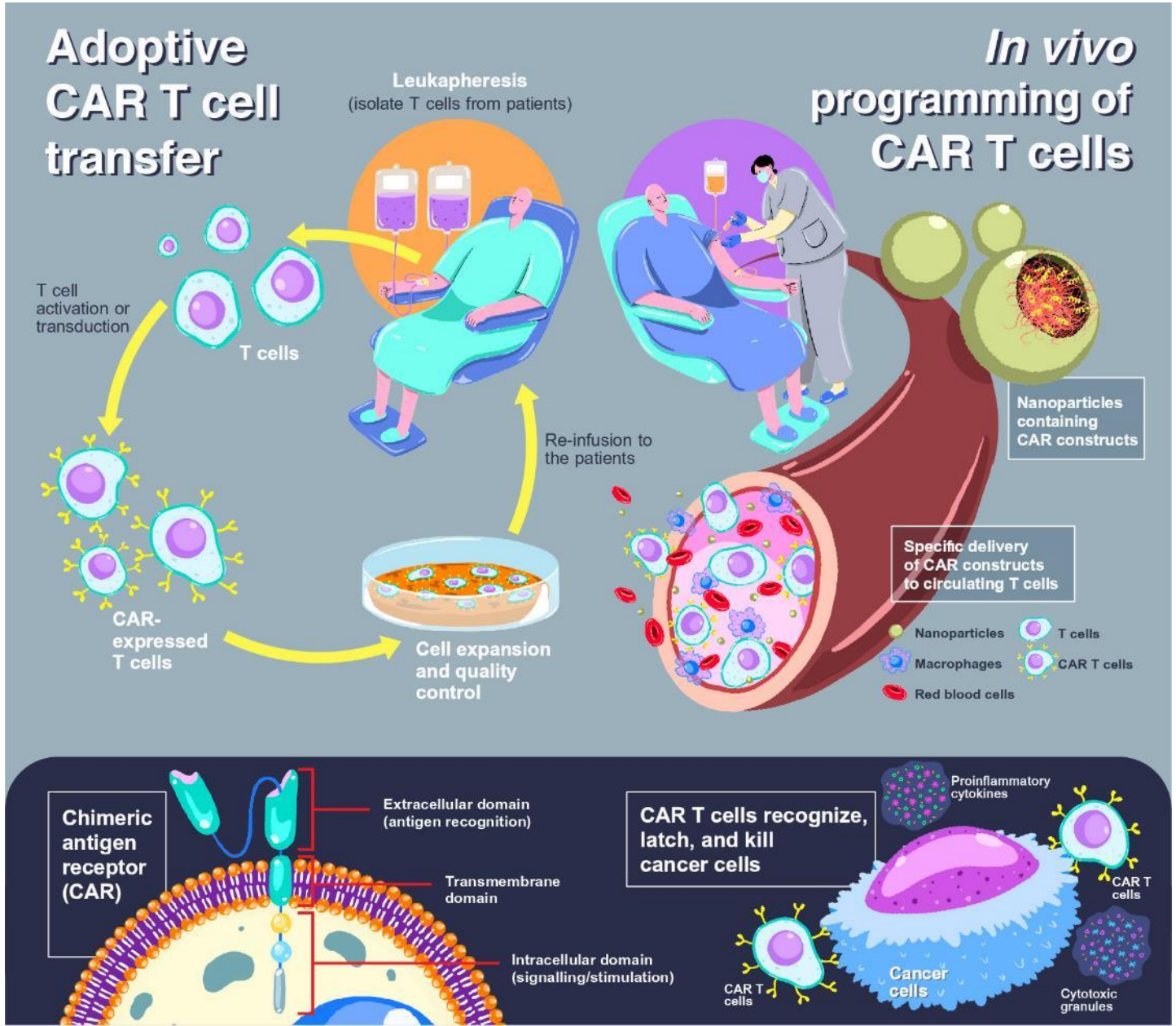
Challenging:
- Graft-versus-host disease (GVHD)



- Using allogeneic CAR T cells derived from a stem cell transplant donor (limited for relapse patients with previous allogeneic SCT).
- Using virus-specific memory T cells
- Using non-αβ T cells (e.g., NK cells, NK T cells, γδ T cells)
- Using gene editing

*Depil, S., Duchateau, P., Grupp, S.A. et al. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. Nat Rev Drug Discov **19**, 185–199 (2020).*

Off-the-shelf: In vivo reprogramming/engineering cells

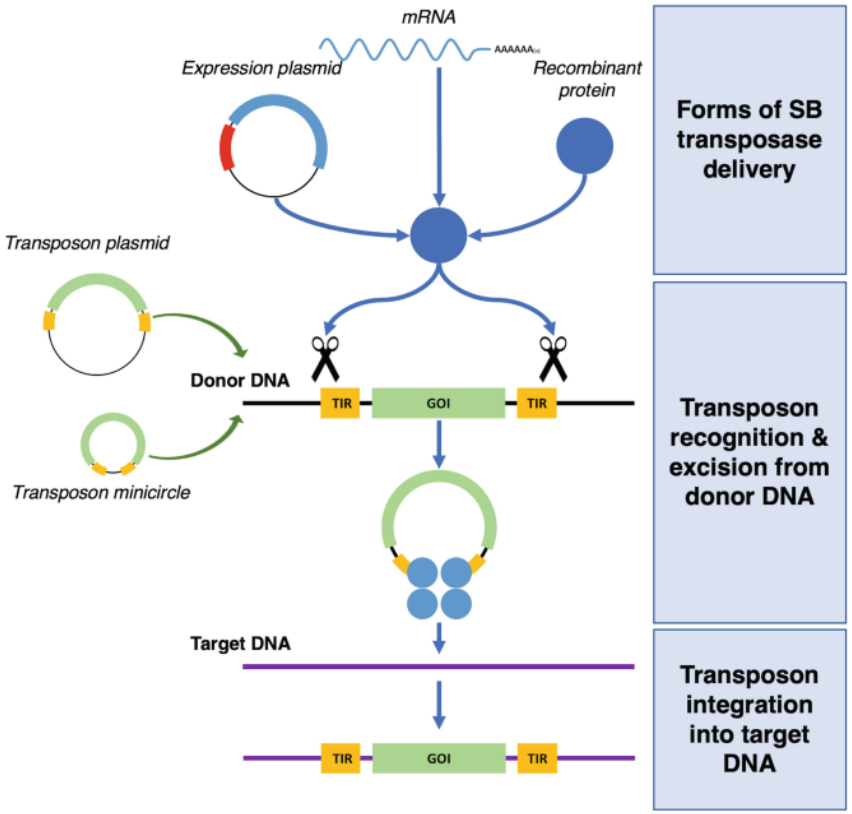


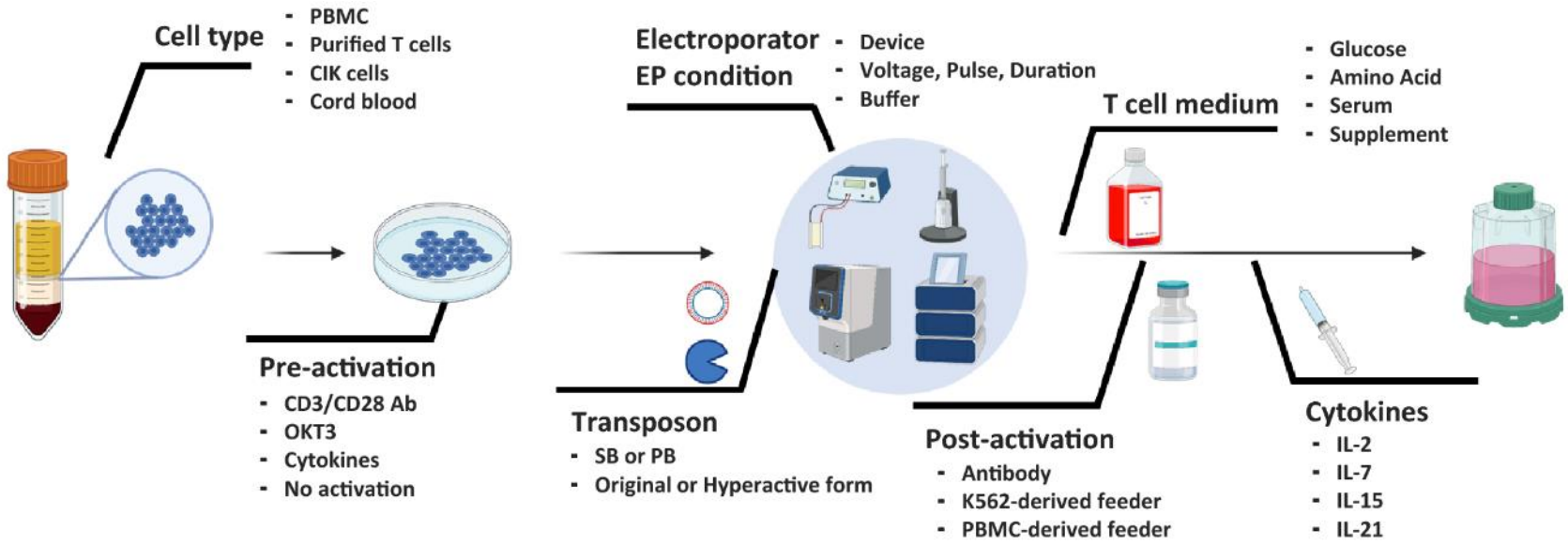
Major concerns:

- Gene delivery system (Integrating viral vector vs non-viral vector)
- Efficiency (limited number of T cells, no T cell selection, persistence of CAR T cells)
- Targetability (cancer in disguise)

Laomeephol C, Areecheewakul S, Tawinwung S, Suppipat K, Chunhacha P, Neves NM, Luckanagul JA. Potential roles of hyaluronic acid in in vivo CAR T cell reprogramming for cancer immunotherapy. Nanoscale. 2022 Dec 15;14(48):17821-17840.

Gene delivery: Targeting gene insertion



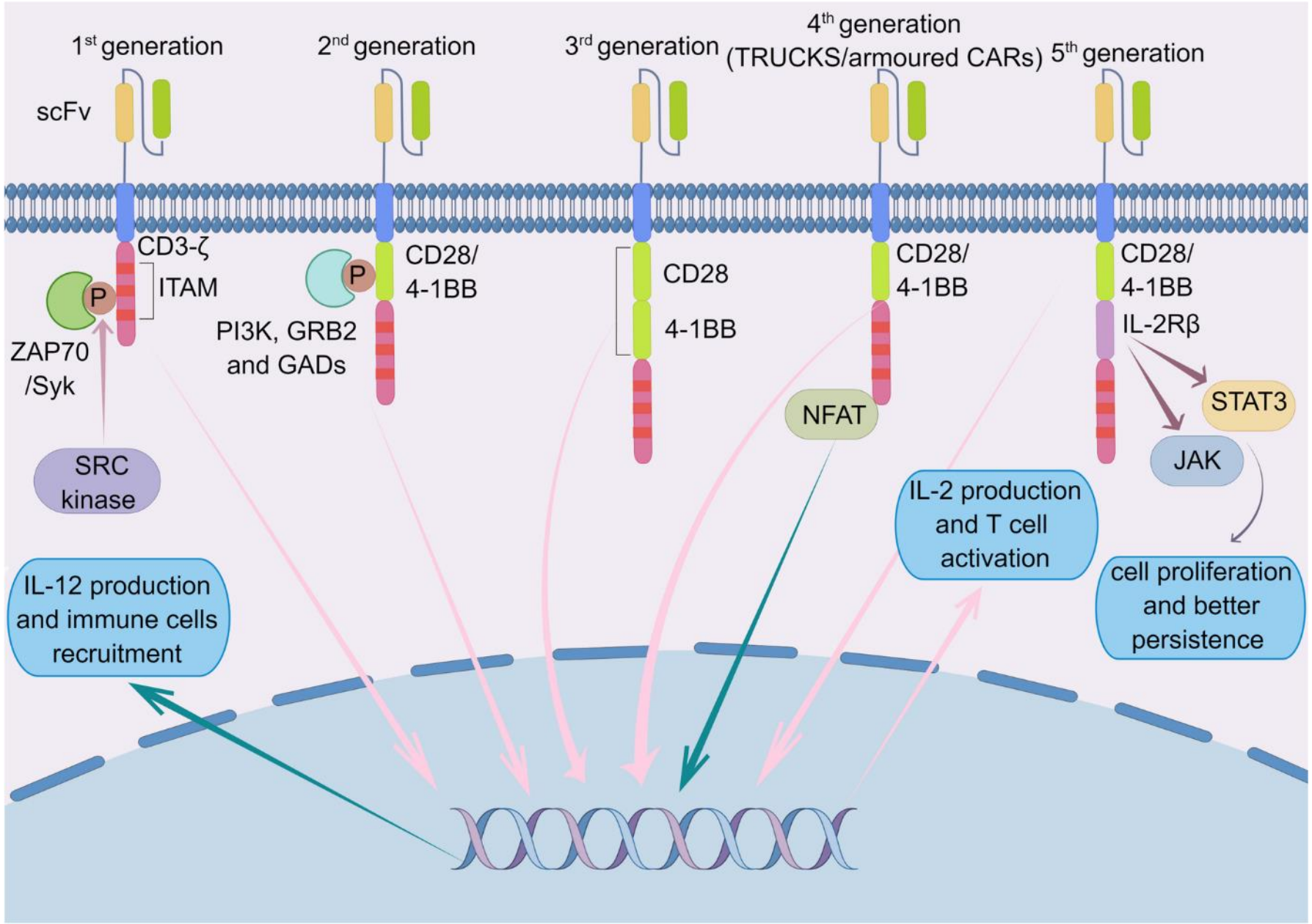


*Yagyu, S., Nakazawa, Y. piggyBac-transposon-mediated CAR-T cells for the treatment of hematological and solid malignancies. Int J Clin Oncol **28**, 736–747 (2023).*

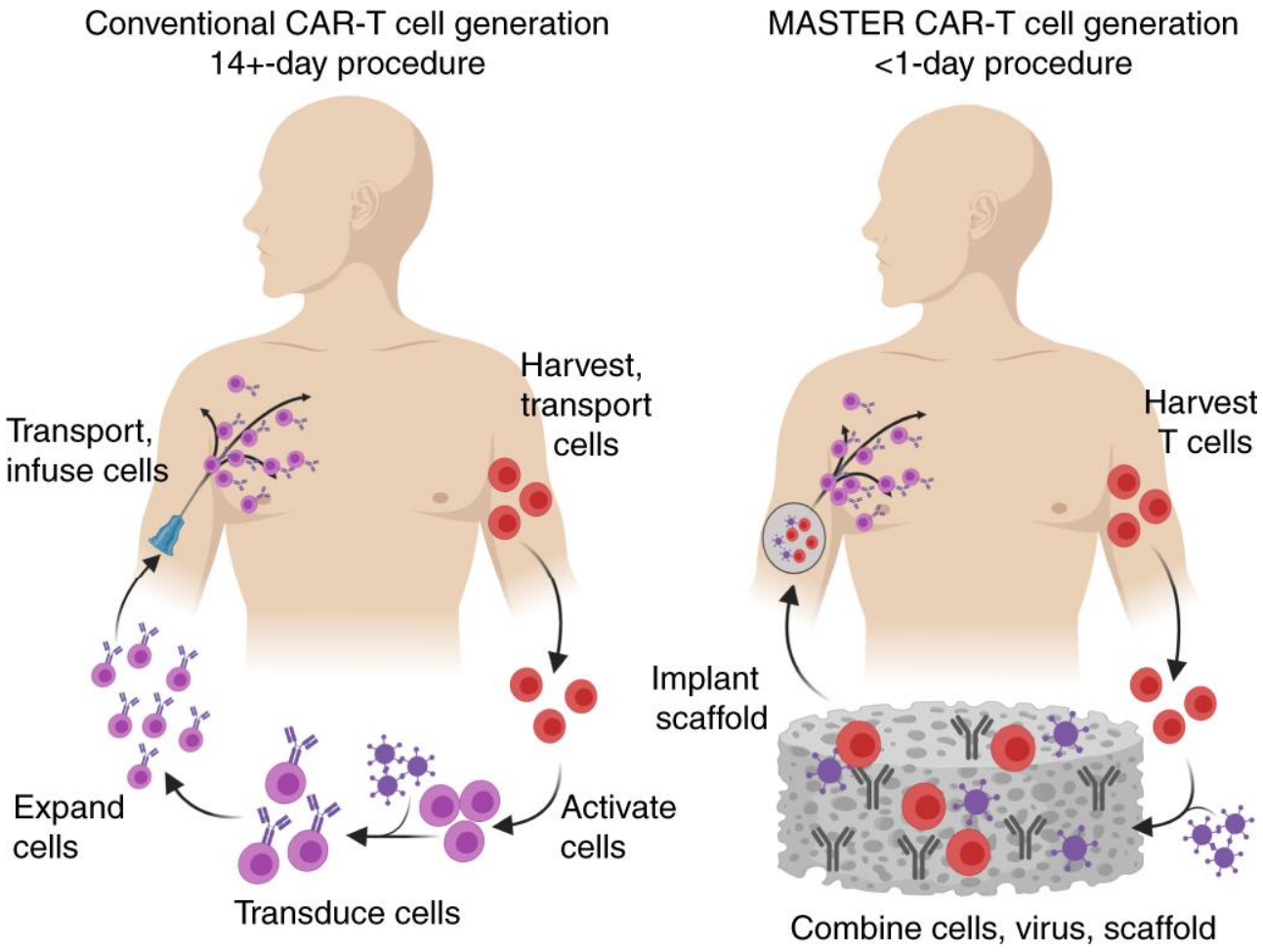
Objectives:

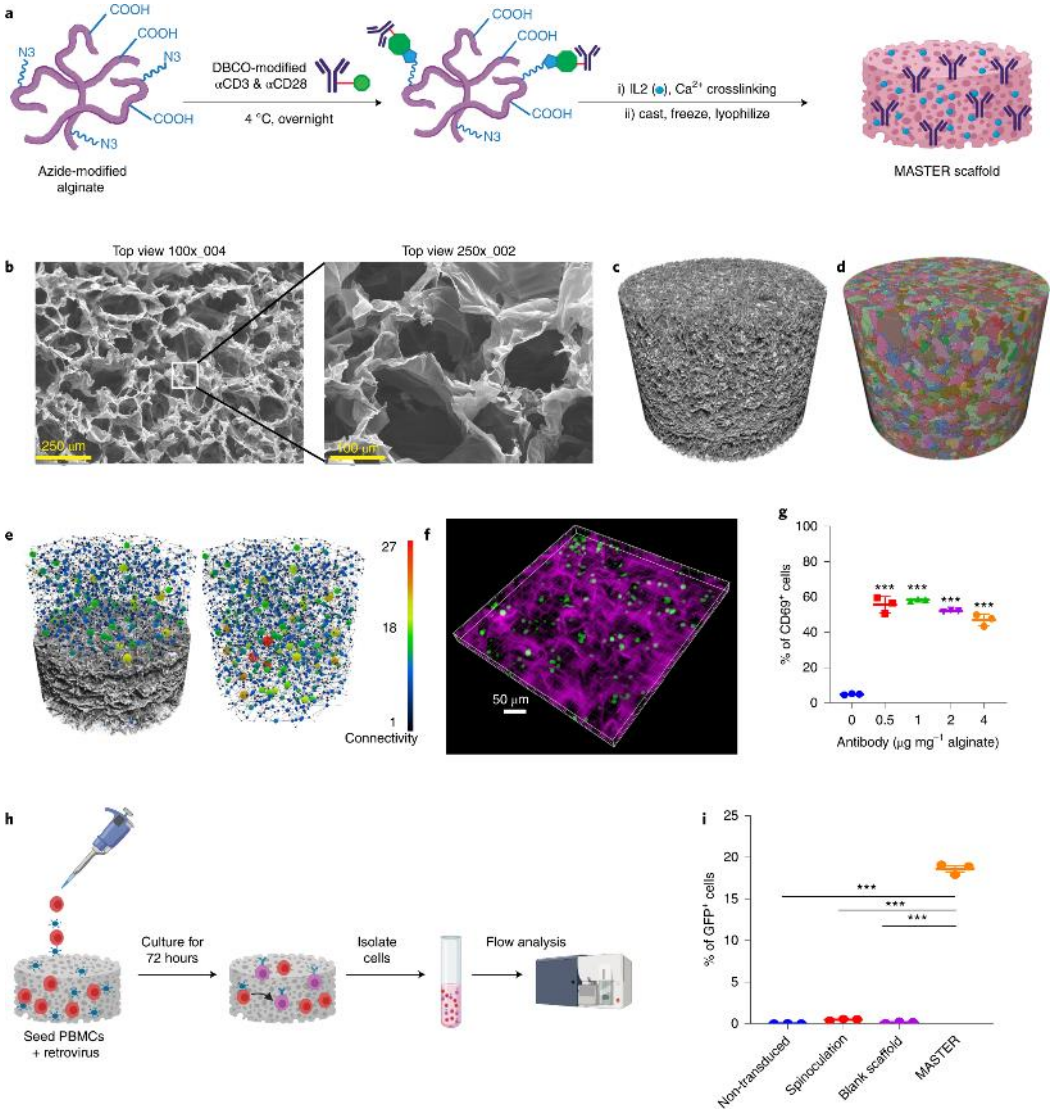
- Non-viral gene integrating delivery
- Insertional mutagenesis

CAR development: New CAR



Manufacturing: Time and cost reduction





*Agarwalla, P., Ogunnaike, E.A., Ahn, S. et al. Bioinstructive implantable scaffolds for rapid in vivo manufacture and release of CAR-T cells. Nat Biotechnol **40**, 1250–1258 (2022).*

Recap: Future of IECs

- Emerging Field: IEC therapies are rapidly advancing in cancer treatment, with numerous innovative products currently under development.
- Key Focus Areas: Efforts are directed toward improving safety and quality, while also prioritizing the development of off-the-shelf products and advancing manufacturing technologies.
- Regulatory Evolution: Regulatory frameworks must remain adaptable to accommodate novel products and evolving industry standards.

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