# กระบวนการผลิตผลิตภัณฑ์ยีนบำบัดชนิด CAR-T cells

## ความรู้พื้นฐานผลิตภัณฑ์ทางการแพทย์ขั้นสูง ชนิดยีนบำบัด

## สำนักยาและวัตถุเสพติด กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข

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2024-05-24

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Chulalongkorn University

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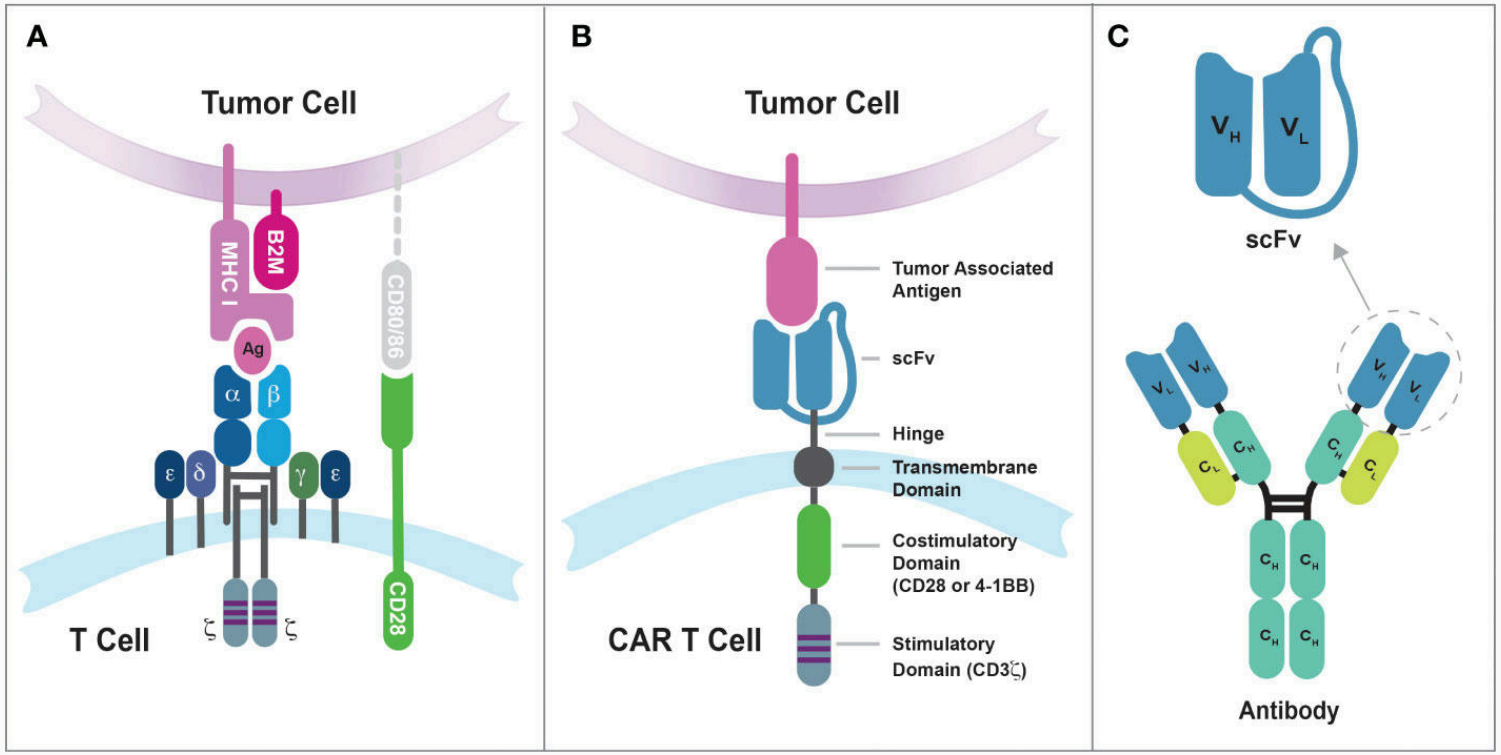
# CAR-T cell

# Chimeric Antigen Receptor

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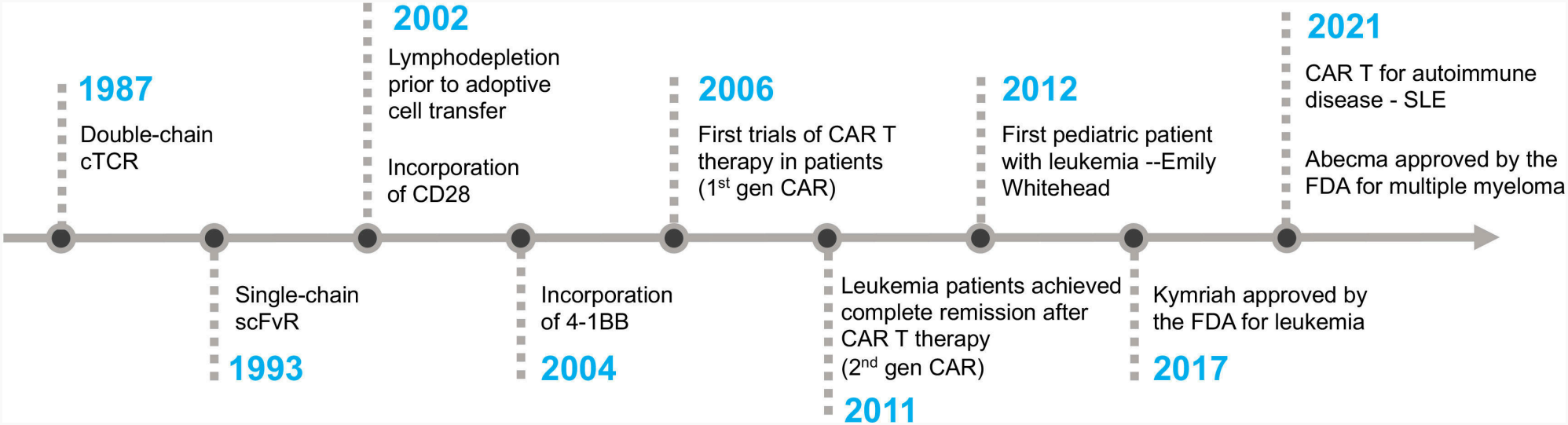
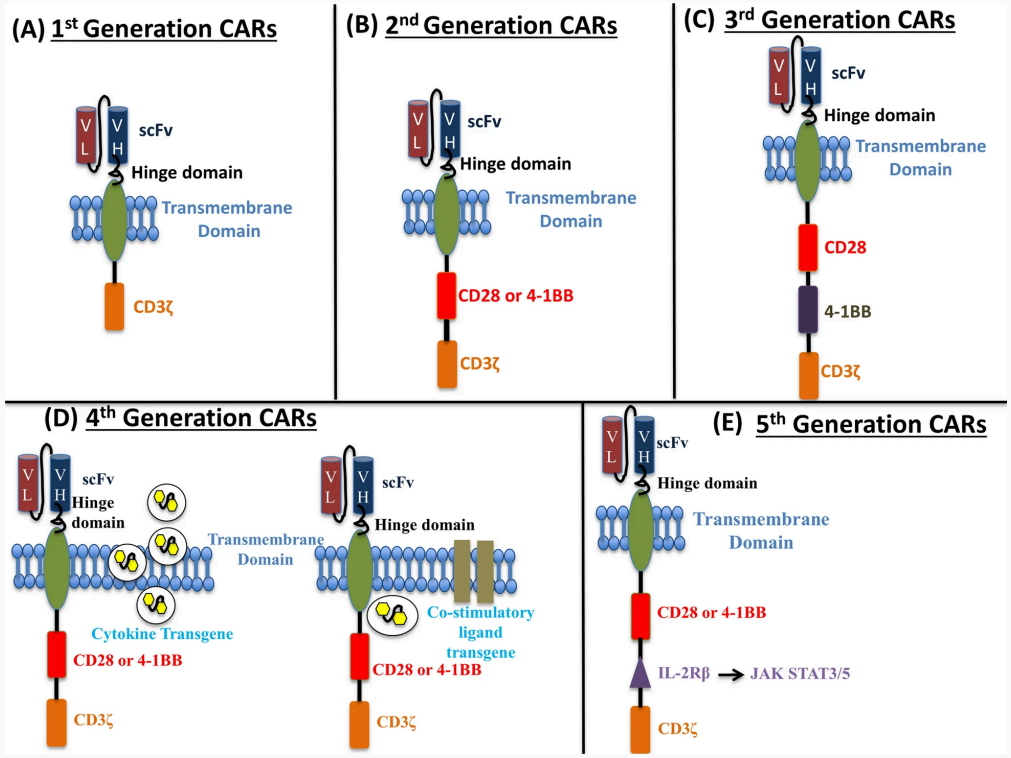
# CAR-T cell timeline



# CAR-T cell generation

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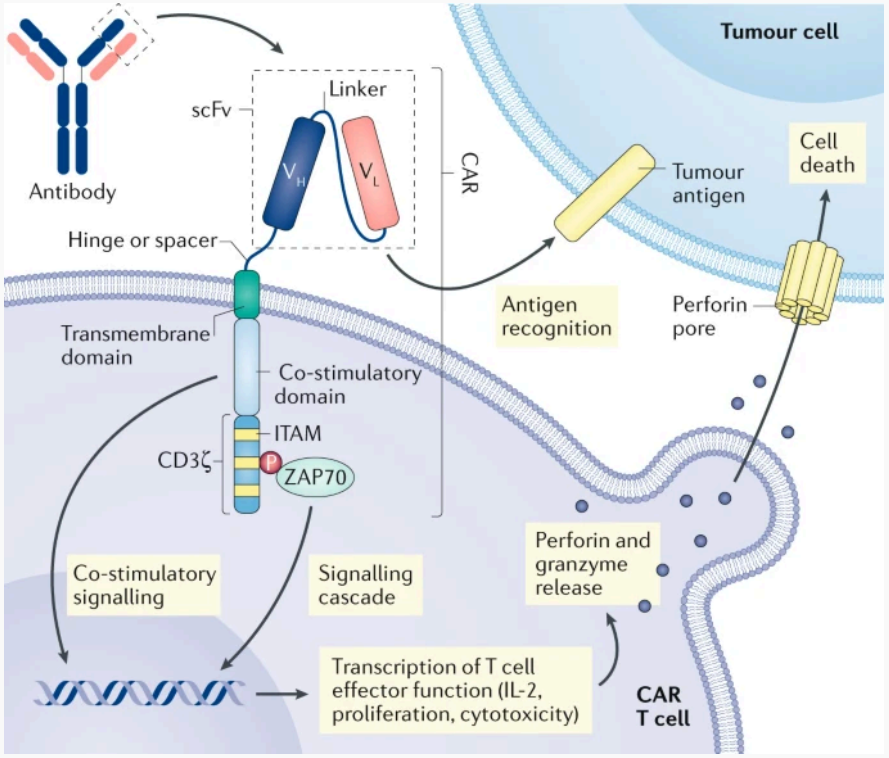
# CAR-T cell in action

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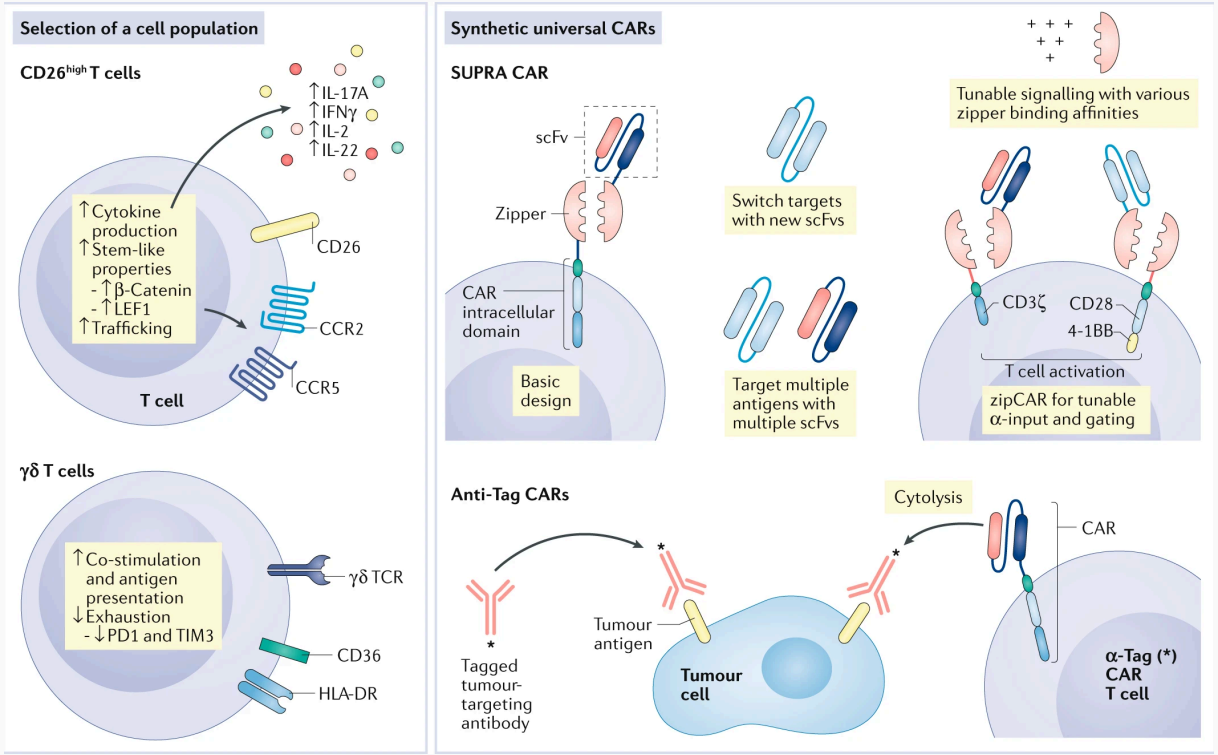
# CAR-T approved product

| Company name<br>Brand name<br>Generic name | Date of approval | Target antigen/ Antibody | Hinge/ transmembrane | Costimulatory domains | Vector/ promoter | Targeted cancers | Pivotal trial | No. of Patients | Outcomes | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Novartis<br>Kymriah<br>Tisagenlecleucel | Aug 30, 2017 | CD19<br>Mouse FMC63 | CD8α/CD8α | 4-1BB + CD3ζ | Lentiviral<br>EF1α | R/R CAYA B-ALL | ELIANA<br>(NCT02228096) | 75 | 81% overall remission rate | (58) |
| Kite<br>Yescarta<br>Axicabtagene ciloleucel | Oct 18, 2017 | CD19<br>Mouse FMC63 | CD8α/CD8α | CD28 + CD3ζ | Gammaretroviral<br>LTR | R/R LBCL | ZUMA-1<br>(NCT02348216) | 108 | 58% complete response | (59) |
| Kite<br>Tecartus<br>Brexucabtagene autoleucel | Jul 24, 2020 | CD19<br>Mouse FMC63 | CD28/CD28 | CD28 + CD3ζ | Gammaretroviral<br>LTR | R/R MCL | ZUMA-2<br>(NCT02601313) | 68 | 67% complete response | (61) |
| Juno<br>Breyanzi<br>Lisocabtagene maraleucel | Feb 5, 2021 | CD19<br>Mouse FMC63 | IgG4/CD28 | 4-1BB+ CD3ζ | Lentiviral<br>EF1α | R/R LBCL | Transcend NHL001<br>(NCT02631044) | 269 | 53% complete response | (60) |
| Bluebird<br>Abecma<br>Idecabtagene vicleucel | Mar 26, 2021 | BCMA<br>Mouse BB2121 | CD8α/CD8α | 4-1BB+ CD3ζ | Lentiviral<br>MND | R/R MM | KarMMa<br>(NCT03361748) | 128 | 33% complete response | (63) |
| J&J and Legend<br>Carvykti<br>Ciltacabtagene autoleucel | Feb 28, 2022 | BCMA<br>dual camel single-domain antibodies | CD8α/CD8α | 4-1BB + CD3ζ | Lentiviral<br>EF1α | R/R MM | CARTITUDE-1<br>(NCT03548207) | 97 | 82.5% complete response | (64) |

R/R, relapsed or refractory. CAYA, children and young adults. LBCL, large B-cell lymphoma. MCL, mantle cell lymphoma. MM, multiple myeloma.

# Future of CAR

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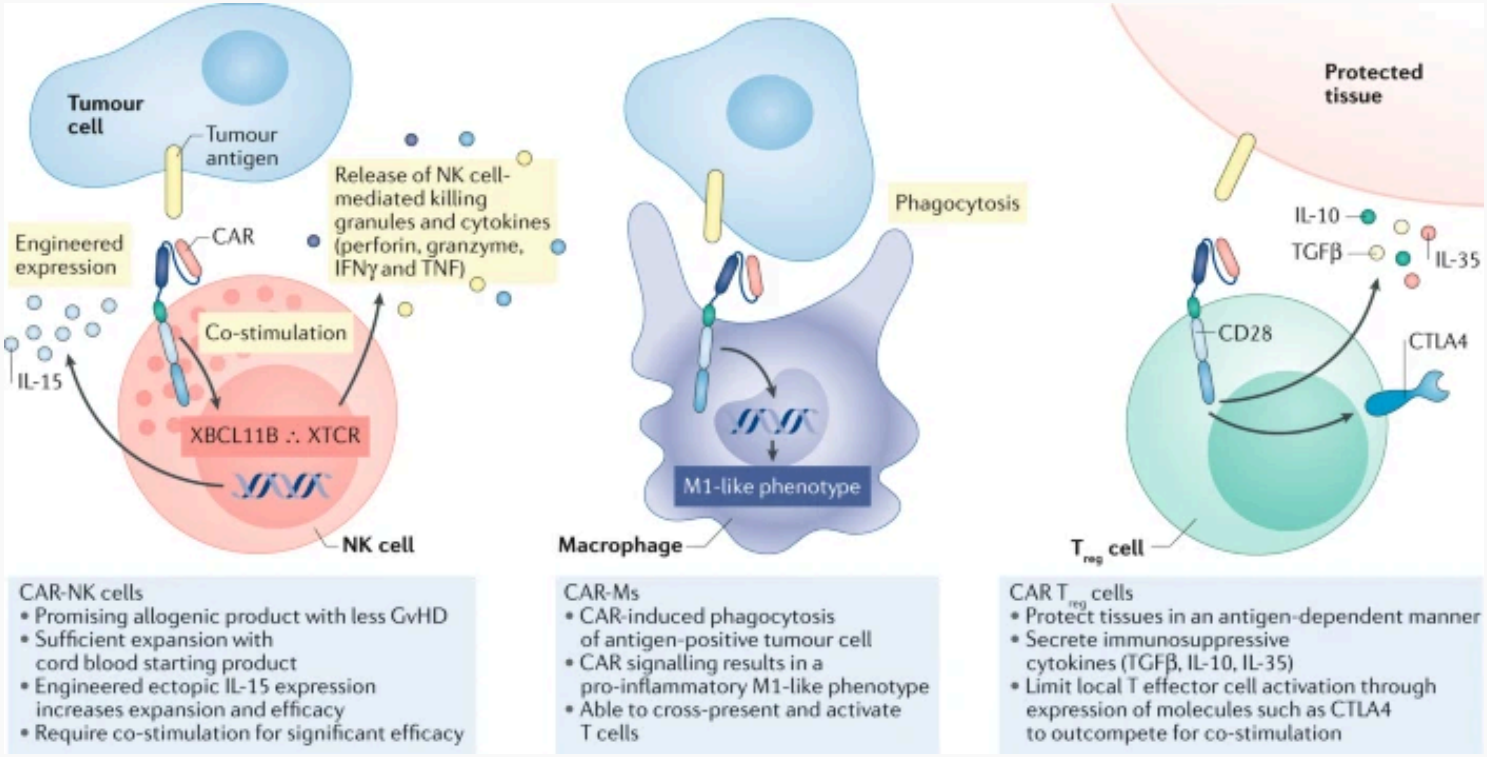
# Future of CAR

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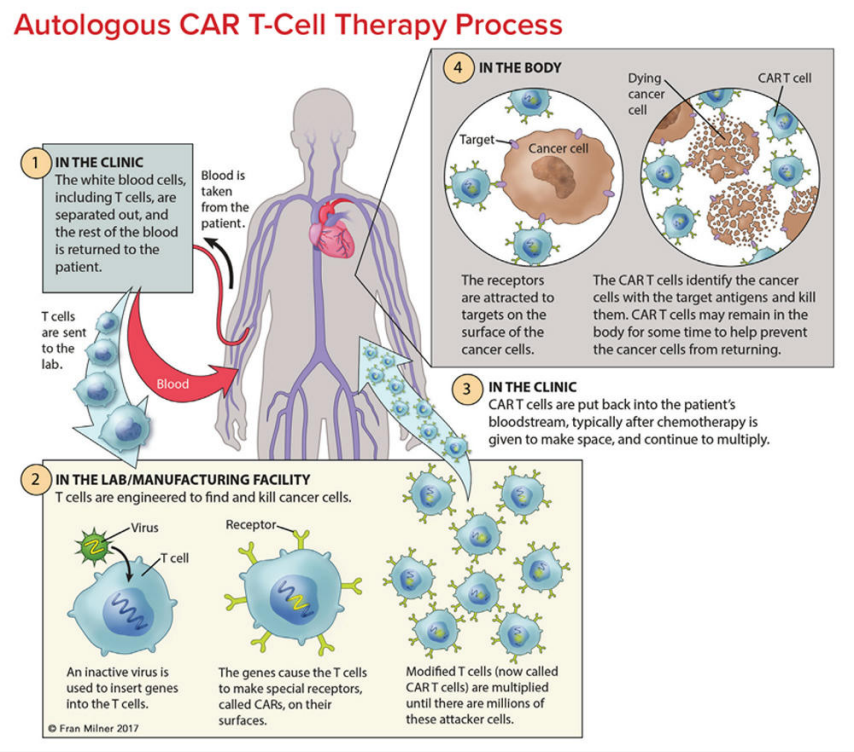
# CAR-T cell therapy process

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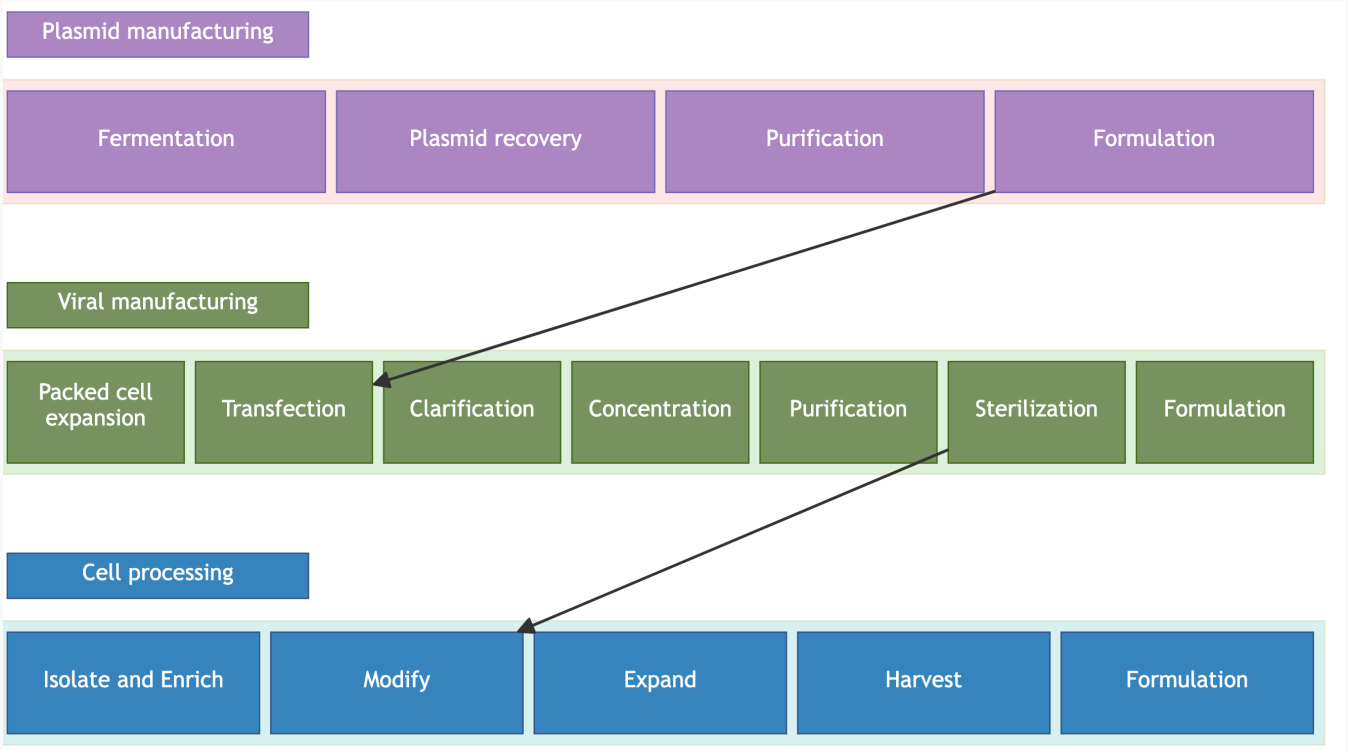
# Typical manufacturing process C&GTP

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# Starting material

# Starting material

- Cell
- Gene editing vector
- Cell culture media
- etc.

# Starting material qualification

- Safety testing:
  - sterility, mycoplasma, adventitious agents
  - other relevant human pathogens not included in donor testing
- Established acceptance criteria
  - e.g., minimum cell number, % $CD3^+$, viability;
- Additional characterization studies such as phenotypic analysis
  - e.g., % of $CD4^+$ and $CD8^+$ cells, % NK cells, % monocytes, % B cells

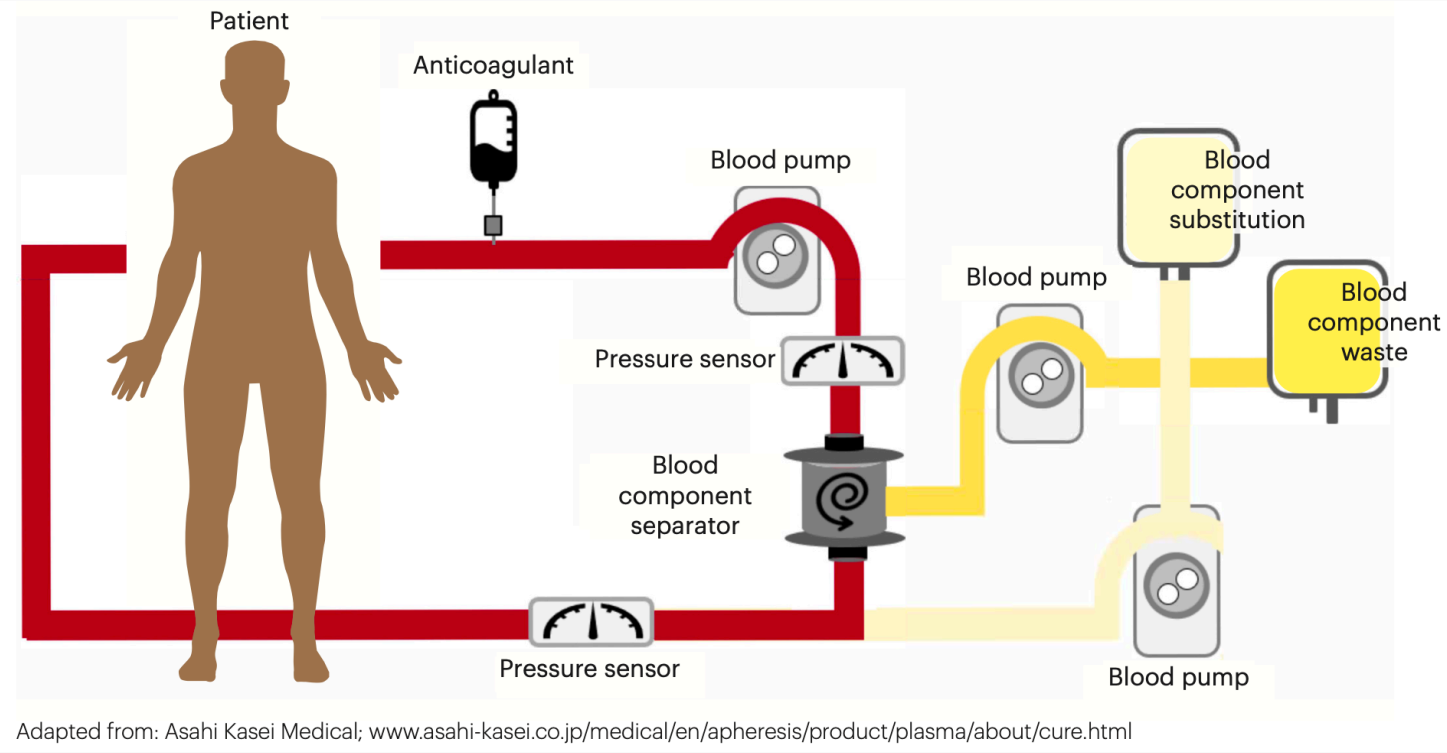
Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications

# Adventitious agents

Adventitious agents are considered to be

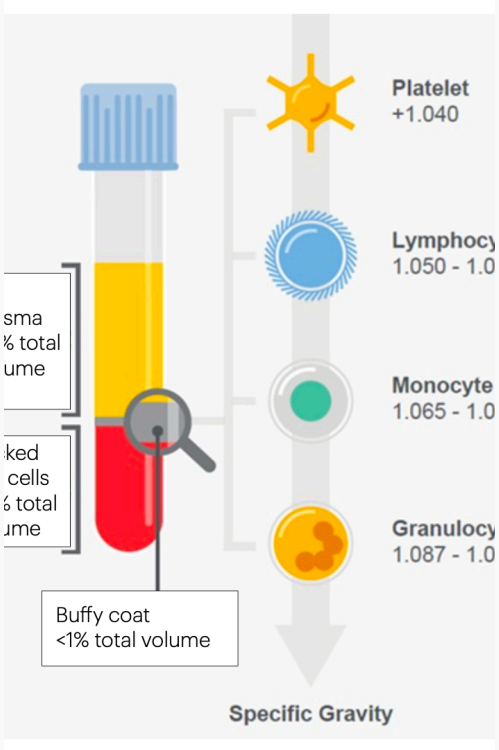
- viruses
- bacteria, mycoplasma/spiroplasma, mycobacteria, rickettsia
- fungi
- protozoa
- parasites
- transmissible spongiform encephalopathy (TSE) agents

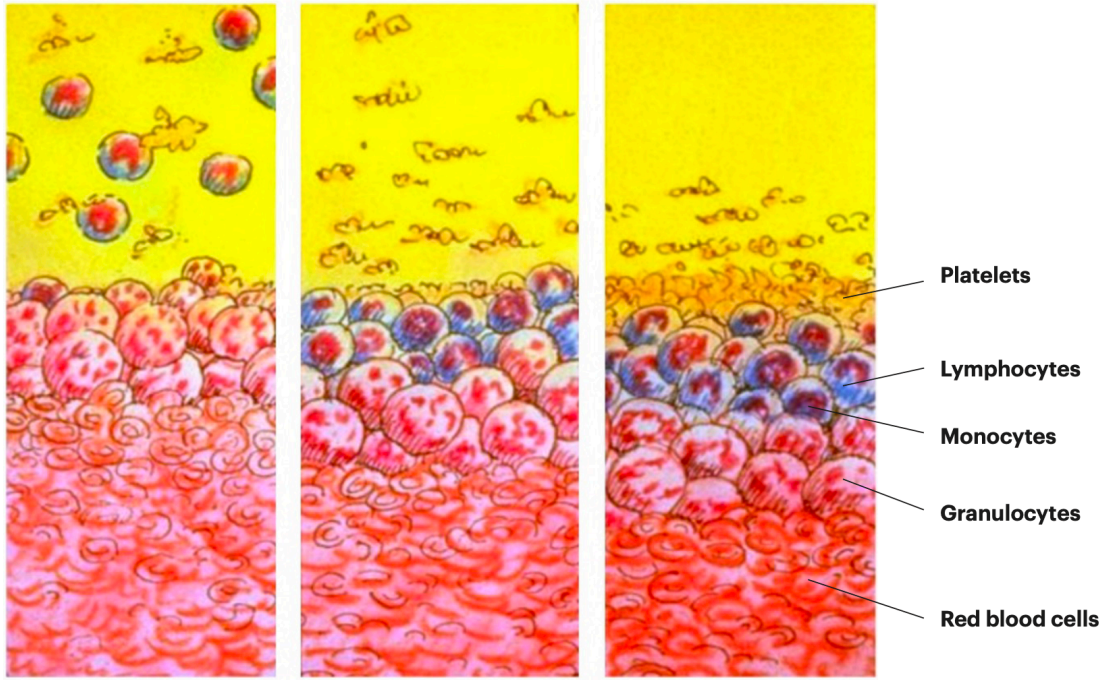
# Apheresis




Adapted from: Asahi Kasei Medical; www.asahi-kasei.co.jp/medical/en/apheresis/product/plasma/about/cure.html

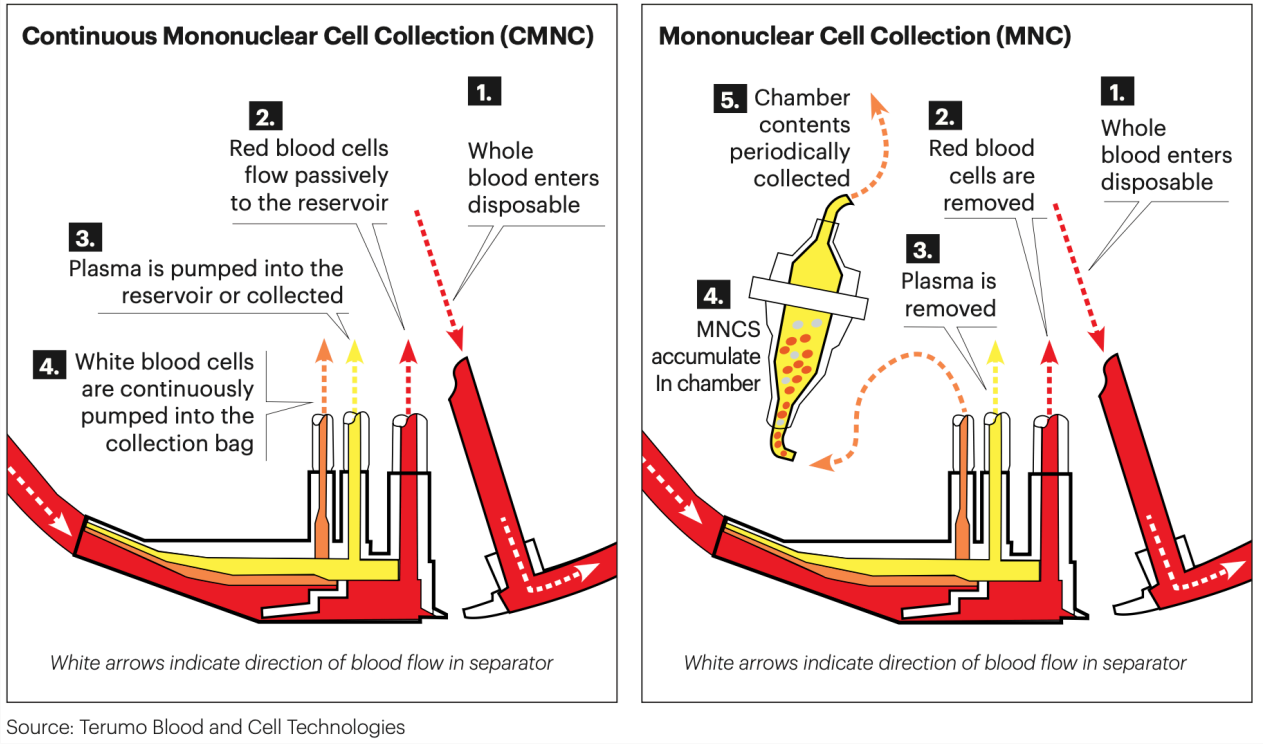
# Blood composition




Adapted from "Principles of Blood Separation and Apheresis Instrumentation"[3]

# Cell collection techniques




Source: Terumo Blood and Cell Technologies

# Donor screening and testing

Donors are defined as individuals as sources for HCT/Ps intended for implantation, transplantation, infusion, or transfer into a human recipient

donor testing and donor screening are meant to address the following three areas:

- Limiting the risk of transmission of communicable disease from donors to recipients;

# Donor screening and testing

- Establishing manufacturing practices that minimize the risk of contamination;
- Requiring an appropriate demonstration of safety and effectiveness for cells and tissues that present greater risks due to their processing or use.

# Donor screening

According to 21 CFR, Parts 1270 and 1271, all donors of cells and tissue must be screened for:

- Risk factors for, and clinical evidence of, Relevant Communicable Diseases and Disease Agents (RCDAD), including:
  - Human immunodeficiency virus (HIV), types 1 and 2
  - Hepatitis B virus (HBV)
  - Hepatitis C virus (HCV)

# Donor screening

  - Human transmissible spongiform encephalopathy (TSE), including Creutzfeldt-Jakob disease (CJD)
  - Treponema pallidum (syphilis)
- Communicable disease risks associated with xenotransplantation

# Donor testing

According to 21 CFR 1271.80(b), general requirements for donor testing are as follows:

- Testing must be done for relevant communicable diseases
- donor specimens must be collected for testing at the time of recovery of cells or tissue from the donor or up to 7 days before or after recovery.
- Appropriate FDA-licensed, approved, or cleared donor screening tests, in accordance with the manufacturer’s instructions, must be conducted

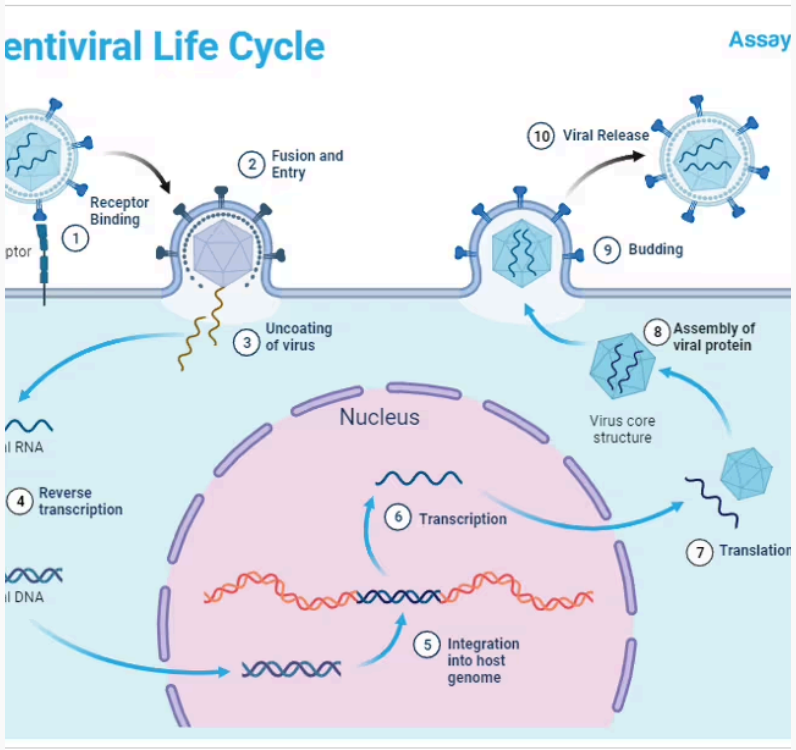
# Ancillary Materials

come into contact with the cellular therapeutic product, however are not intended to be in the final product

- Monoclonal antibodies: used in cell selection/depletion
- Cytokines, growth factors, and other supplements: used to regulate/activate/differentiate cells in culture
- Antibiotics, serum culture media, and enzymes: used to passage cells

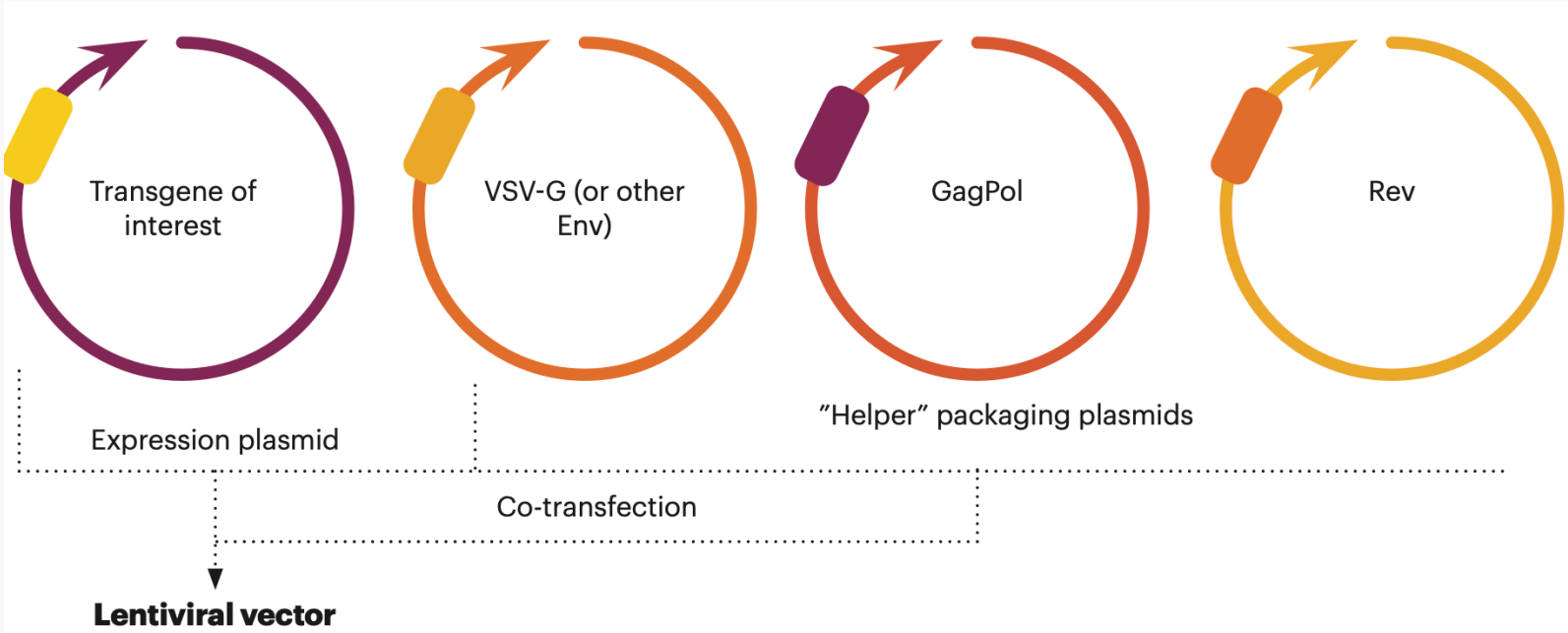
# Vector Production

# Lentivirus



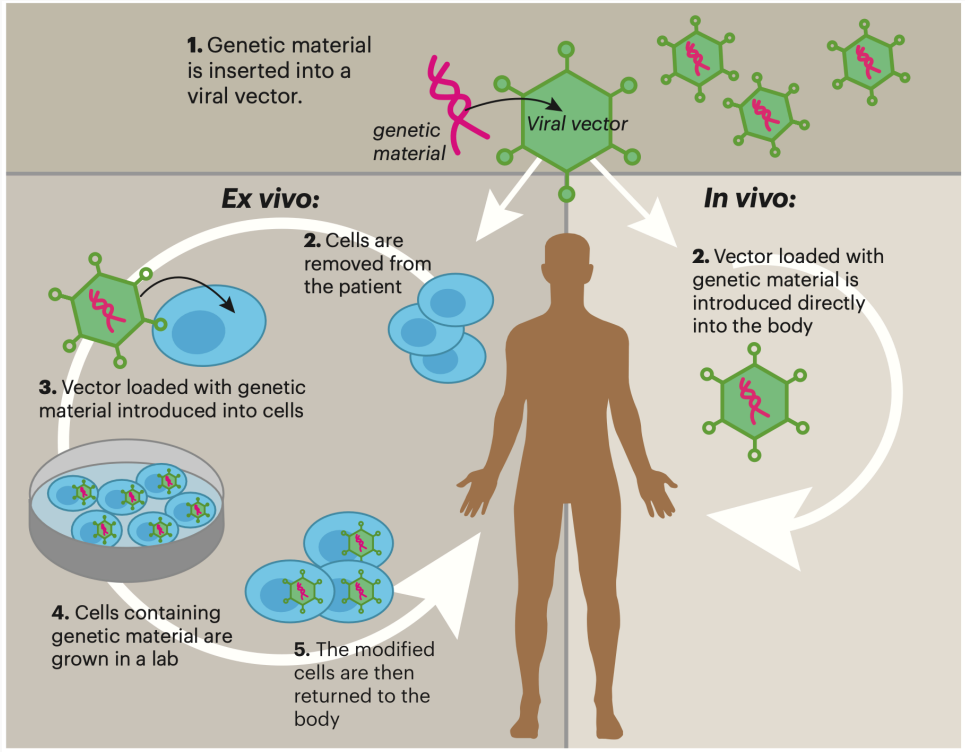

- efficiently introduce genetic material into a broad range of dividing and non-dividing cells, low immunogenicity
- Potential Risks
  - Insertional Mutagenesis
  - Immunogenicity
  - Limited Control of Expression
  - Off-Target Effects
  - Risk of Vector Mobilization

# Lentivirus vector




Adapted from: Brown J. Supporting AAV and lentiviral vector development and commercialization. Pharma's Almanac. May 24, 2019. https://www.pharmasalmanac.com/articles/supporting-aav-and-lentiviral-vector-development-and-commercialization[15]

# Vecter transfection

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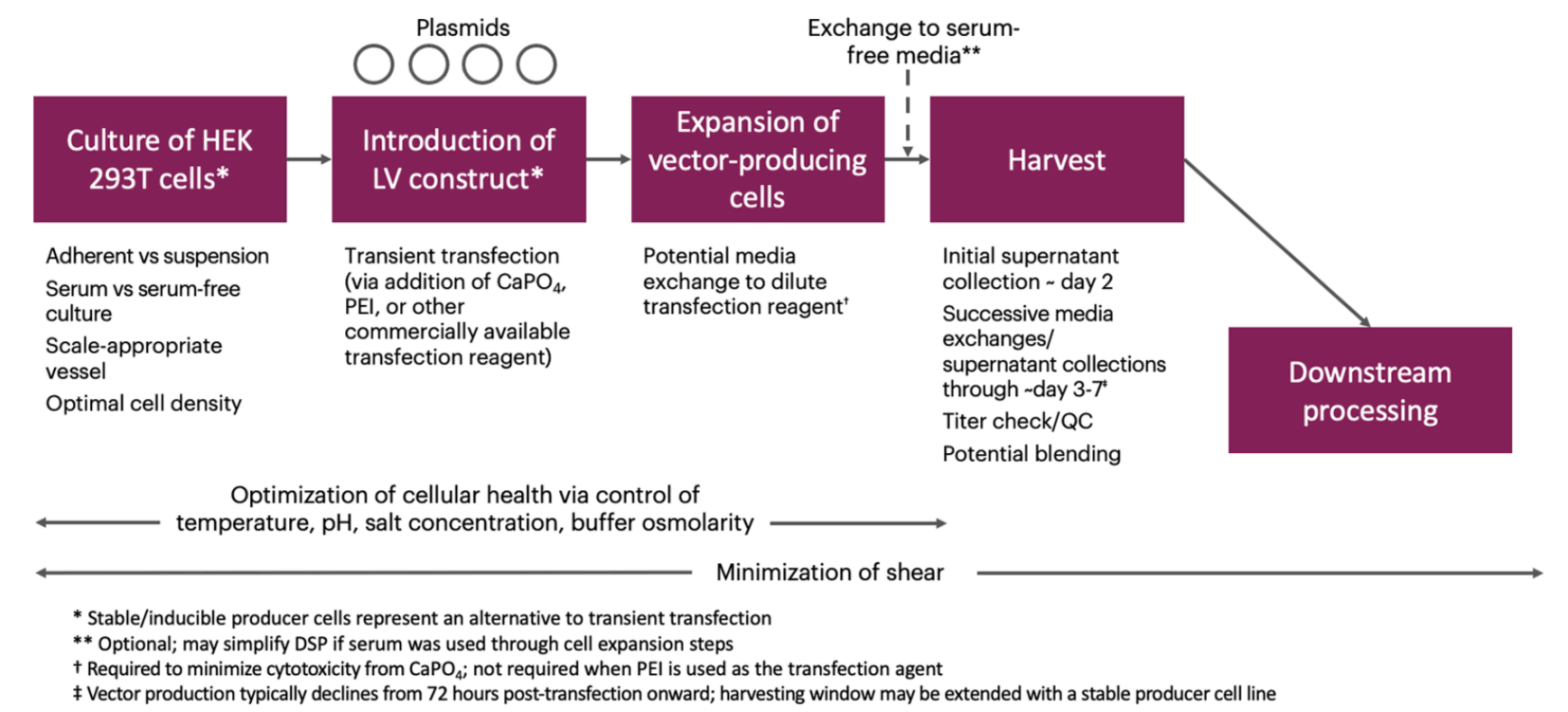
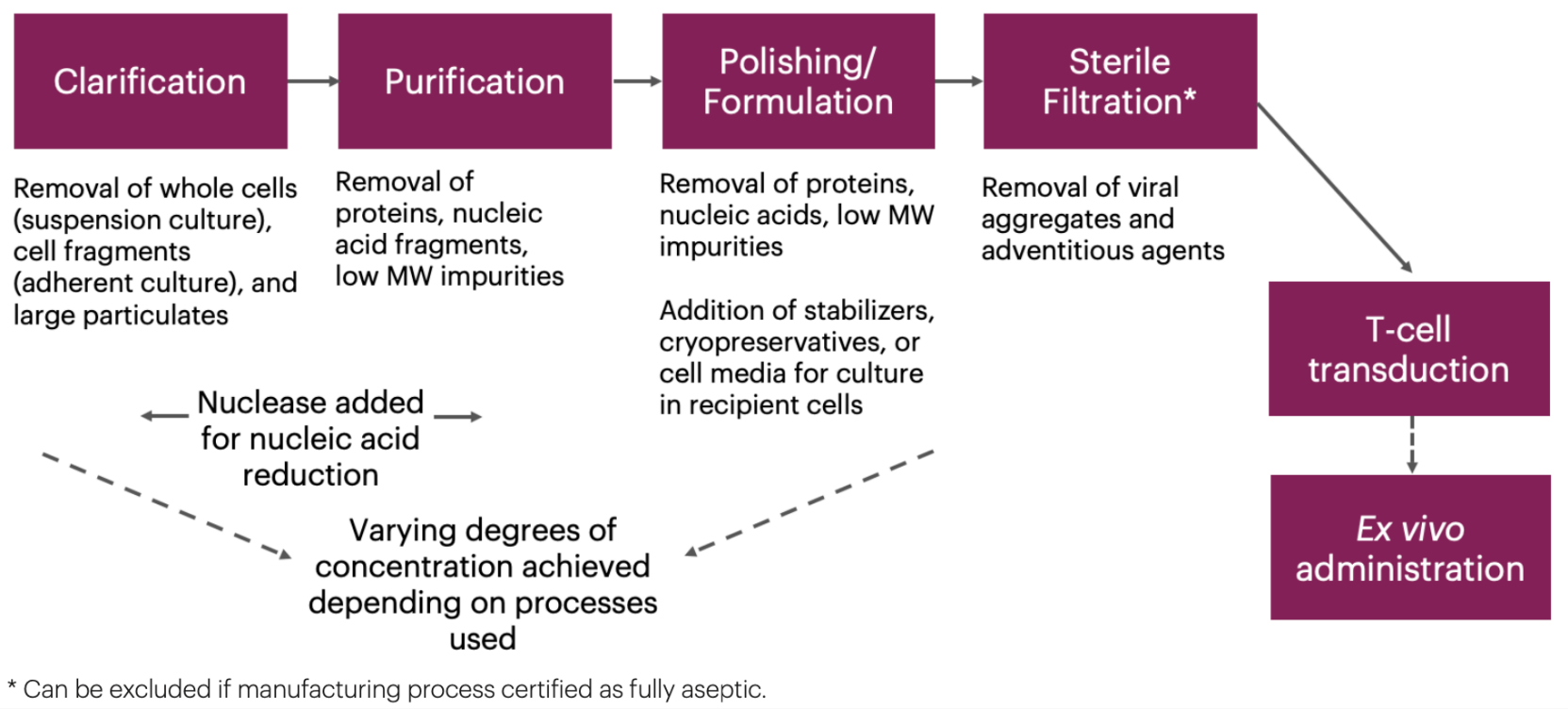
# General upstream processing for LV production



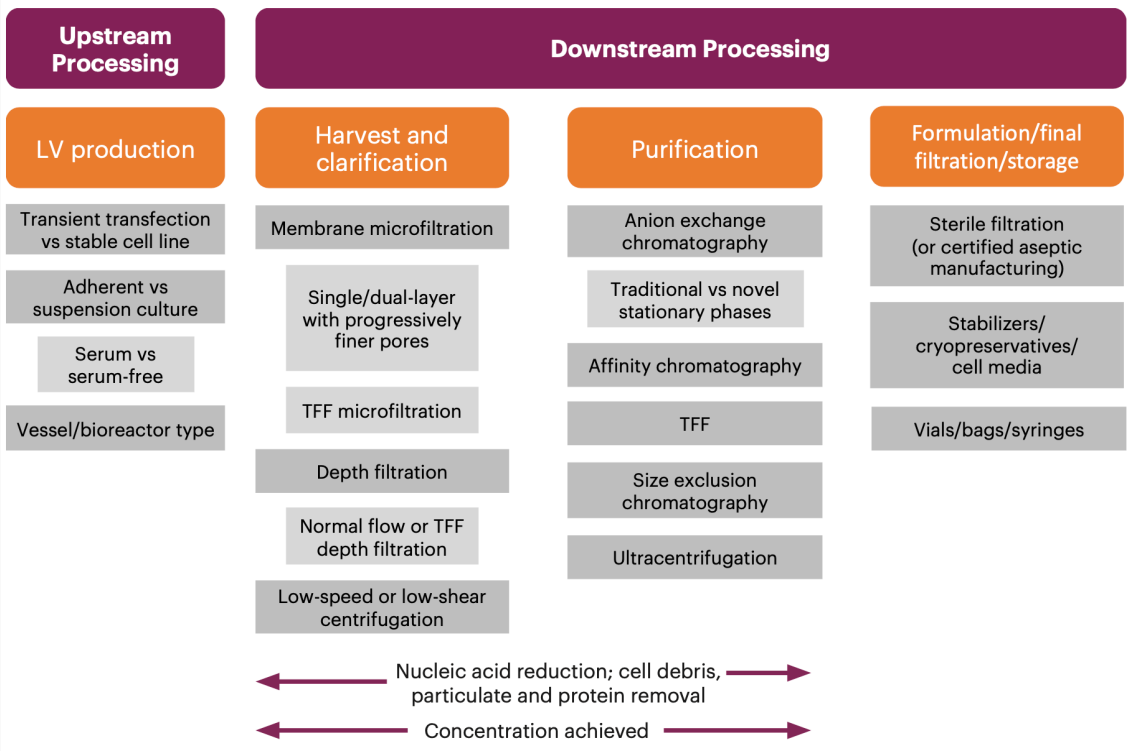
# inducible and stable cell lines for LV production

| Cell line | LV generation | Induction system | Envelope | Reported titer | Adherent or Suspension | Reference |
|---|---|---|---|---|---|---|
| GPRG-TL20-IL2RG | 3rd | Tet-off | VSV-G | $5x10^7$ TU/mL | Adherent | Throm 2009[35] |
| 293SF-PacLV | 3rd | Tet-on, Cumate | VSV-G | $3.4x10^7$ TU/mL/day | Suspension | Broussau 2008[36] |
| 293TsaGLOBE | 3rd | Tet-on | VSV-G | $1.4x10^7$ TU/mL | Suspension | Chen 2020[37] |
| LentiPro26 | 3rd | NA | MLV Amphotrophic Envelope | $10^6$ TU/mL/day | Adherent | Tomas 2018[31] |
| WinPac | 3rd | NA | RD114-PR | $1x10^6$ TU/mL | Adherent | Sanber 2015[34] |

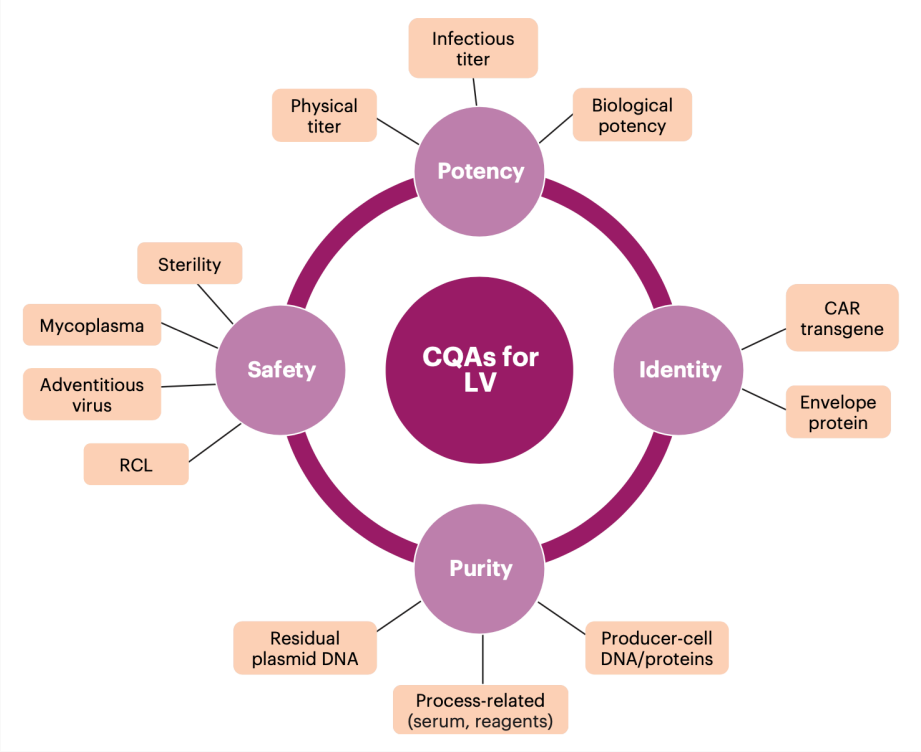
# General downstream processing for LV for ex vivo



# Options for upstream and downstream processing of LVs



# CQAs for LV in the development of CAR T-cell



# Release testing for key LV attributes

| | | | |
|---|---|---|---|
| **Identity** | Transgene presence | PCR, ddPCR, NGS, Sanger Sequencing | 21 CFR 610.14[73] |
| | Envelope | SDS-PAGE, MS, immunoblotting, ELISA | 21 CFR 610.14[73] |
| **Safety** | Adventitious virus (human, bovine, and porcine if animal-derived materials used) | *in vivo* and *in vitro* assays | ICH Q5A (R1)[24]<br>9 CFR 113.53[74]<br>9 CFR 113.46[75]<br>9 CFR 113.47[76] |
| | Replication-competent LV | qPCR, PERT, cytopathology | Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up. FDA Guidance for Industry, January 2020[77] |
| | Mycoplasma | PCR, RT-PCR, cell culture-based assays | USP <63>[78] |
| | Sterility | Culture-based assays | USP <71>[79] |
| | Endotoxin | LAL method: gel-clot, chromogenic, and turbidimetric | USP <85>[80] |
| **General** | pH | pH meter (potentiometry) | USP <791>[81] |
| | Osmolality | Osmometer | USP <785>[82] |
| | Appearance (color and clarity) | Visual | USP <631>[83] |

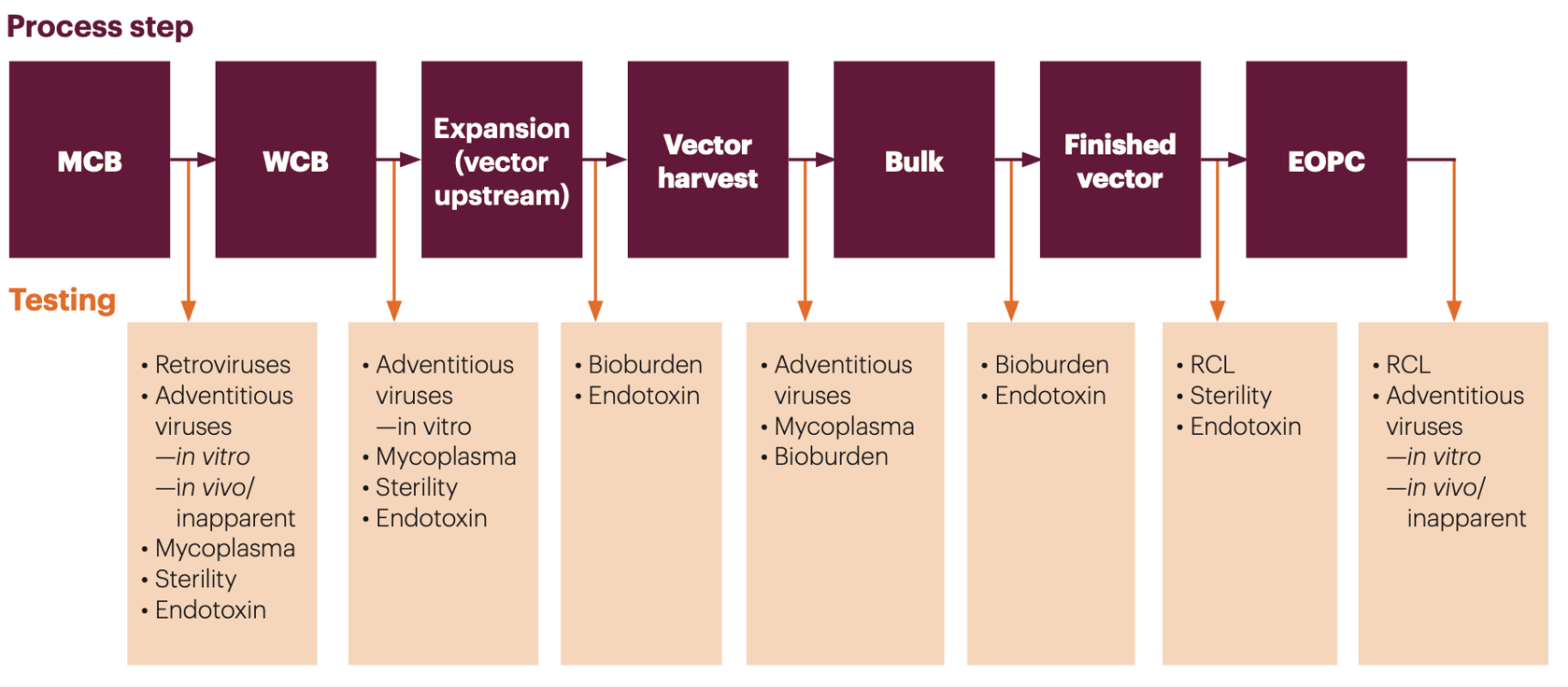
# Release testing for key LV attributes (cont)

| | | | |
|---|---|---|---|
| **Purity** | Residual plasmid | qPCR | Guidelines on the quality, safety, and efficacy of biotherapeutic protein products prepared by recombinant DNA technology: Replacement of Annex 3 of WHO Technical Report Series, No. 814. (2013)[84] |
| | Residual host cell DNA, total DNA | qPCR, Picogreen, DNA Threshold assay | Guidelines on the quality, safety, and efficacy of biotherapeutic protein products prepared by recombinant DNA technology: Replacement of Annex 3 of WHO Technical Report Series, No. 814. (2013)[84]<br>USP <509>[85] |
| | Host cell protein | ELISA, MS | USP <1132>[86] |
| | Residual serum/ nuclease/ transfection reagent/ solvent | ELISA, MS, chromatography | Serum: 21 CFR 610.15(b)[87]<br>Nuclease: ICH M7(R1)[88]<br>Transfection reagents: ICH M7(R1)[88]<br>Solvent: USP <467>[89] |
| | Product-related impurities: Interfering particles, non-infectious particles | ELISA, MS | Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). FDA Guidance for Industry, January 2020[17] |
| | Visible particulates | Visual inspection** | USP <788>[90]<br>USP <790>[91]<br>USP <1790>[92] |

# Release testing for key LV attributes (cont)

| | | | |
|---|---|---|---|
| **Strength/ potency** | Physical/genomes titer | ELISA (p24), qPCR, RT-PCR, HPLC | Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). FDA Guidance for Industry, January 2020[17] |
| | Infectious/functional titer | Transduction of cells followed by quantification of the pro-viral DNA copy number by qPCR or by immunofluorescence with flow cytometry | Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). FDA Guidance for Industry, January 2020[17] |
| | Physical titer: infectious titer ratio | Calculation | Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). FDA Guidance for Industry, January 2020[17] |
| | Functional/biological potency (transduced primary cells) | Cell proliferation, cytotoxicity, cytokines | Potency Tests for Cellular and Gene Therapy Products. FDA Guidance for Industry, January 2011[93] |

# LV production process timepoints for safety testing



# stability studies for bulk vector

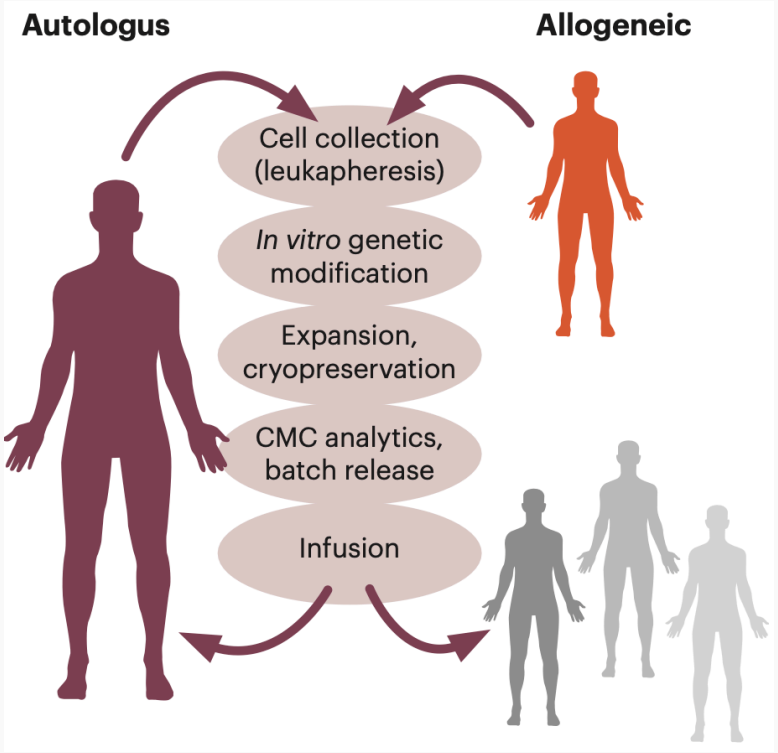
| Phase | Study type | Temperature | Quality | Lots | Method Status | Study duration |
|---|---|---|---|---|---|---|
| Preclinical (IND-FIH)[106] | Long-term | Nominal | GMP (Dev OK) | 3 | Qualified | 12 months[17,109] |
| Phase 1[106] | Long-term | Nominal | GMP | 1 to 3 | Qualified | 12 months[17,109] |
| Pivotal (registration lots)[110] | Long-term | Nominal | GMP | All PPQ | Validated | 12 months[17,109] |
| Pivotal (registration lots)[110] | Stressed & accelerated | ˚C -20, +5 | GMP | 3 | Validated | 12 months |
| Commercial | Long-term | Nominal | GMP | Up to 3 | Validated | 12 months[109] |

# stability studies for finished vector

| Material | Phase | Study Type | Temperature | Quality | Lots | Method Status | Study Duration |
|---|---|---|---|---|---|---|---|
| Finished vector | Preclinical (IND-FIH)[106] | Long-term | Nominal | GMP (Dev OK) | 3 | Qualified | 3 to 5 years[17,106,109] |
| Finished vector | Phase 1[106] | Long-term | Nominal | GMP | 1 to 3 | Qualified | 3 to 5 years[17,106,109] |
| Finished vector | Pivotal (registration lots)[107] | Long-term | Nominal | GMP | All PPQ | Validated (PPQ) | 3 to 5 years[17,106,109] |
| Finished vector | Pivotal (registration lots)[106,107] | Stressed & accelerated | ˚C -20, +5, room temp, +36 | GMP | 3 | Validated | 72 hr to months |
| Finished vector | Pivotal (registration lots)[106,107,111] | CCIT | Nominal | GMP | 3 | Validated | 3 to 5 years |
| Finished vector | Pivotal (registration lots)[107] | In-use conditions: Stability of manufacturing intermediates during process characterization | | GMP | 3 | Validated | In-use conditions and holds |
| Finished vector | Commercial | Long-term | Nominal | GMP | Up to 3 | Validated | 3 to 5 years[110] |

# CAR-T Production

# Autologous and allogeneic generation

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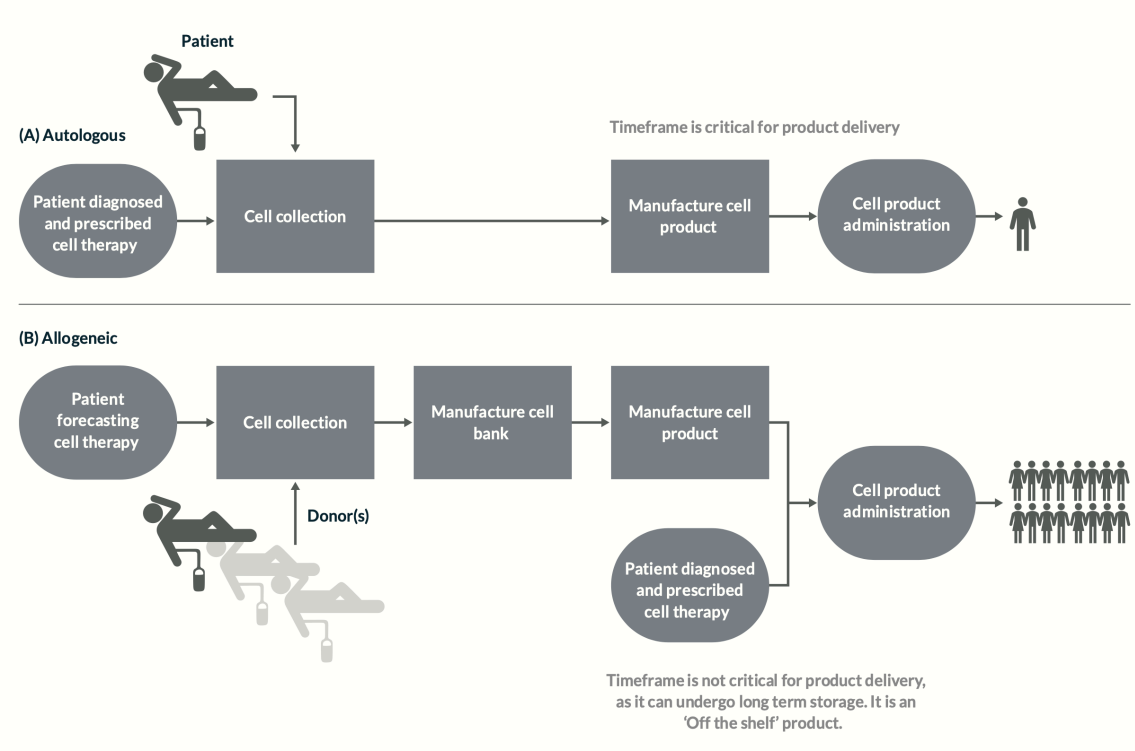
# Autologous and allogeneic in production

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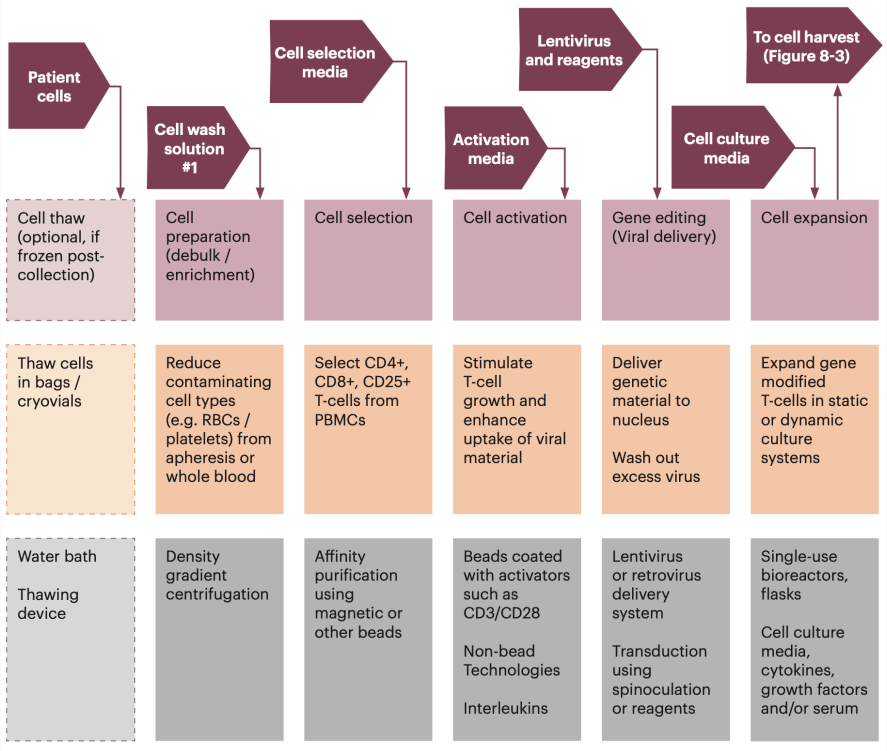
# Unit Operations for an Example CAR-T

The main manufacturing steps in CAR-T therapy production are:

- Cell isolation (leukapheresis)
- Activation
- Transduction and/or genetic editing
- Expansion
- Harvest and formulation
- Cryopreservation
- Reinfusion (post-chemo depletion)

# Autologous cell therapy manufacturing process

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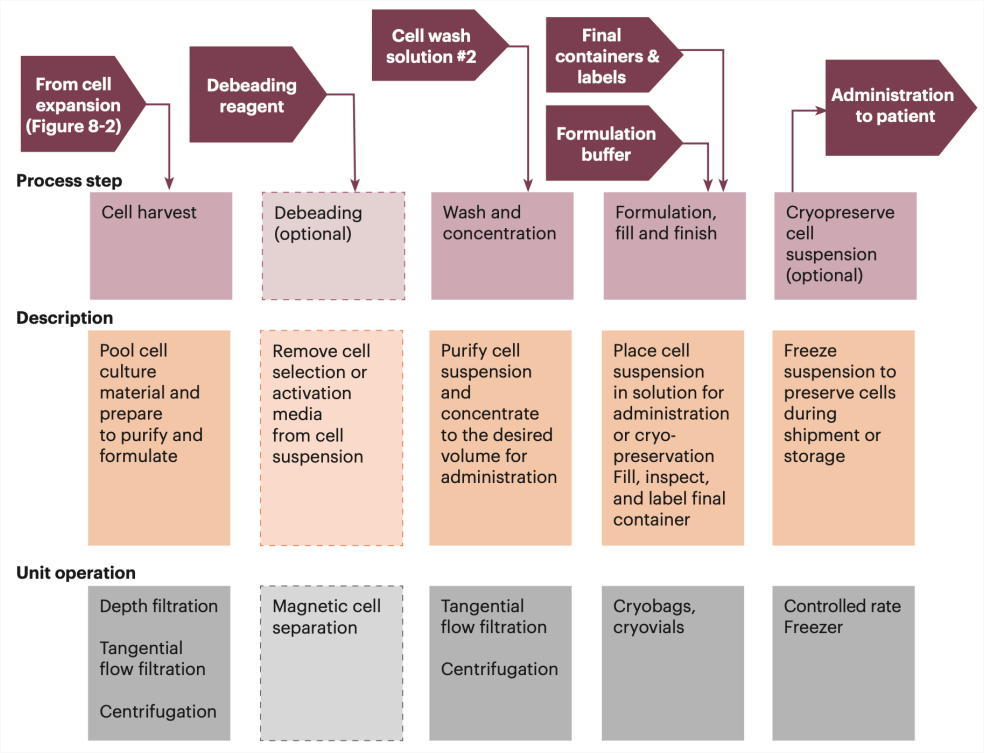
# Autologous cell therapy manufacturing process

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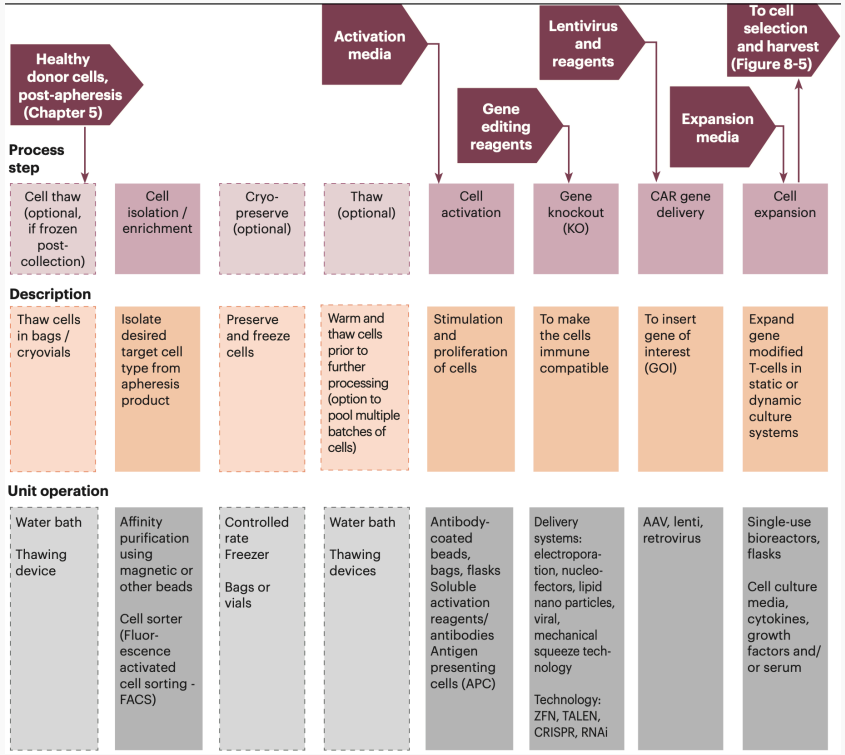
# Allogeneic cell therapy manufacturing process

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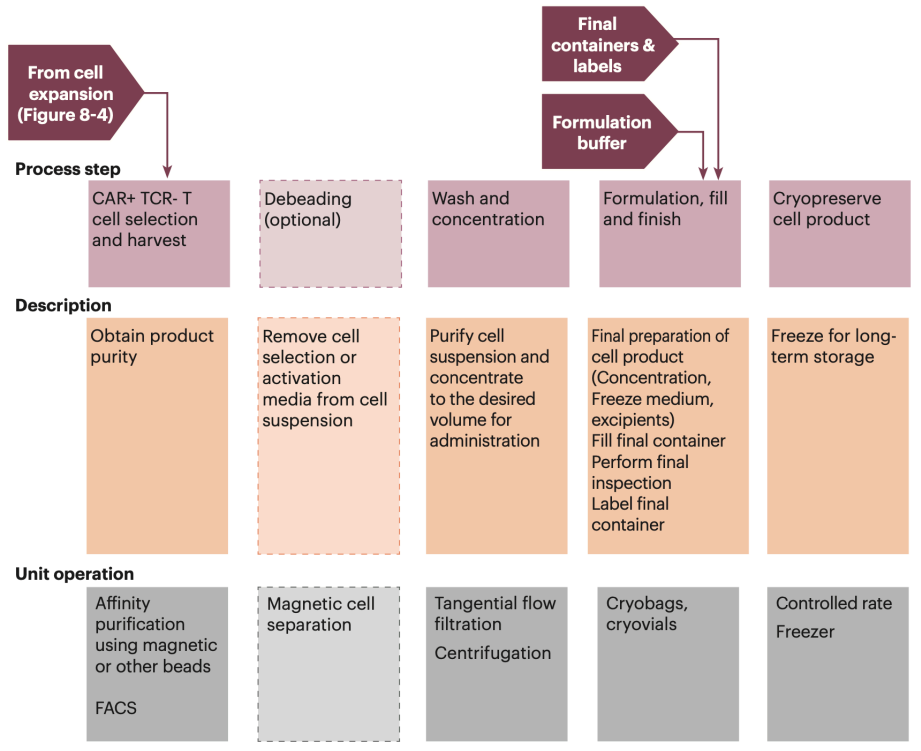
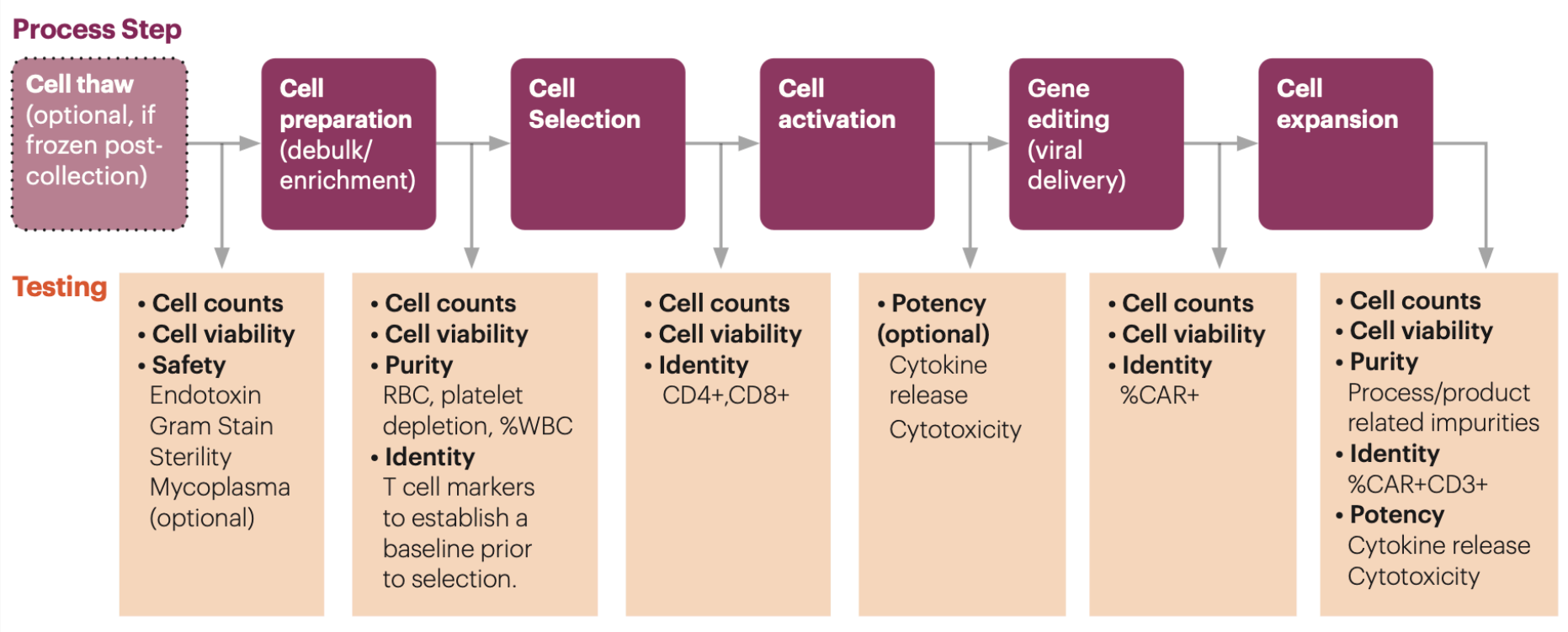
# Allogeneic cell therapy manufacturing process

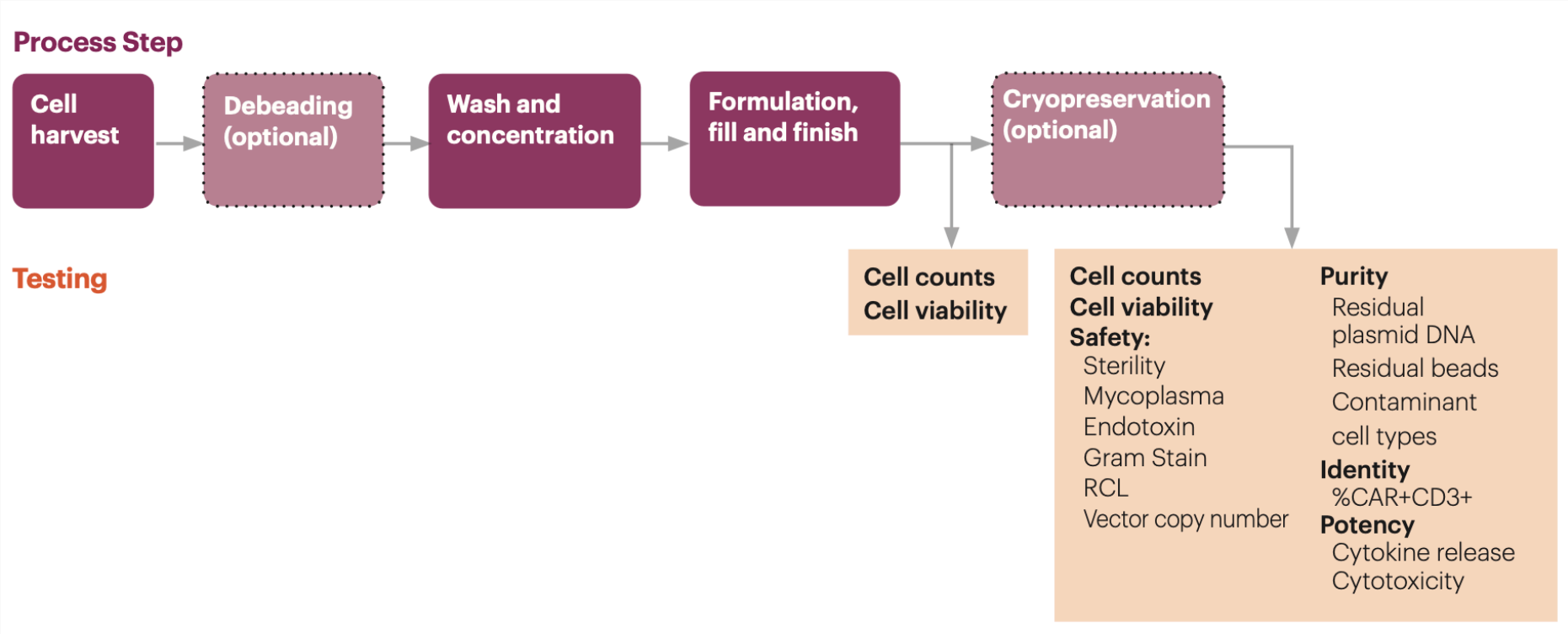



กระบวนการผลิตผลิตภัณฑ์ยีนบำบัดชนิด CAR-T cells

# Autologous cell therapy testing paradigm



# Autologous cell therapy testing paradigm



# stability for an autologous CAR-T product to determine shelf life

| Attribute category | Test | Time points following lot release (months) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 3 | 6 | 9 | 12 | 18 | 24 | 36 |
| Viable cell count | Cell counting | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Cell viability | Cell counting | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Safety | Sterility (USP <71> / EUR Ph 2.6.27) | ✓ | | | | ✓ | | ✓ | ✓ |
| Identity | % CAR+ CD3+ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Potency | Cytokine release (ELISA) | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

# in-line and at-line PAT for cell therapy process monitoring

| Technology | Measurement |
|---|---|
| NIR spectroscopy | Cell culture metabolites (glucose, glutamine, lactate, ammonia), viable and total cell density, osmolality |
| Raman spectroscopy | Cell culture metabolites (glucose, glutamine, lactate, ammonia), viable and total cell density, osmolality |
| Fluorescent sensors | pH and dissolved oxygen |
| Refractive index | Compositional changes |
| Multi-wavelength fluorimetry | Amino acids |
| Holographic imaging | Cell shape/size, cell viability |
| Impedance | Biomass/cell viability |
| Turbidimetry | Biomass |
| HPLC | Media components (amino acids, sugars, proteins, metabolites) |
| LC-MS | Media components (amino acids, sugars, proteins, metabolites) |
| Coulter counter | Biomass/cell viability |
| Imaging | Cell size/shape, cell viability |
| Photometric analyzers | Cell culture metabolites (glucose, glutamine, lactate, ammonia) |

# early-phase autologous CAR-T cell product specifications

| Quality Attribute | Parameter | Methodology | Specification |
|---|---|---|---|
| **Appearance** | Color | Compendial | Description of color |
| | Clarity | Compendial | Description of turbidity |
| **Identity** | Confirmation of identity | Flow cytometry | Anti-XXX CAR+ cells detected (identity confirmed) |
| **Purity** | Cell viability | Fluorescent microscopy and image analysis | Initial specifications based on platform knowledge (product, process), patient population, health authority guidance, and risk-based approach |
| | T-cell purity/ immunophenotype | Flow cytometry | |
| | Product-related impurities | Flow cytometry | |
| | Process-related impurities | ELISA or other suitable methodologies | Initial specifications based on historical process understanding, initial impurity risk assessment or tox assessment |
| **Strength** | CAR + viable T cells | Flow cytometry | > XX CAR+ cells/mL (strength may be used in lieu of potency) |
| **Safety** | Transduction controls | qPCR | Initial specifications based on platform knowledge (product, process), patient population, health authority guidance, and risk-based approach. Strength alternative orthogonal control, develop in later phase of development |
| | Endotoxin | Compendial | XX EU/mL |
| | Mycoplasma | Compendial | Not detected |
| | Sterility | Compendial | No growth |

# commercial-phase autologous CAR-T cell specifications

| Quality Attribute | Parameter | Methodology | Specification |
|---|---|---|---|
| **Appearance** | Color | Compendial | Description of color |
| | Clarity | Compendial | Description of turbidity |
| **Identity** | Confirmation of identity | Flow cytometry | Anti-XXX CAR+ cells detected (identity confirmed) |
| **Purity** | Cell viability | Fluorescent microscopy and image analysis | Meaningful specification established per clinical correlative analysis |
| | T-cell purity/ immunophenotype | Flow cytometry | |
| | Product-related impurities | Flow cytometry | |
| | Process-related impurities | ELISA or other suitable methodologies | Meaningful specification established per process characterization/ impurity risk assessment |
| **Strength** | CAR + viable T cells | Flow cytometry | > XX CAR+ cells/mL |
| **Potency** | Antigen-specific function | Bioassay | Product-specific acceptance criteria established per clinical correlative analysis (potency, transduction controls) |
| **Safety** | Transduction controls | qPCR | |
| | Endotoxin | Compendial | XX EU/mL |
| | Mycoplasma | Compendial | Not detected |
| | Sterility | Compendial | No growth |

# cell-based therapy product QTPP

| | Category of Attributes | Examples |
|---|---|---|
| General Property | Therapeutic Indication | Relapsed or Refractory Diffuse Large B-Cell Lymphoma, r/r Acute Lymphoblastic Leukemia |
| | Shelf life | years |
| | Storage Conditions | 2-8°C, cryopreserved in vapor phase $LN_2$ <-130°C |
| | Container Closure System | Bag, vial, sterile-sealed |
| | Dosage Form | Liquid suspension, tissue equivalent, cryopreserved, fresh |
| | Dose Regimen | Daily, monthly, single infusion |
| | Delivery volume per dose | mL, mL/kg |
| | Method of administration | IV administration |
| Drug product Quality Attributes | General attributes | pH, osmolality |
| | Appearance | Color, opalescence, visible particulates |
| | Safety | Microbial testing that, depending on the nature of the product, is likely to be based on a multidimensional approach encompassing in-process and final-product testing |
| | Identity | Tests to distinguish the specified cells through physical or chemical characteristics of the cell line (i.e., phenotype, genotype, or other markers; qPCR of transgene; tissue-specific gene expression) |
| | Content | Total cell number, cell concentration (cells/ml), active (transduced) cells/ml |
| | Purity and impurities | Tests to assess product purity, considering the product (e.g., live cells, dead cells, cellular impurities, residual vector, process-related impurities such as residual media components, DMSO, anticoagulant) |
| | Potency | Measure of the relevant product biological functions.<br>Methods to assess product biological activity are based on the different elements involved with the mechanism of action (MoA), often multiple tests evolving from specific markers in early stage to more functional assays at later stage. |

# Challenge on ATMP development

- ATMPs are complex pharmaceuticals
  - gene therapy: transgene, type of vector, genetically modified cells
  - cell therapy: autologous, allogeneic, complex process, combination products
  - assessment requires expertise from several areas e.g. tissue engineering, gene therapy, cell therapy, biotechnology, surgery, pharmacovigilance, risk management, medical devices and ethics

# Challenge on ATMP development

- Specific administration of certain ATMPs (catheters, surgery etc.)
- Specific safety issues (e.g. integrational mutagenesis of GTMPs, biodistribution/ectopic tissue formation of cell-based MPs)
- Nature of disease: monogenetic vs multifactorial
- Mode of action: treatment of disease to repair/regeneration
- Special challenges concerning manufacturing/quality, safety and efficacy studies
- manufacturing constraints

# Challenge on ATMP development

  - GMP requirements for production
  - starting and raw materials; continuity of material supply
  - immature production technologies, comparability
  - variability and process validation
- characterization, potency testing (related to clinical outcome)
- non-clinical challenges
  - proof of concept, safety aspects (species specificities)
- clinical aspects

# Challenge on ATMP development

  - feasibility of dose finding and biodistribution studies in humans, concomitant medication/surgical procedures, efficacy!
- Product-related challenges:
  - safety: dose, tumorigenicity, biodistribution, integration
  - efficacy: inter-individual variability, administration

Questions?