Quality Control of the Raw Materials for Cell and Gene Therapy Products

Atsushi NISHIKAWA

Principal Reviewer, Office of Cellular and Tissue-based Products,

Pharmaceuticals and Medical Devices Agency (PMDA), JAPAN

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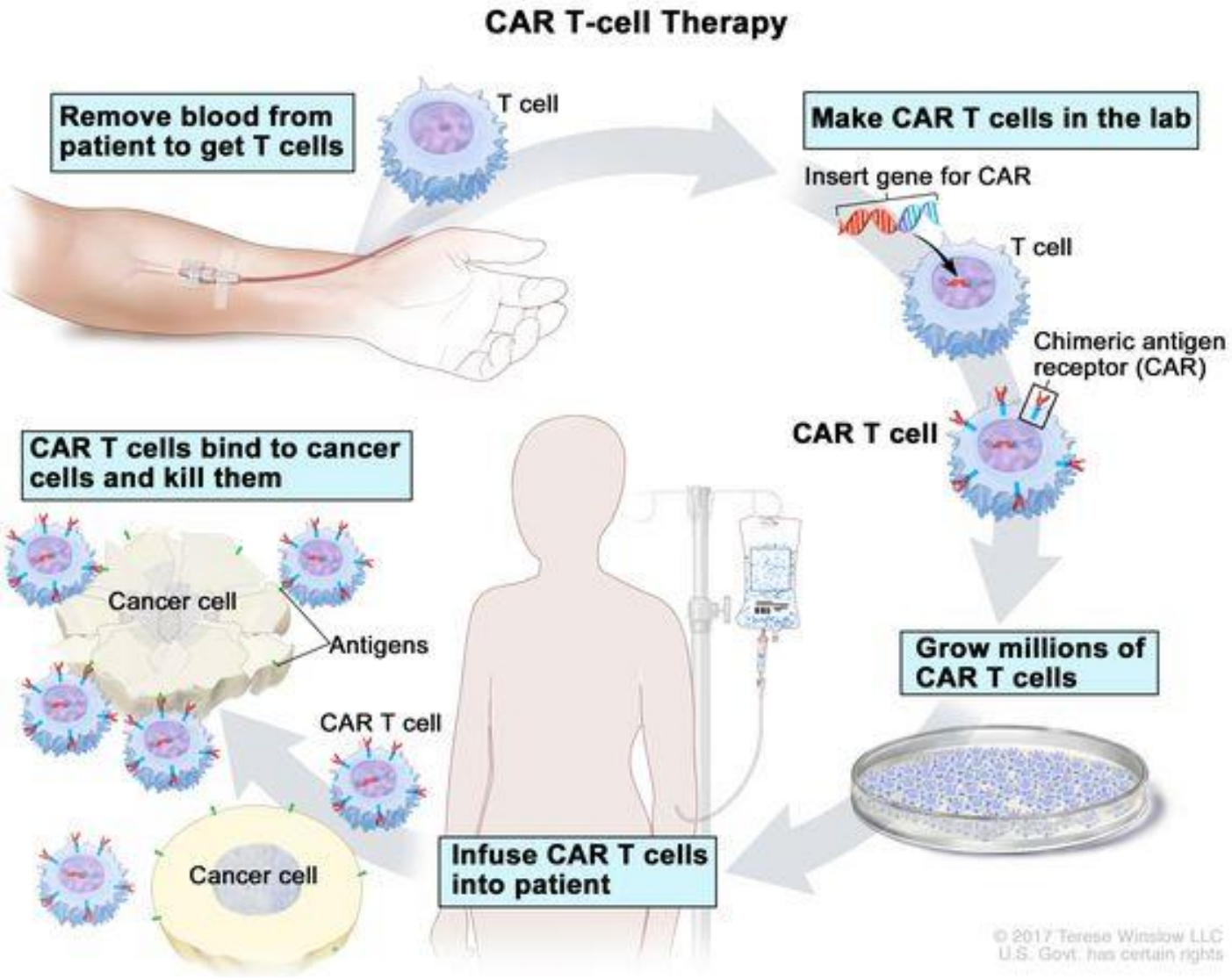
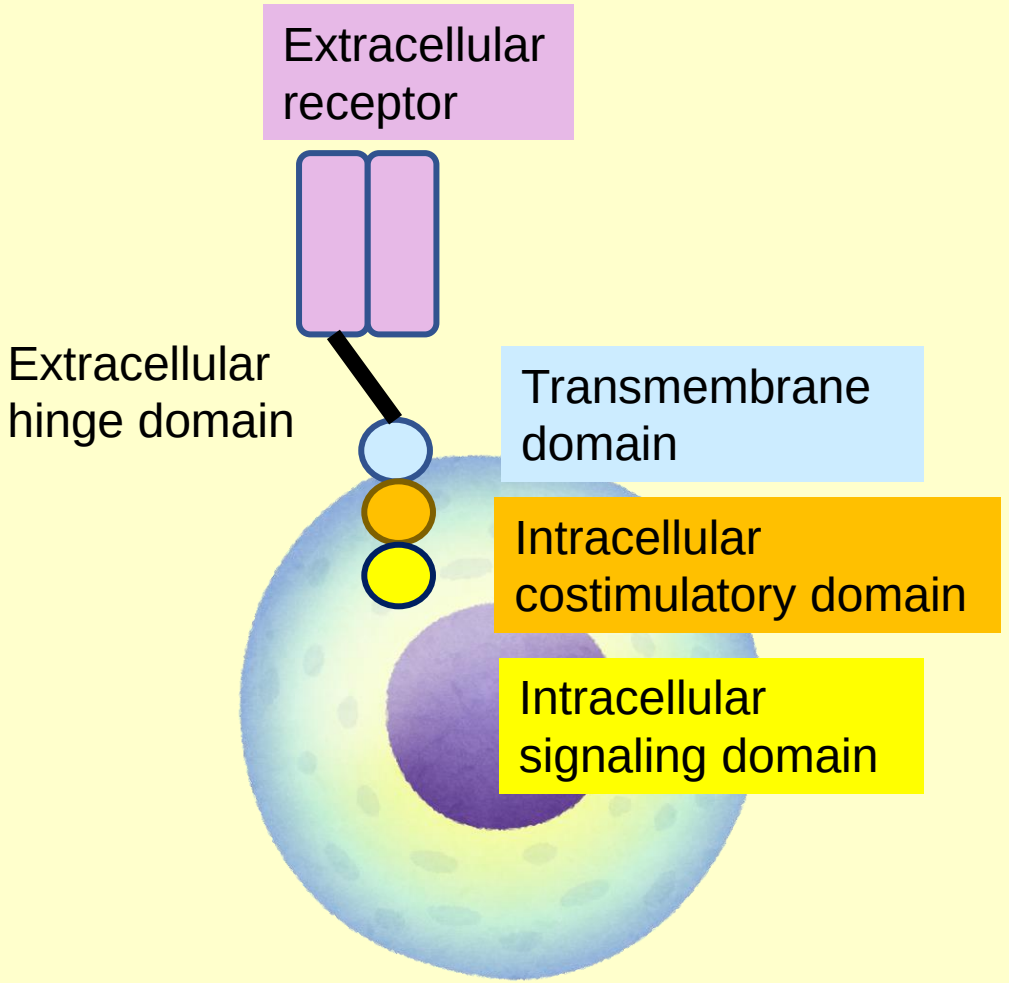
Outline

1. **Overview of CAR-T Products**
2. **Quality control of raw materials for each type of modality – regulatory situation in Japan**
   - Control of starting materials
   - Control of raw materials (FBS, trypsin, etc.)
   - Control of transgenic reagents
   - Risk management of adventitious agents
3. **Regulatory differences between regions**

CAR-T Therapy



CAR:
Chimeric Antigen Receptor



https://www.cancer.gov/publications/dictionaries/cancer-terms/def/car-t-cell-therapy

Approved auto CAR-T Products in JAPAN

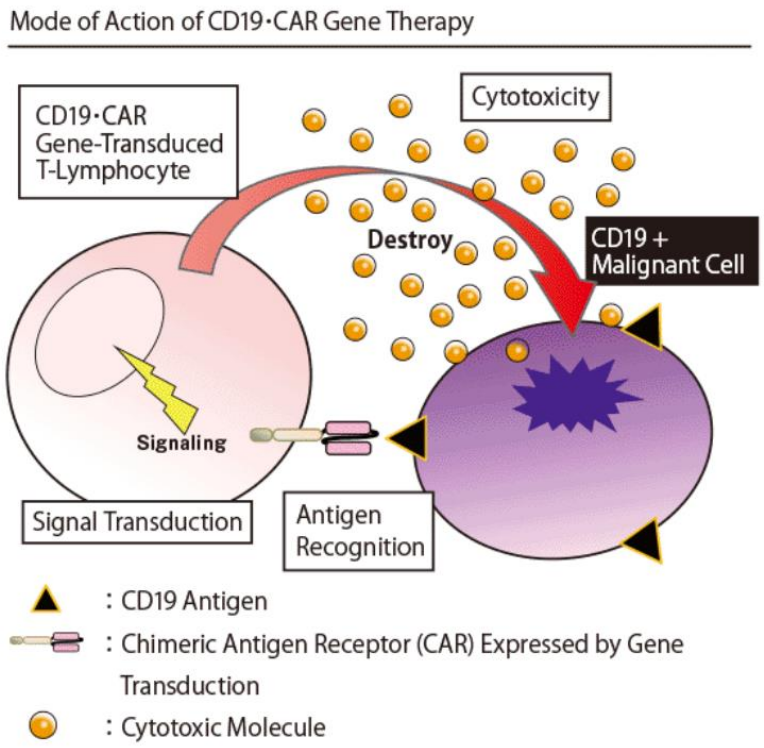
B-Cell Maturation Antigen (BCMA) CAR-T

Abecma (2022)

Carvykti (2022)

Multiple myeloma

CD19 CAR-T



Kymriah (2019)

Yescarta (2021)

Breyanzi (2021)

Large B-Cell Lymphoma, etc.

http://www.takara-bio.com/medi_e/car.html

Novel types of CAR-T cell products

Currently approved types of CAR-T products

- Autologous CAR-T
- Genetically modified with viral vectors (lentiviral vectors, retroviral vectors)

Novel types of CAR-T cell products

- Allogeneic CAR-T
- Method of CAR gene transfer
  - Non-viral vectors (piggy-BAC, etc.)
  - Genome editing
    - Genome editing tools are also used to knockout TCR, PD-1, CTLA-4, etc. to enhance CAR-T cell function.

Depending on the type of modality, appropriate quality control should be considered.

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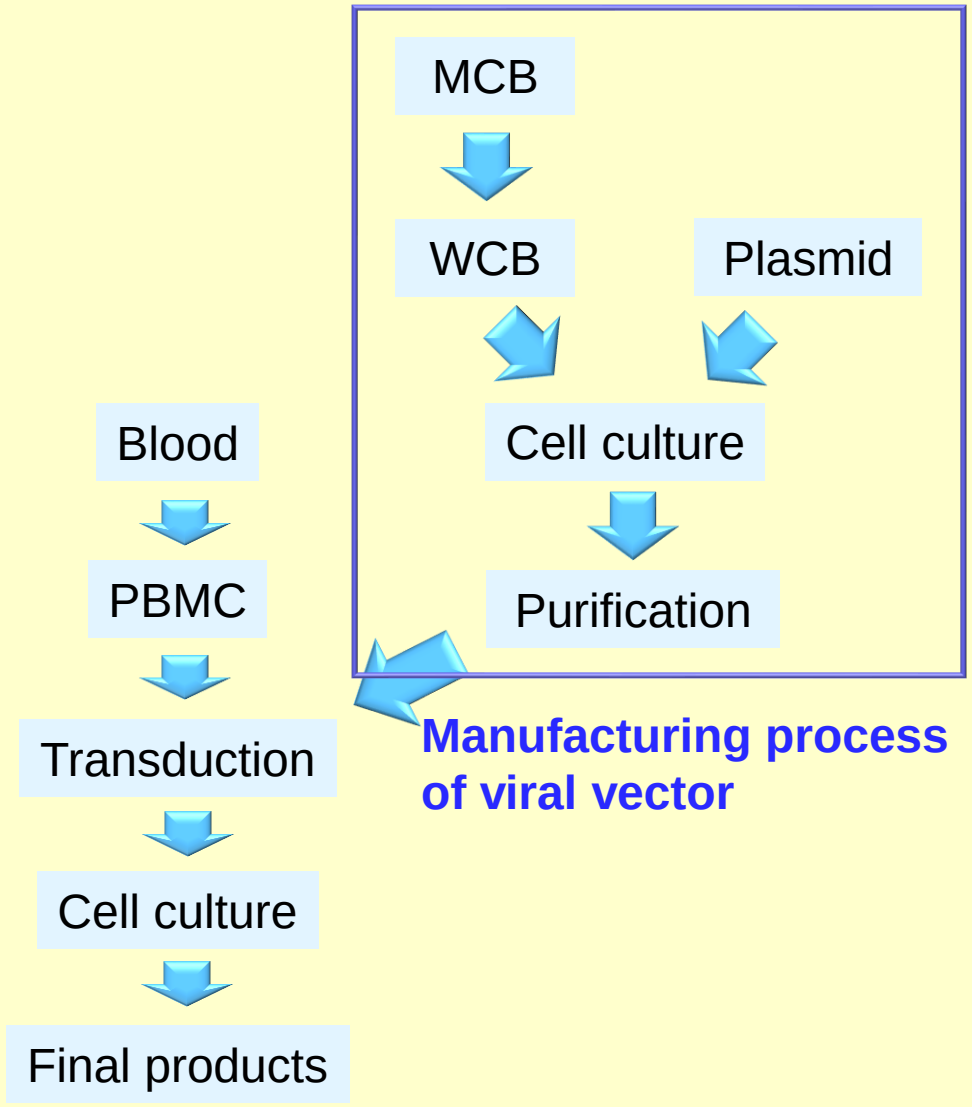
Manufacturing process of CAR-T products using viral vectors



- Viral vector is a critical intermediate important for quality control of the final product
  - ✓ Quality control should be conducted like the drug substance of biological products (monoclonal antibodies, etc)

- Manufacturing process, specifications, stability of the viral vector are reviewed.
- Manufacturing sites are required to undergo GCTP(GMP) inspections.

Control of starting materials

- Starting materials for the production of viral vectors are cell substrates and plasmids.

① Control of the cell substrate (HEK293 cells, etc)
  - ➢ Virus testing and characterization for MCB and WCB should be performed in accordance with the ICH Q5A and Q5D guideline.

② Control of the plasmids
  - ➢ Test items and acceptance criteria should be established.
    - ✓ Appearance, pH, Osmolality, Identity (restriction-enzyme analysis, sequencing, etc.), Quantity, Purity ($A_{260/280}$), Topology (% of supercoiled form), Residual host-cell DNA and RNA, HCP, Endotoxin, Bioburden, etc.

- Control of the apheresis material will be mentioned later.

Control of ancillary raw materials (serum, trypsin, etc.)

Control of ancillary raw materials used in the manufacturing process

① Compendial raw materials
- ➢ Test items and acceptance criteria should be established in accordance with, but not limited to, USP, EP, JP, etc.

② Non-compendial raw materials
- ➢ Appropriate test items and acceptance criteria should be established.
  - e.g. Customized medium
    - ✓ Sterility, Mycoplasma, Endotoxin, virus testing, and others.

- **Ancillary raw material specifications are not usually approval matters in Japan, but quality control of these materials should be conducted at the manufacturing site in accordance with GMP.**

The above concept applies not only to products using viral vectors, but also to products using piggy-BAC, CRISPR/Cas9 and others.

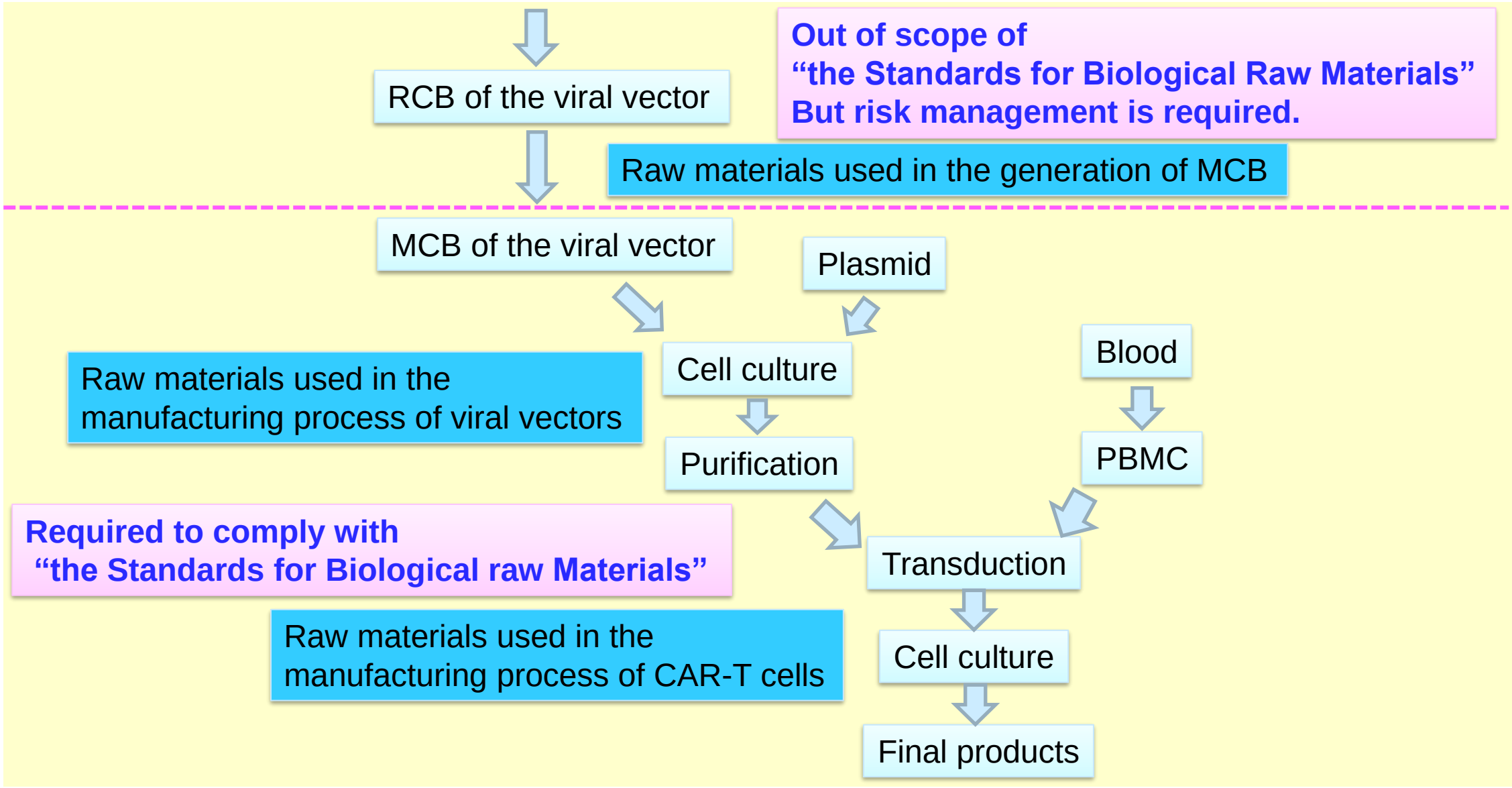
Control of viral vector

Examples of specifications for viral vectors

Category	Specifications or Analytical Procedures
Identity	Genome integrity
Purity	Process-related impurities, Host cell protein, Host cell DNA, Plasmid DNA
Safety	Endotoxin, Sterility, Testing for bulk harvest (*in vitro* assay、Replication competent virus、Mycoplasma)
Potency	Testing to confirm that functional CAR T cells can be generated when the vector is transduced into T cells
Quantity	Vector titer, Infectious titer (Used to determine the amount of vector to be added during CAR-T cell production)
Others	Appearance, Osmolality, pH

The specifications of the viral vector should include tests performed at the DP for biological products (e.g. sterility tests).

Risk management of adventitious agents when using viral vectors



Risk management of adventitious agents when using viral vectors

Risk management of biological raw materials

① Materials used in the manufacturing process of viral vectors and CAR-T cells
- Required to comply with "the Standards for Biological Raw Materials" (MHLW Notification No. 210, 2003.)
- "Secondary materials" should also comply with the Standards.

② Risk management during the manufacturing process
- Virus testing for MCB, WCB, etc in accordance with ICH Q5A guideline.

③ Materials used in the manufacturing process of viral vector MCB
- Out of scope of "the Standards for Biological Raw Materials"
- But risk management is required
  - ✓ Check whether the materials have undergone a process of virus inactivation or removal
  - ✓ If not, conduct appropriate virus testing for MCB

What is secondary materials?

- Benzonase
  - Used in the manufacturing process of viral vectors
    - (Primary) raw material of the product
- Casamino acids used in the manufacturing process of Benzonase
  - Material of the primary raw material of the product
  - Not contained in the Benzonase itself
    - Secondary raw material

Standards for Biological Raw Materials

- FBS, trypsin and other animal derived materials must comply with the following “Standards for Animal-Derived Raw Materials.”

Examples of how to comply with the “Standards for Animal-Derived Raw Materials” for FBS

Requirement	How to deal with it
(1) Healthy animal	To demonstrate that 'the source animals were healthy' or 'the source animals were fit for human consumption following ante- and post-mortem inspection in accordance with the appropriate national legislation’ with supporting COA, COO, etc.
(2) If derived from a cell bank, Virus testing for MCB, WCB	-
(3) If the whole of a living animal is used as the starting material to manufacture products, donor animal eligibility	-
(4) Virus inactivation/removal	To indicate the treatment conditions of the virus inactivation or removal process applied to the material with supporting COA, COO, etc. In addition, viral clearance data under these treatment conditions should be provided.
(5) Traceability	To ensure that records of collection sites, collection dates, test results and lot numbers are maintained. It is acceptable to explain that this information is recorded in the COO or COA.
(6) (2) to (4) are applied to the biologically derived products	-

Standards for Biological Raw Materials

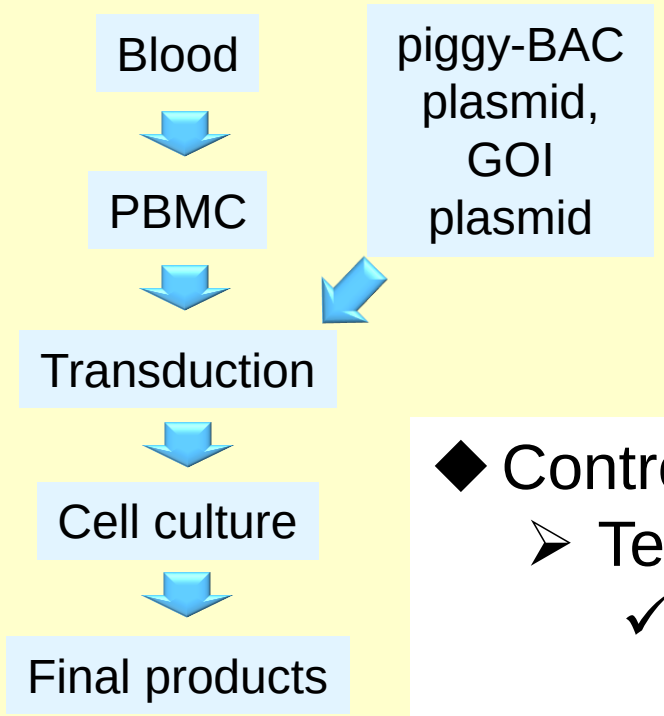
- FBS and other ruminant derived materials must comply with not only the “Standards for Animal-Derived Raw Materials” but also following “Standards for Ruminant-Derived Raw Materials.”

Examples of how to comply with the “Standards for Ruminant-Derived Raw Materials” for FBS

Requirement	How to deal with it
(1) Animal parts	To indicate that prohibited body parts are not used with supporting COO, etc.
(2) Geographical sourcing	To indicate that the country where the material was collected is on the list of countries considered to have a negligible BSE risk with supporting COO, etc.
(3) Traceability	To ensure that records of origin country, collection dates, breeding or slaughter conditions, measures to prevent the spread of TSE and lot numbers are maintained. It is acceptable to explain that this information is recorded in the COO or COA.
(4) (5) Exceptions for non-compliance with standards.	-

Manufacturing process of CAR-T products using piggy-BAC

- Starting materials are plasmids and apheresis material.



- The piggy-BAC plasmid and the GOI (e.g. CAR gene) plasmid are important for quality control of the final product, but quality control is simple.
- So it is considered sufficient to establish the plasmid specifications.

◆ Control of the plasmids

➢ Test items and acceptance criteria should be established.

✓ Appearance, pH, Osmolality, Identity (restriction-enzyme analysis, sequencing, etc.), Quantity, Purity ($A_{260/280}$), Topology (% of supercoiled form), Residual host-cell DNA and RNA, HCP, Endotoxin, Bioburden, etc.

Risk management of adventitious agents when using piggy-BAC

Risk management of biological raw materials

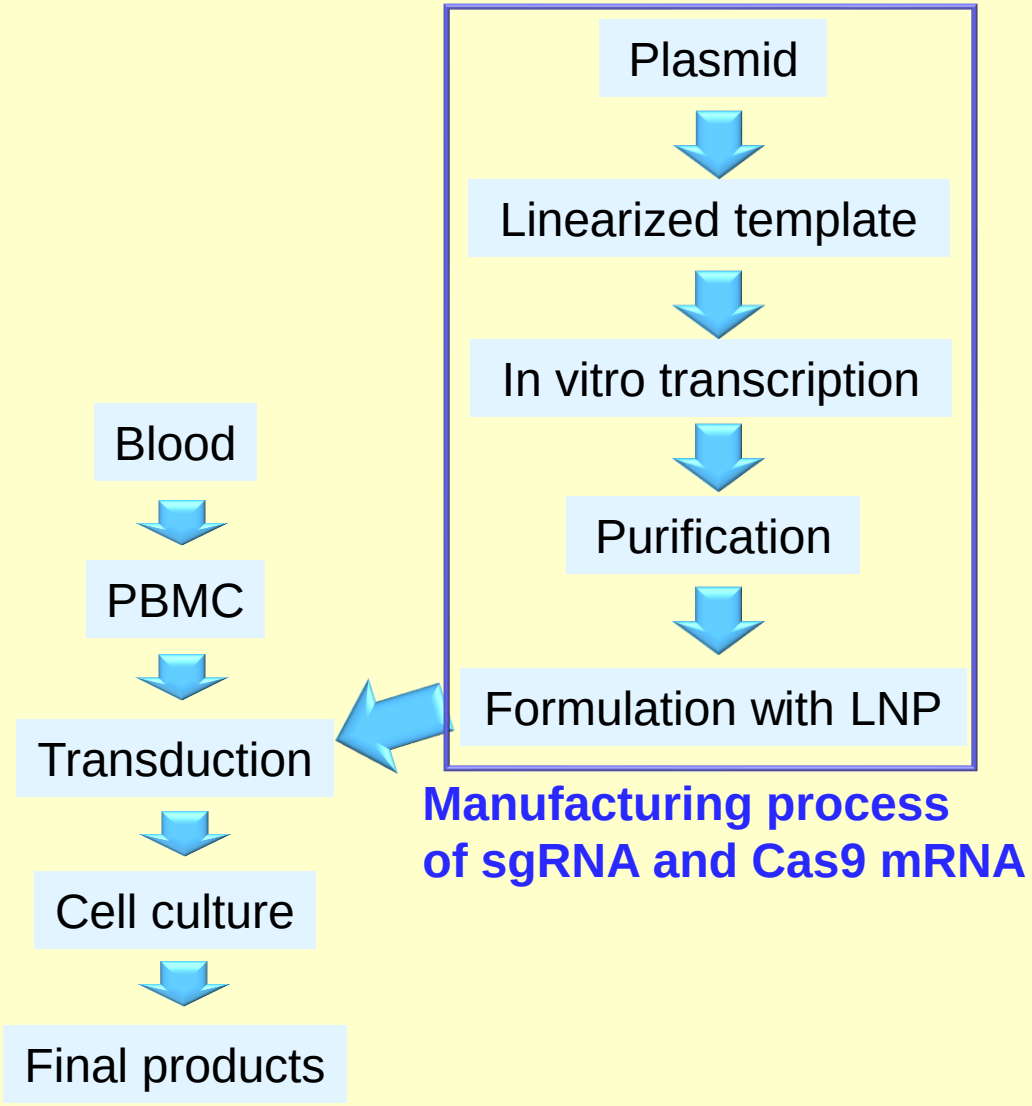
① Materials used in the manufacturing process of CAR-T cells

- Required to comply with "the Standards for Biological Raw Materials" (MHLW Notification No. 210, 2003.)
- "Secondary materials" should also comply with the Standards.

② Risk management during the manufacturing process

- Virus testing for in-process control, if necessary.

Manufacturing process of CAR-T products using CRISPR/Cas9



Manufacturing process of sgRNA and Cas9 mRNA

- sgRNA and Cas9 mRNA are critical intermediates important for quality control of the final product
  - ✓ Quality control should be conducted like the drug substance of biological products (monoclonal antibodies, etc)

- Manufacturing process, specifications, stability of the mRNA are reviewed.
- Manufacturing sites are required to undergo GCTP(GMP) inspections.

Control of starting materials

- Starting materials are plasmids and apheresis material.
  - ➢ Since there are still few examples of products using CRISPR/Cas9, the quality control regulation may be revised in the future.

◆ Control of the plasmids
  - ➢ Test items and acceptance criteria should be established.
    - ✓ Appearance, pH, Osmolality, Identity (restriction-enzyme analysis, sequencing, etc.), Quantity, Purity ($A_{260/280}$), Topology (% of supercoiled form), Residual host-cell DNA and RNA, HCP, Endotoxin, Bioburden, etc.

- Control of the apheresis material will be mentioned later.

Control of mRNA

Examples of specifications for mRNA

Category	Specifications or Analytical Procedures
Identity	Genome integrity
Purity	% 5’ Capped, % PolyA tailed RNA, Product-related impurities, Process-related impurities, Residual DNA template
Safety	Endotoxin, Sterility
Potency	Testing to confirm that functional CAR T cells can be generated when CAR gene is transduced into T cells, Protein reduction by gene KO
Quantity	Contents
Others	Appearance, Osmolality, pH, Identity (lipid), Lipid content, Particle size

✓ **Since there are still few examples of products using CRISPR/Cas9, the quality control regulation may be revised in the future.**

Risk management of adventitious agents when using CRISPR/Cas9

Risk management of biological raw materials

① Materials used in the manufacturing process of mRNA and CAR-T cells

- Required to comply with "the Standards for Biological Raw Materials" (MHLW Notification No. 210, 2003.)
- "Secondary materials" should also comply with the Standards.

② Risk management during the manufacturing process

- Virus testing for in-process control, if necessary.

Apheresis material (autologous and allogeneic CAR-T)

- Quality control of PBMCs collected by apheresis
  - CD markers, viability, etc.
  - Acceptable if necessary test items are established to ensure the quality of the final product.

Apheresis material (allogeneic CAR-T)

- Donor eligibility for allogeneic CAR-T
  - HIV, HBV, HCV, HTLV
    - NAT or serological test must be conducted taking into account the window period
    - Denial of history of infection by interview
  - PVB19
    - Denial by interview and tests
  - Treponema pallidum, Chlamydia trachomatis, Neisseria gonorrhoeae, Tubercle bacillus
    - Denial by interview, medical examinations, tests, etc.
  - EBV, CMV, WNV
    - Denial by tests as needed

Apheresis material (autologous CAR-T)

Additional points to consider for quality control of PBMCs for autologous CAR-T

- Equipment and reagents
  - Same as used in clinical trials?
  - If it's different from the equipment/reagents used in the clinical, it is necessary to evaluate the suitability.
- Stability of the apheresis material during transport
  - Proper packaging form
  - Storage conditions
  - Time limit for transport

Manuals should be provided to medical institutions, including recommended apheresis equipment and reagents, transport temperature, and the time limit for transport.

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Donor eligibility of allogeneic human cells

Requirement of donor screening test

	HIV	HBV	HCV	HTLV	EBV	PVB19	CMV	WNV	Treponema pallidum	Chlamydia trachomatis	Neisseria gonorrhoeae	Tubercle bacillus
US	✓	✓	✓	✓*1			✓*1	✓	✓	✓*2	✓*2	
EU	✓	✓	✓	✓*3	✓*2		✓*2		✓			
Japan	✓	✓	✓	✓	✓*2	✓	✓*2	✓*2	✓*4	✓*4	✓*4	✓*4

*1：For viable, leukocyte-rich cells or tissue.
*2：As needed.
*3：HTLV-I antibody testing must be performed for donors living in, or originating from, high-prevalence areas or with sexual partners originating from those areas or where the donor's parents originate from those areas.
*4：Denial by medical interviews is also acceptable.

Modified from Regenerative Therapy 2020; 15: 265-73 Table 2

- For each region, the main viruses and bacteria with infectious cases are designated.
  - The donor's area of residence may need to be considered. (e.g. There have been no cases of WNV infection in Japan.)
- The severity of the disease caused by the virus should also be considered.
  - Importance of the denial of HIV, HBV, HCV and HTLV

Donor eligibility of allogeneic human cells

Requirement of testing kit, testing site, window period, traceability, etc.

	Negation of Infectious diseases by interview	Informed Consent for commercial Use	Appropriate site for collection	Testing kit and testing site	Window period consideration	Traceability of cell collection and preparation
US	✓	✓	✓	✓*1	✓*2	✓
EU	✓	✓	✓	✓*3	✓*4	✓
Japan	✓	✓	✓		✓	✓

*1：FDA-approved testing, at CLIA certified labs
*2：Anonymous semen donors only.
*3：CE marked testing
*4：If samples from a living donor undergo serology testing and are also tested by NAT for HIV, HBV, and HCV, retesting after a time interval is not required.

Modified from Regenerative Therapy 2020; 15: 265-73 Table 3

- There are differences between regions in terms of test kit, test site, window period consideration.
  - Harmonization of test kits and testing sites may not be practical.
  - Is it possible to discuss the mutual acceptability of each region's regulatory requirements?

Comparison of requirements for bovine serum

Requirements for viral and TSE risk management, specs, etc.

	US USP90	US USP1024	EU EMA GL*1	EU EP2262	EU EP5.2.8	Japan Standard for Animal Materials	Japan Standard for Ruminant Materials
Healthy animal		✓	✓	✓		✓	
Material collection		✓		✓	✓		✓
Virus inactivation/removal process(condition, validation)		✓	✓	✓		✓	
Specification	✓	✓	✓	✓			
Storage condition		✓		✓			
COA/COO	✓	✓	✓			✓	✓
Geographical sourcing (TSE)		✓			✓		✓
Animal parts (TSE)					✓		✓
Age (TSE)		✓			✓		✓*2

*1：EMA/CHMP/BWP/457920/2012 rev 1
*2：It is desirable to pay attention to the restrictions on the age of cattle for each country of origin. [Standards for biological raw materials, Operational guideline, PFSB/ELD Notification No. 1002-1, PFSB/MDRMPE Notification No. 1002-5, 2014.]

Modified from Cytotherapy 2023; 25: 220-8 Table 3

- There are differences between regions in terms of specification and TSE risk assessment.
  - Specifications and storage conditions are not specified in the Japanese pharmacopoeia.
  - Japanese developers may need to change materials when developing in the US or EU?

Comparison of requirements for bovine serum

Requirements for viral testing.

	US USP1024	EU EP2262
Bovine viral diarrhea virus	✓	✓
Bovine parvovirus	✓	✓
Bovine adenovirus	✓	✓
Bluetongue virus	✓	✓
Bovine respiratory syncytial virus	✓	✓
Rabies virus	✓	✓
Reovirus	✓	✓
Other viruses (if necessary)	✓*1	✓*2

*1：Bovine herpesvirus 1, Parainfluenza 3 virus, Bovine leukemia, Bovine rotavirus, Bovine circovirus, Bovine polyomavirus and many others.
*2：It is described that “depending on the country of origin, specific tests for other viruses may be needed.”

Modified from Cytotherapy 2023; 25: 220-8 Table 5

- Specific list of viral testing is not described in the standard for biological raw materials in Japan.
- In Japan, viral risk management focuses on the requirement to check the health status of the animals of origin and to carry out a viral inactivation or removal process on the material.
  - Japanese developers may need to change materials when developing in the US or EU?

Summary

1. Overview of CAR-T Products

- Following the successful development of auto CAR-T products, the development of allogeneic CAR-T cell products is also underway.
- In addition to viral vectors, non-viral vectors and genome editing technology have emerged as methods of CAR gene transfer in recent years.

2. Quality control of raw materials – regulatory situation in Japan

- Appropriate quality control should be in place for each type of modality.
- Risk management of adventitious agents from the biological raw materials should be conducted. Risk management methodologies are common regardless of the modality.

3. Regulatory differences between regions

- There are regulatory differences between regions in terms of virus testing and specifications for biological raw materials.
- It seems realistic to sort out what can be harmonized and what is difficult, and to consider those that can be harmonized first.

Thank you for your attention!

http://www.pmda.go.jp/ (Japanese)
http://www.pmda.go.jp/english/index.html (English)