

Reverse translational research of CAR-T therapy

- Analysis of some 500 cases of CAR-T therapy for B-cell lymphoma in Japan -



Comprehensive Development of CAR-T Cell Therapy in Taiwan
Recent Advances and Future Perspectives in CAR-T Cell Therapy
Industry in Asian

CEO, Cyto-Facto Inc.
Professor, Kobe Univ.
Dept of Science, Technology and Innovation

Shin Kawamata



Agenda

1. Introduction of the CMO/CDMO company Cyto-Facto Inc.

2. Role of the CDMO in supporting cell therapy through collaboration with academia, medical sites and pharma.

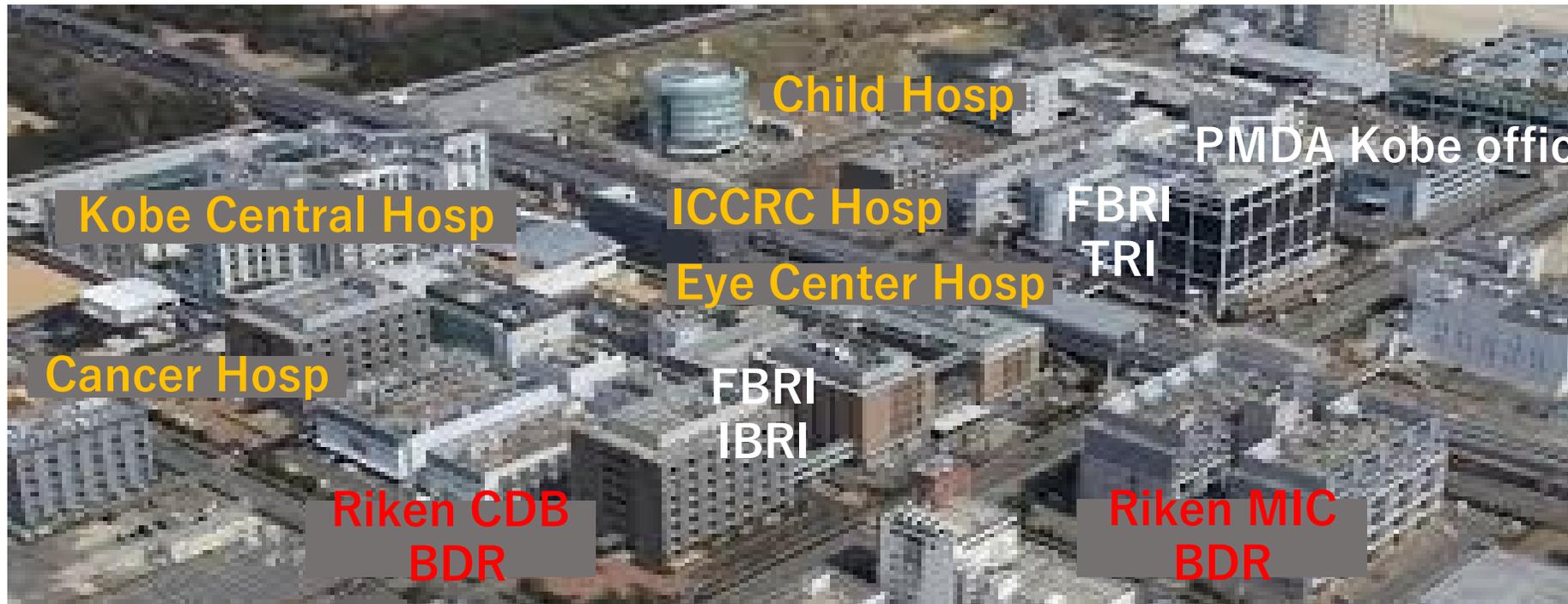
Introduction of CMO/CDMO company Cyto-Facto Inc.



Kobe Port Island

Cyto-Facto Inc. is a spinoff company

from Foundation for Biomedical Research and Innovation (FBRI) that aims to support Kobe BioMedical Innovation Cluster project



Kobe BioMedical Innovation Cluster in 2023

2 Riken basic research centers

5 Hospitals with 1600 beds

→ Translational Institution FBRI

PMDA branch office

5 universities and collages

360 Biomedical companies

FBRI organization chart

Until 2023 March



Tasuku Honjo

Foundation for Biomedical Research and Innovation

Atsuhiko Kawamoto

Medical Innovation Center



Translational research and data center for clinical study

Tadaaki Hanatani

Pro-cluster Center



Foster medical cluster in Kobe and international networking

Shin Kawamata

Center for Cell Therapy



Regulatory science for cell therapy and cell manufacturing

Est 2015



Ext 2023

Toshio Kitamura

Research Center



Research for aging, immune therapy and regenerative medicine

FBRI RDC until March 2023

Clinical manufacturing CDMO



MSC iPSC-NK



PM

CDMO

Commercial manufacturing CMO



CMO

CAR T

FBRI RDC FTE 105



research/inspection

Software for cell manufacturing control

New CAR T design
CQA for MSC
CQA for iPSC

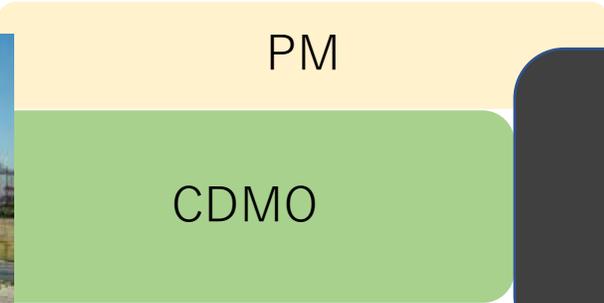


Spin off company Cyto Facto after April 2023

Clinical manufacturing CDMO



MSC iPSC-NK



Software for cell manufacturing control

Commercial manufacturing CMO



CAR T



Cyto-Facto Inc.

FTE 85
CEO Kawamata

research/inspection



New CAR T design
CQA for MSC
CQA for iPSC

Kobe Univ,
Prof Kawamata
FTE 5

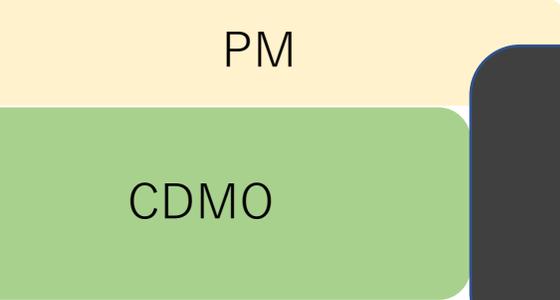


Spin off company Cyto Facto after April 2024

Clinical manufacturing CDMO



MSC iPSC-NK



Software for cell manufacturing control

Commercial manufacturing CMO



CMO

CAR T



Cyto-Facto Inc.

FTE 85
CEO Kawamata

research/inspection



New CAR T design
CQA for MSC
CQA for iPSC

Kobe Univ,
Prof Kawamata
FTE 5



CDMO new co

Keio Univ.
Tonomachi

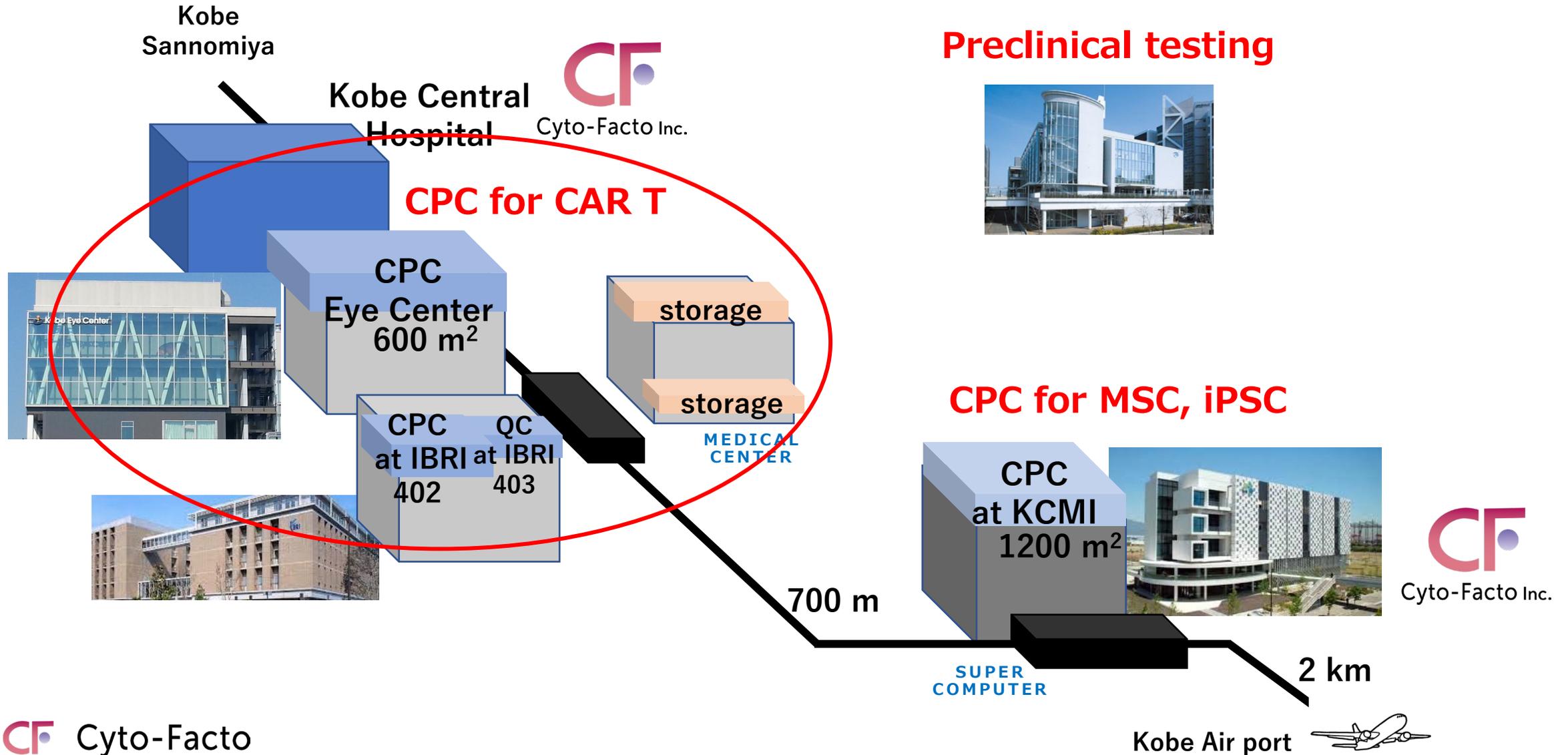


Main services

Specialized in manufacturing cell and gene products

- research, process development and sales of products
- CMO/CDMO service
- analytical service and QC testing
- consultation, organizing seminar, training course

Cell manufacturing facilities of Cyto-Facto Inc.



Preclinical testing



CPC for MSC, iPSC



Introduction of Cell Processing Center

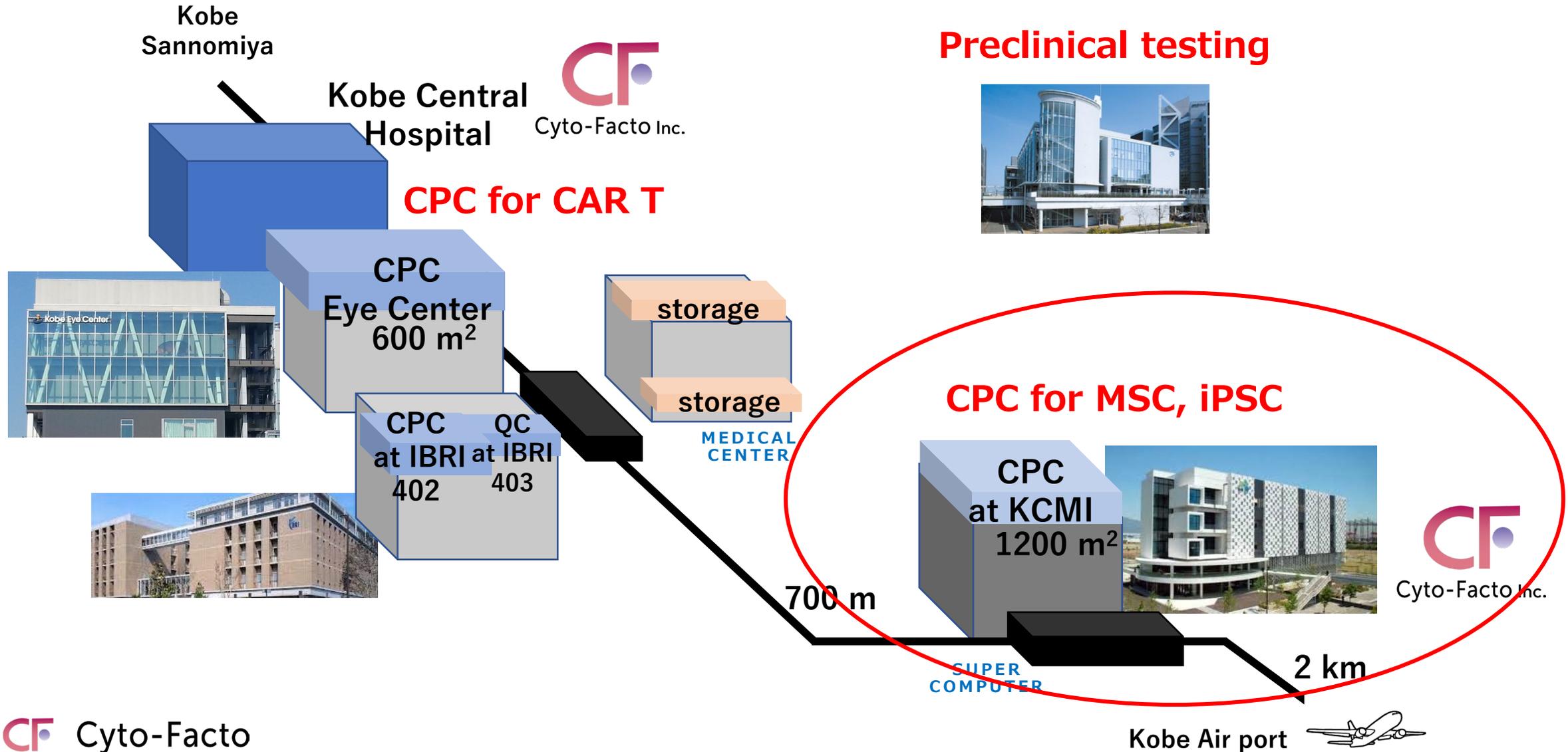


**PIC/S GMP compliance manufacturing facility
That allows international shipment
Shipping record to Australia**

Introduction of Cell Processing Center



Cell manufacturing facilities of Cyto-Facto Inc.



CPC facility in KCM I 5F



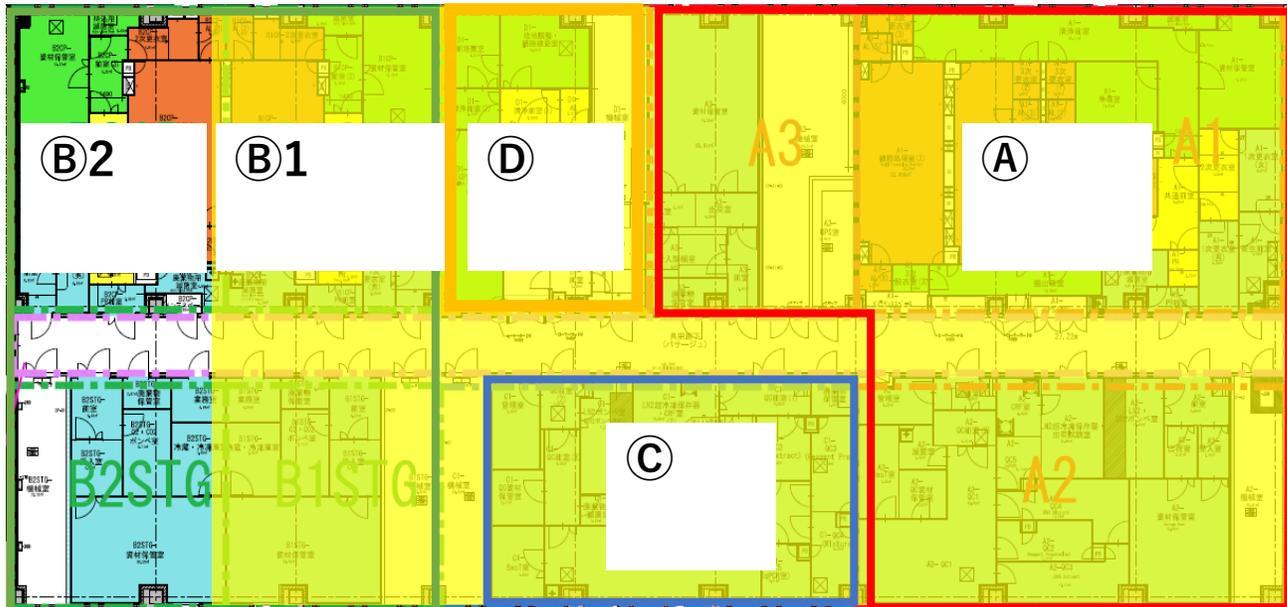
FUJIFILM
Value from Innovation

Healios

SINFONIA

5CPCs
Central QC Room
Material Storage Room

Total 1,200m²

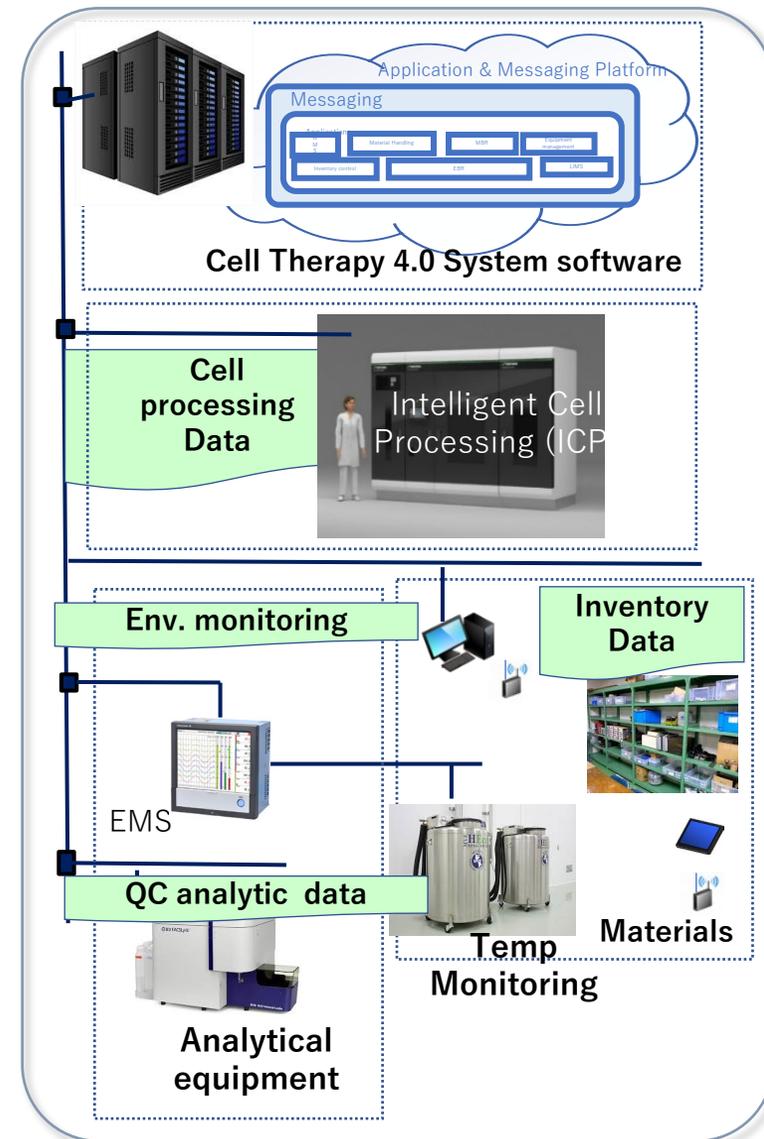
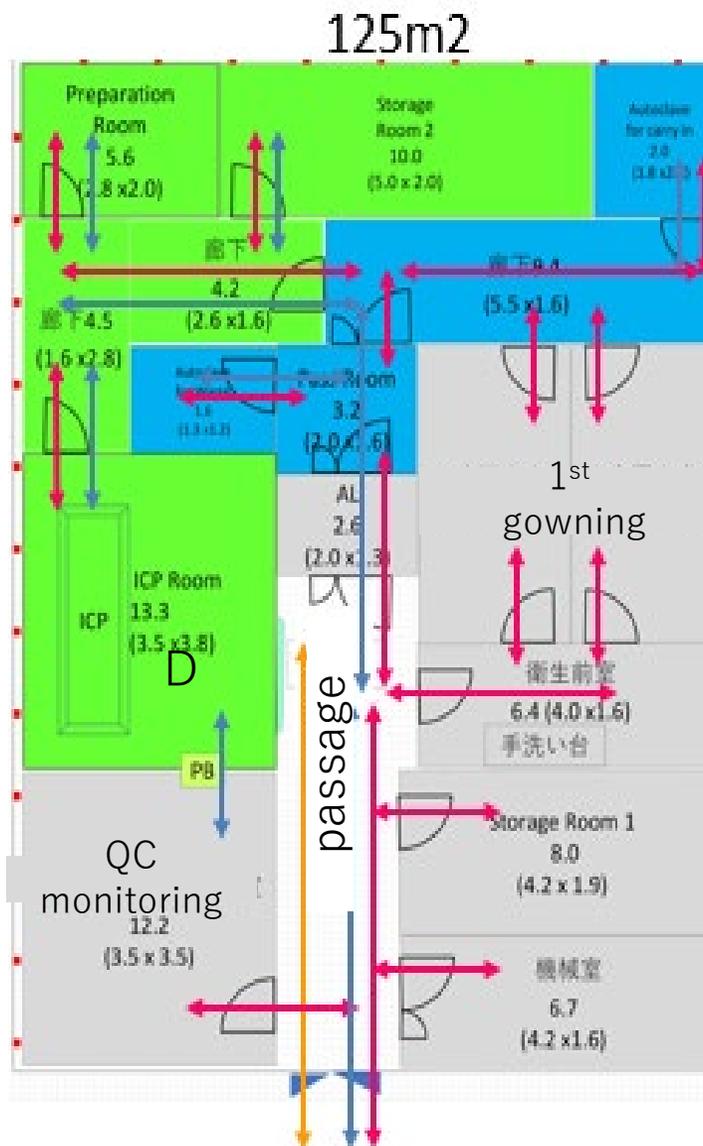


- Clinical Trial of **Autologous MSC**
- Clinical Trial of **eNK derived from iPSC**
- Central QC Room

QbD based-cell manufacturing Solution Lab in KCMCI



Cloud AMP C&G system

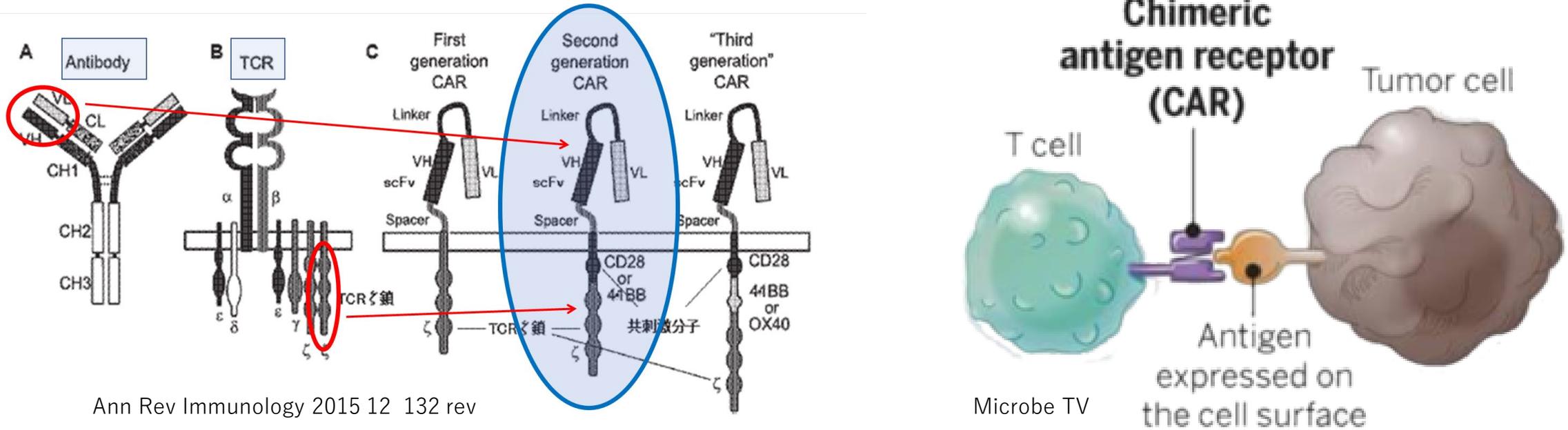


Agenda

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Genetically modified T cells (CAR-T) for the treatment of cancer



CMO service for global pharmaceutical companies offers us to define a global standards for cell manufacturing and supply chain.

Genetically modified cells for gene and cell therapy

HER2-CAR T

Target: Sarcoma, Glioblastoma
Baylor Med Collage

Claudin 18.2-CAR T

Target: gastric and pancreatic cancer
Carsgen Therapeutics

GD2-CAR T

Target: Neuroblastoma
Baylor Med Collage

MMG49 (Integrin β)-CAR T

Target: MM
Osaka Univ/Otsuka Pharma

CD116-CAR T

Target: AML
Shinshu-Univ.

NKR-2-CAR T

Target: solid tumor
Celyad/Ono

SeFv(anti-PD-1)-CAR T

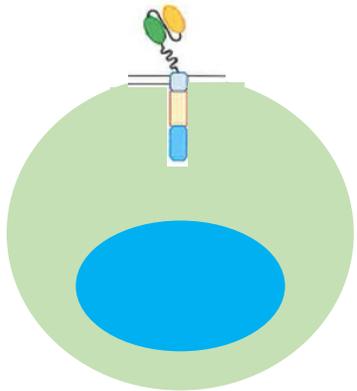
Target: Lung cancer
Sarwish Nat. Biotech 2018

Anti-mesothelin-CAR T

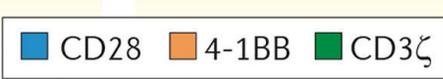
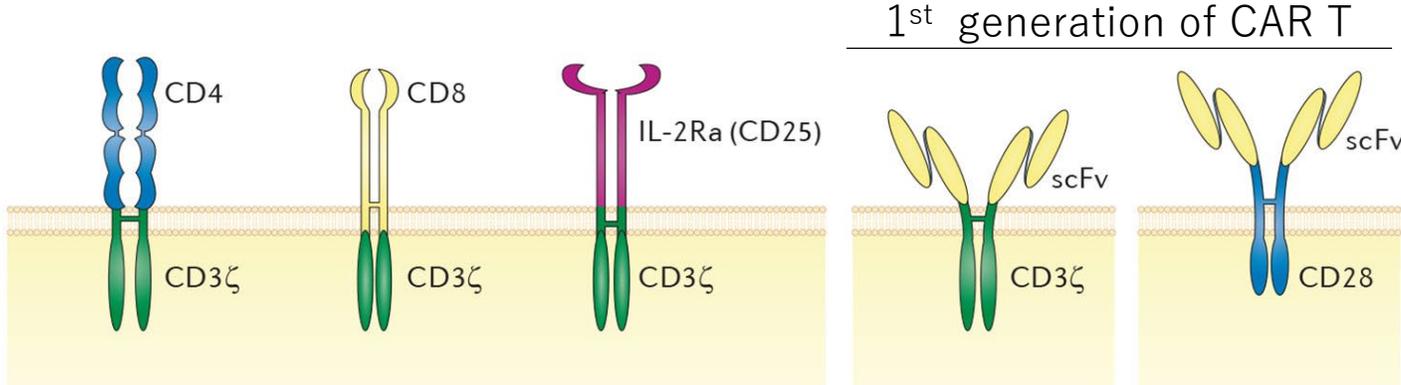
Target: mesothelial Ca
Memorial Sloan Kettering Cancer Center

BCMA/CS1 bispecific-CAR T

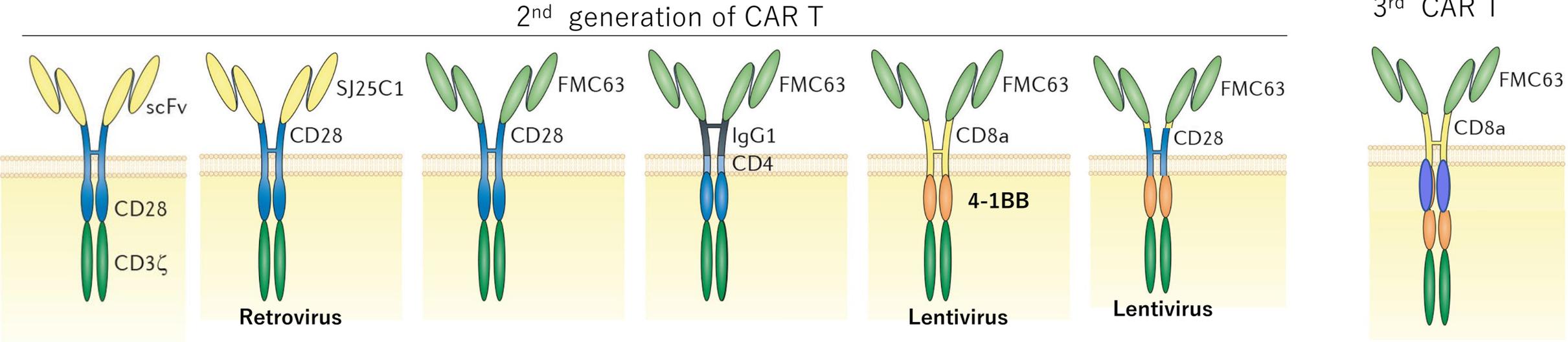
Target: MM
Nat. comm. 2020



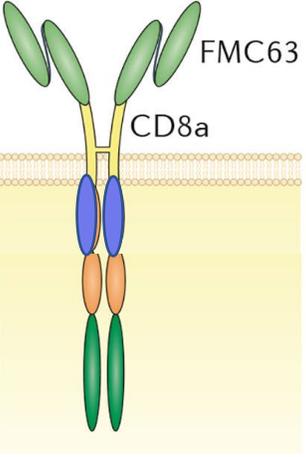
Evolution of Chimeric Antigen Receptor for CAR-T



Rev. Nat Rev Drug Discovery 2019 van der Stegen et al.



3rd CAR T



Kite/ZUMA-1

Neelapu NEJM 2017
Locke Lancet Oncol 2019

Novartis/JULIET Juno/TRANSCEND

Schuster 2019 NEJM Abramson 2020 Lancet

Multi-center CD19 CAR T cell trials

| Study/Sponsor | ZUMA-1/Kite | JULIET/Novartis | TRANSCEND/Juno |
|------------------------|--------------------------------|--------------------------------|--|
| CAR T dose | 2 x10 ⁶ kg | Up to 0.6-6x10 ⁸ | 0.5-1.5x10 ⁸ |
| Conditioning therapy | Cy/Flu | Cy/Flu or Bendamustine | Cy/Flu |
| Lymphoma subtypes % | DLBCL/PMBCL/TFL 78 / 7 / 15 | DLBCL/TFL/Other 78 / 19 / 2 | DLBCL/PMBCL/TFL/Other 64 / 6 / 22 / 8 |
| Relapsed/Refractory | Refractory | Relapsed or refractory | Relapsed or refractory |
| Relapse post-ASCT | 23% | 49% | 35% |
| Bridging therapy | None | Allowed | Allowed |
| Treated/Enrolled | 108/120(90%) | 111/166(67%) | 269/344(78%)* |
| PFS progression-free | 39% at 27.1 months | 34% at 14 months | 44% at 12 months |
| ORR | 83% | 52% | 73% |
| CR | 58% | 40% | 53% |
| OS | 47% alive at 39.1 months | not found | not found |

CMO/CDMO business experience of Cyto-Facto (Its predecessor, the RDC of the FBRI)

- 2014** Launched CAR-T CMO project as R&D Center for Cell Therapy (RDC) of the FBRI. Technology transfer of CAR-T production (Kymriah®) by Novartis.
- 2017** Cell Processing Center in compliance with PIC/S GMP in the EC building in service.
- 2018** Began production of the clinical product Kymriah® for the Japanese market.
- 2020** Obtained approval for manufacture and sale of regenerative medicine products from HA. Commercial production of Kymriah® started.
- 2021** Technology transfer of MSC production (FF-31501) by Fujifilm.
- 2022** Completion and operation of the Cell Processing Center (CPC) on the 5th floor of KCM I
- 2023** FF-31501 investigational product manufacturing began. Several process development projects for CAR-T, MSC manufacturing started.

Cyto-Facto (its predecessor, the RDC of the FBRI) is **the first Asian company to manufacture commercial CAR-T products in compliance with PIC/S GMP**. Cyto-Facto has accumulated experience in manufacturing technology know-how and has launched several cell & gene manufacturing pipelines, including CAR-T, iPSC-based cells and mesenchymal stem cells (MSCs) of various developmental stages.



CPC in the Eye Center (EC) building. 6F



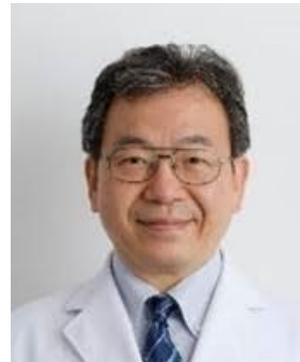
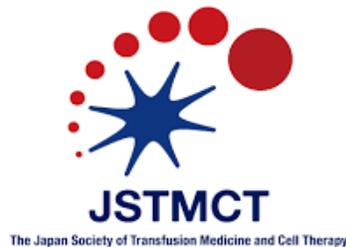
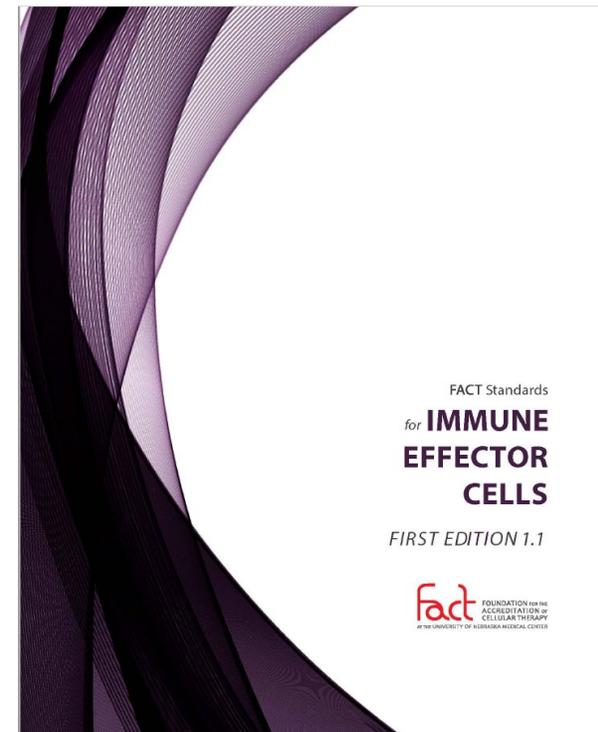
CPC in the Kobe Center for Medical Innovation (KCM I) building. 5F

The CAR-T therapy in Japan was started in 2018 through the network of JSTMCT

Introduce FACT - Standards for IMMUNE EFFECTOR CELLS to Japanese apheresis sites

Set up and open apheresis sites in Japan for Kymriah manufacturing through the network of Japan Society of Transfusion Medicine and Cell Therapy under the technical support from NPKK.

All of the apheresis sites opened in Japan were set up based on the recommended plans stated in the Q&A in the journal of JSTMCT and web HP.



Keio Univ. Hemato
Dr. Tanozaki



Kyoto Univ. Hemato
Dr. Arai



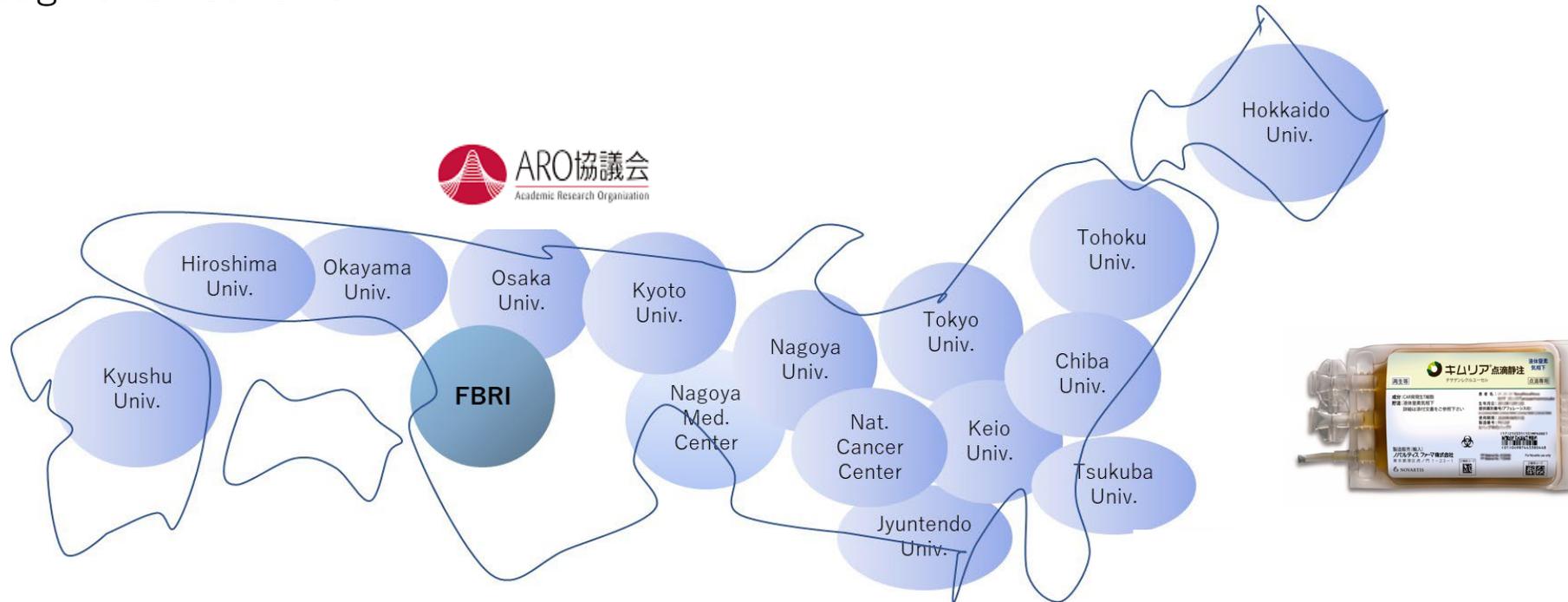
Kyoto Univ. Hemato
FBRI
Kawamata

Network of ARO was utilized to organize the apheresis/hospital sites for CAR-T therapy in Japan

FBRI chairs the Academic Research Organization Liaison Council for Cell Processing Center.

The 17 major academic/governmental medical centers for cell therapy participate Liaison Council.

FBRI promotes the supply of Kymriah and collect the therapy-related issues need to be fixed through this network.



Chaired by FBRI
Kawamata

Cyto-Facto has a prestigious Kymriah manufacturing record, with manufacturing success rate of 95% (123 batches/129 batches)

ORIGINAL PAPER

Risk factors for CAR-T cell manufacturing failure among DLBCL patients: A nationwide survey in Japan



British Journal of Hematology
2023 April 13 Jo et al

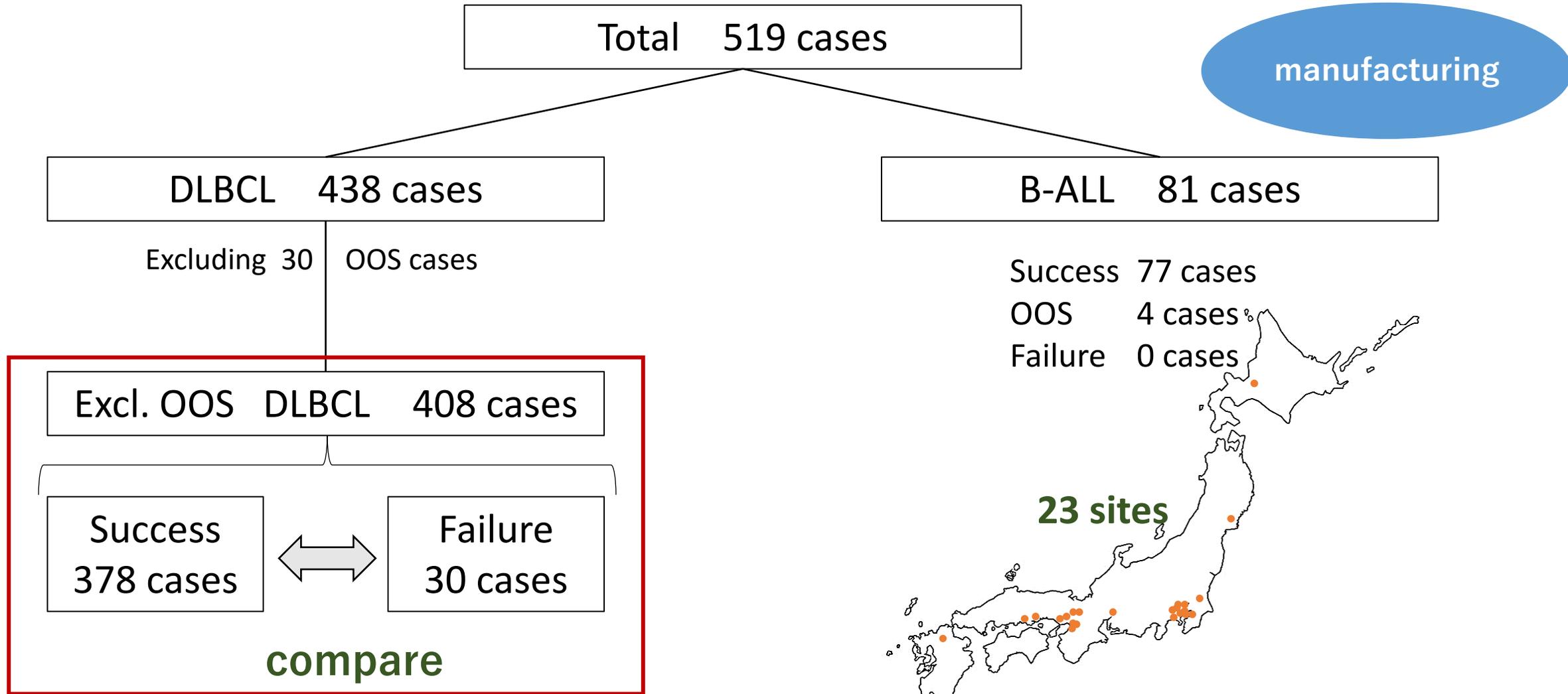
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Ryuji Tanosaki⁸ | Yasuyuki Arai^{1,2}

JO ET AL.

Table 1 revised

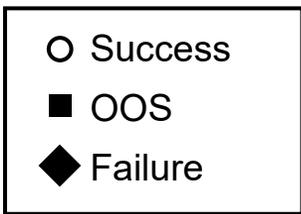
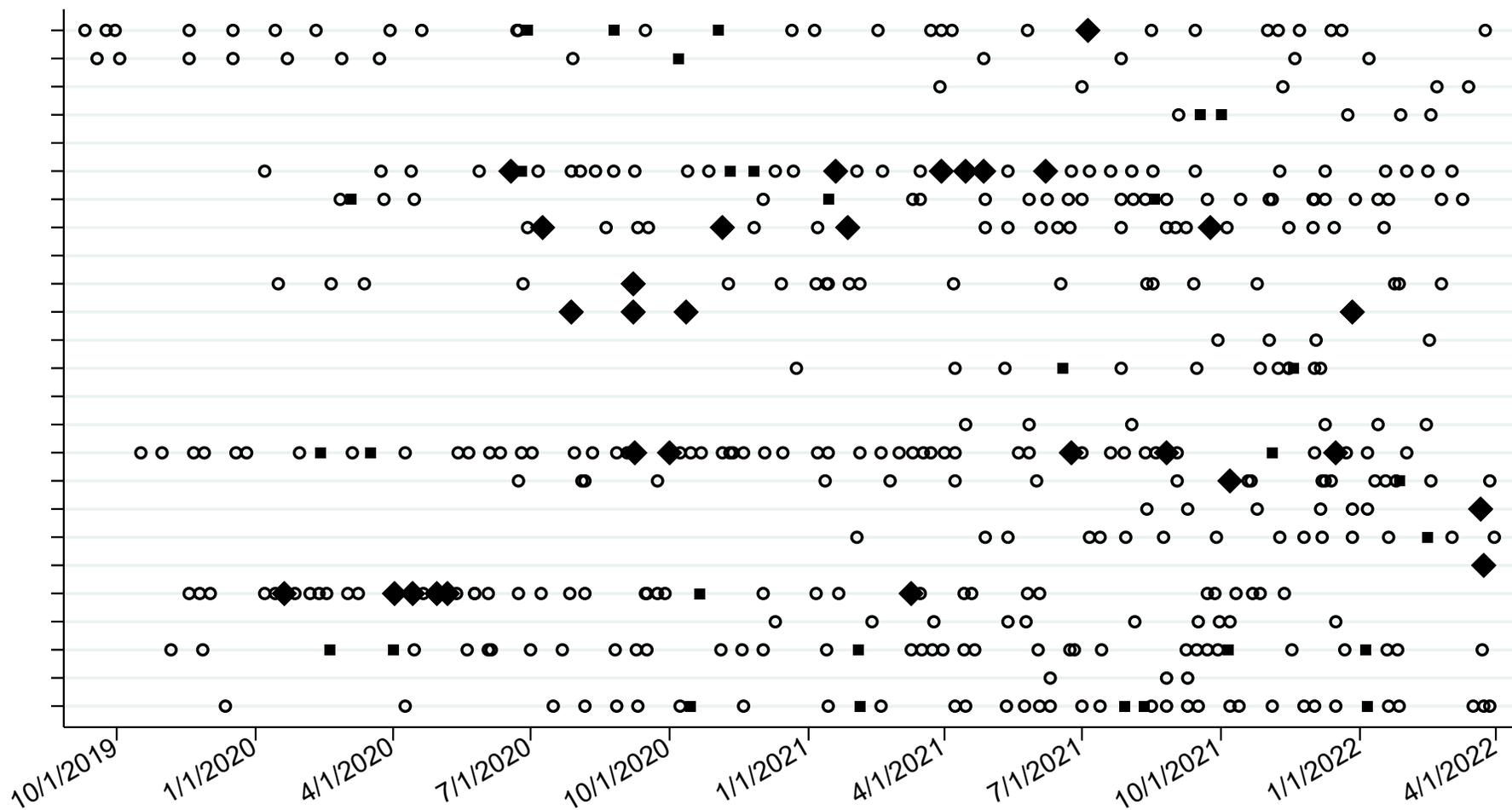
| Factory | 2019-2021 Dec | Successful | Failed | successful rate |
|------------------------------|---------------|------------|--------|-----------------|
| Novartis Morris Plains NJ US | | 255 | 24 | 91% |
| Cyto-Facto (FBRI) Kobe Japan | | 123 | 6 | 95% |

CAR-T manufactured and infused to patients



Kymriah product score for 23 Japanese sites (DLBCL)

Facility



Strictly Confidential

一般社団法人
 日本輸血・細胞治療学会

委員会名: 免疫・再生医療関連委員会
 ワーキンググループ(WG)・タスクフォース

manufacturing



23 sites
408 cases

Explore the factors that affect the success rate of CAR-T manufacturing

Patient episodes for success or failure of CAR-T therapy

Female 20s

- Primary mediastinal Large B cell lymphoma
- Treatment
 - DA-EPOCH-R 6 courses : CR
 - Recurrence → R-ESHAP : no CR
 - R-GCD → Pola BR : no CR
 - **Apheresis → manufacturing OK**
 - IVAC → radiation : PR
 - **CAR-T : CR**
 - No event

Male 60s

- Diffuse large B lymphoma
 - Treatment
 - R-CHOP 1 course : PR
 - BR 4 courses : no CR
 - R-CHASE 1 course : PR
 - **Apheresis → manufacturing failure**
 - R-CHASE 2 courses
 - **Apheresis 2 → manufacturing failure again**
- Additional chemotherapy is not feasible.

Pretreatment conditioning

Factors determine the success of CAR-T manufacturing

Bendamustine risk category High risk : > 3 courses, last Tx conducted within 3 M, Middle risk : > 6 courses last Tx conducted 3 after or within 24 M

| | | Odds ratio | 95%CI | p value |
|-----------------------------|--|---------------|-------------------|---------|
| Risk by Bendamustine | Low | | Reference | |
| | Int | 5.520 | (1.436 - 21.215) | 0.013* |
| | High | 57.088 | (3.370 - 966.996) | 0.005* |
| | Every 10 × 10 ⁴ /μL reduction | 2.020 | (1.107 - 3.690) | 0.022* |
| | CD4/CD8 ratio | | | |
| | ≥1/3 | | Reference | |
| | <1/3 | 3.249 | (1.314 - 8.036) | 0.011* |

Pretreatment conditioning

Platelet

CD4/CD8 ratio

Interpretation

Case 1

- ✓ Bendamustine : no use (Low)
- ✓ Platelet : 20.0 X 10⁴ /μL
- ✓ CD4/CD8 ratio: 1.0 (≥1/3)

Odds ratio

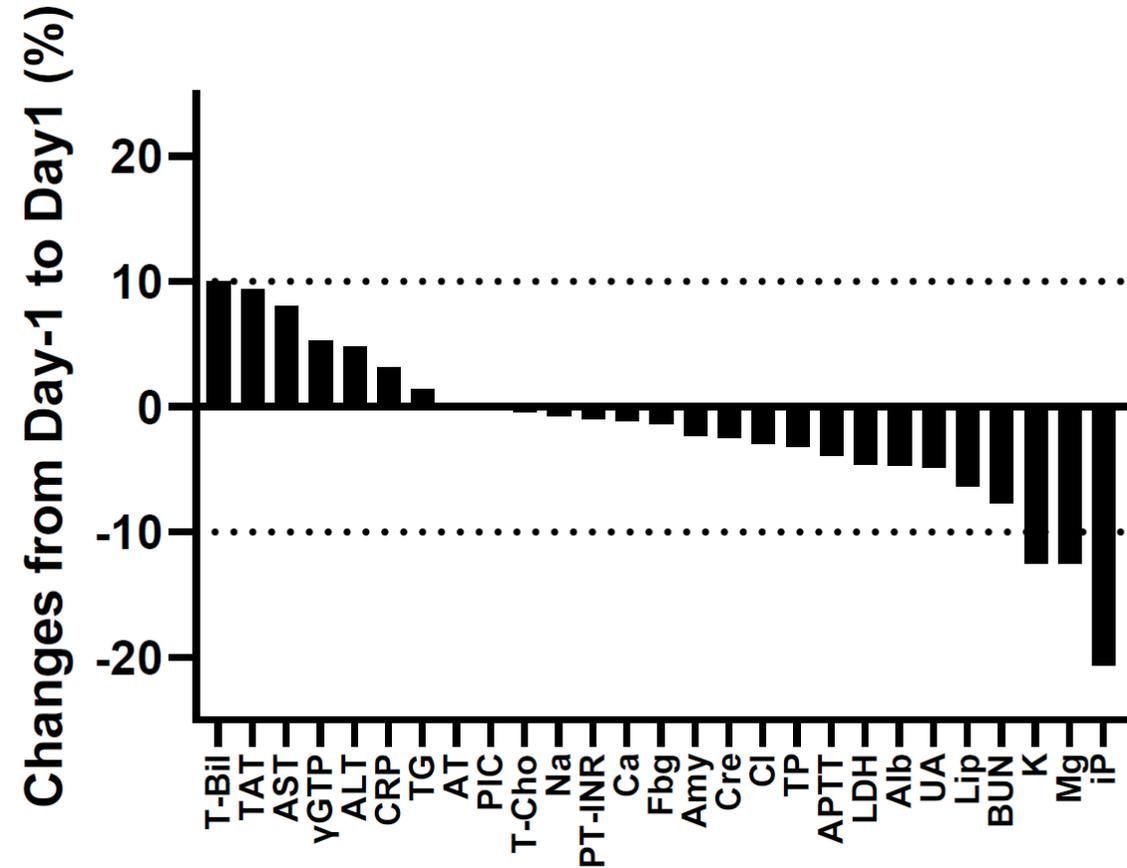
$$\begin{aligned} &\times 57.1 \\ &\times 2.0 \\ &\times 3.2 \\ &= \times \mathbf{365} \end{aligned}$$

Case 2

- ✓ Bendmustine : 6 cycles & rest 2 months (High)
- ✓ Platlet : 10.0 X10⁴/μL
- ✓ CD4/CD8 ratio: 0.2 (<1/3)

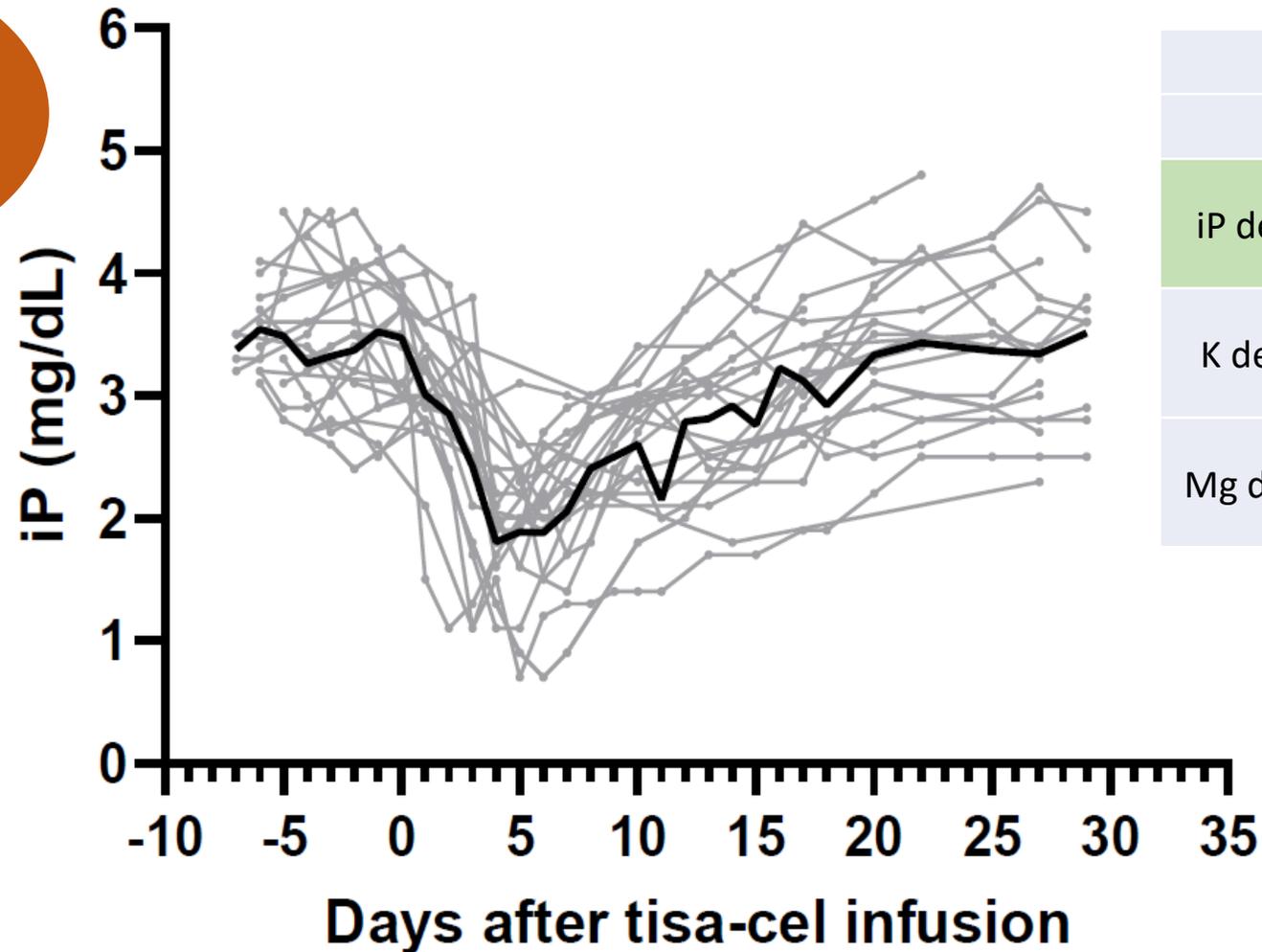
Low phosphate level in serum is observed just after CAR-T therapy

Adverse event after infusion



Low phosphate in serum prior to sever CRS

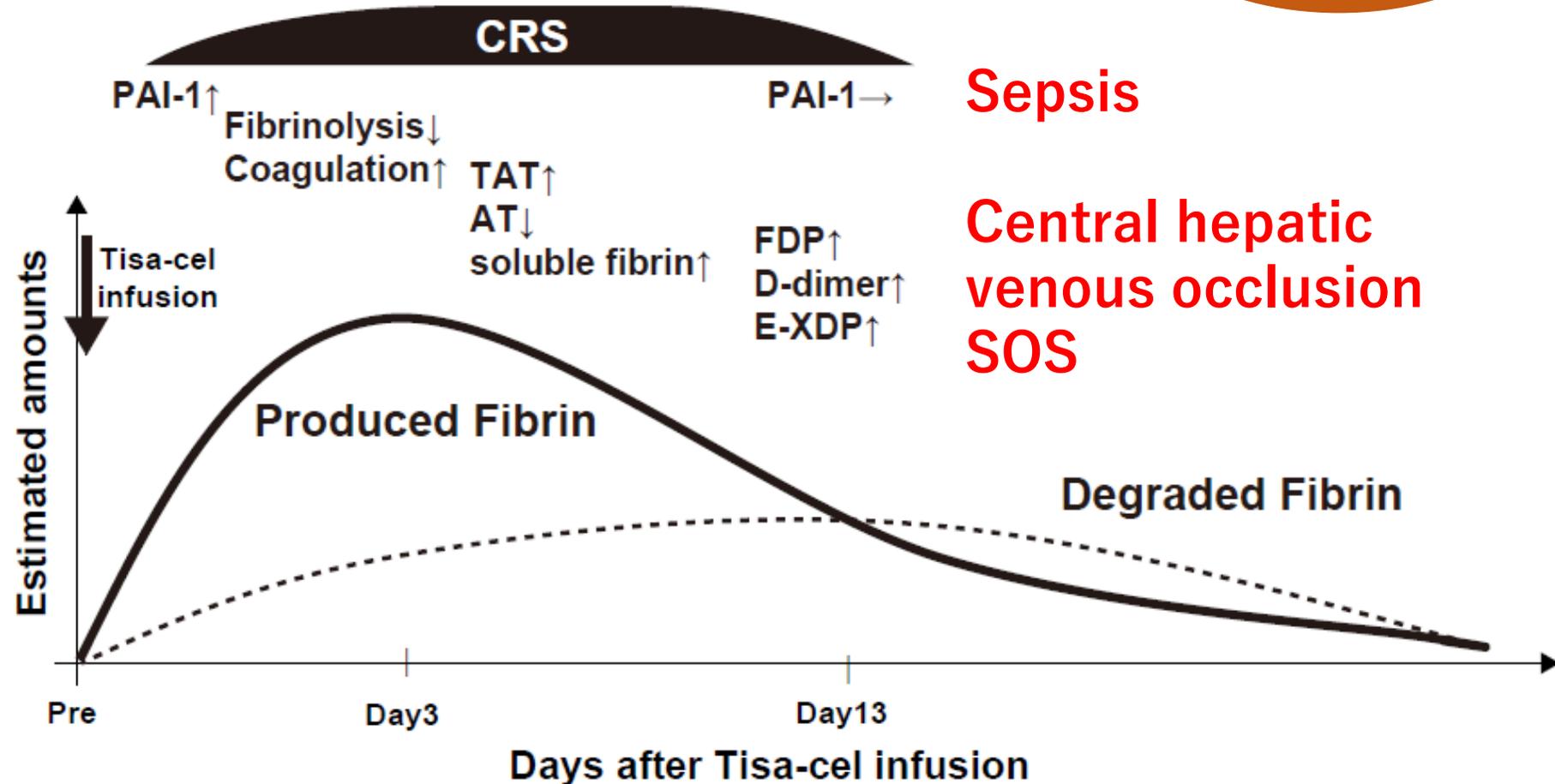
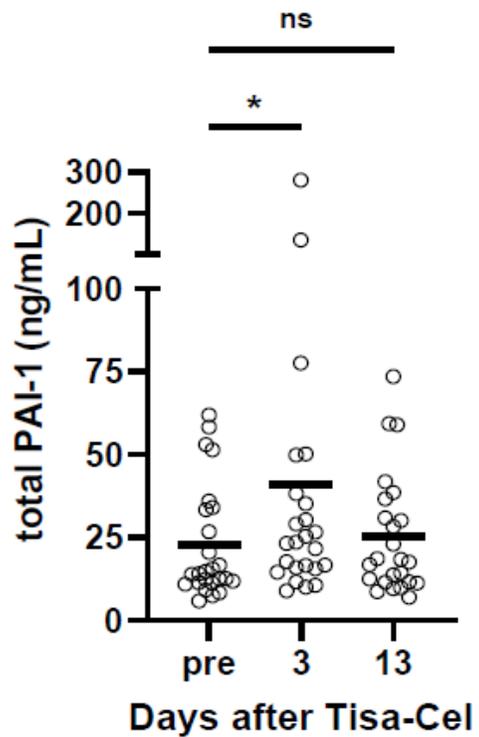
Adverse event after infusion



| | | CRS grade | | | | p value |
|-------------|---|-----------|----|---|---|---------|
| | | 0 | 1 | 2 | 3 | |
| iP decrease | N | 2 | 21 | 0 | 0 | 0.001 |
| | Y | 0 | 15 | 8 | 2 | |
| K decrease | N | 2 | 34 | 6 | 1 | 0.117 |
| | Y | 0 | 2 | 2 | 1 | |
| Mg decrease | N | 2 | 36 | 8 | 1 | 0.083 |
| | Y | 0 | 0 | 0 | 1 | |

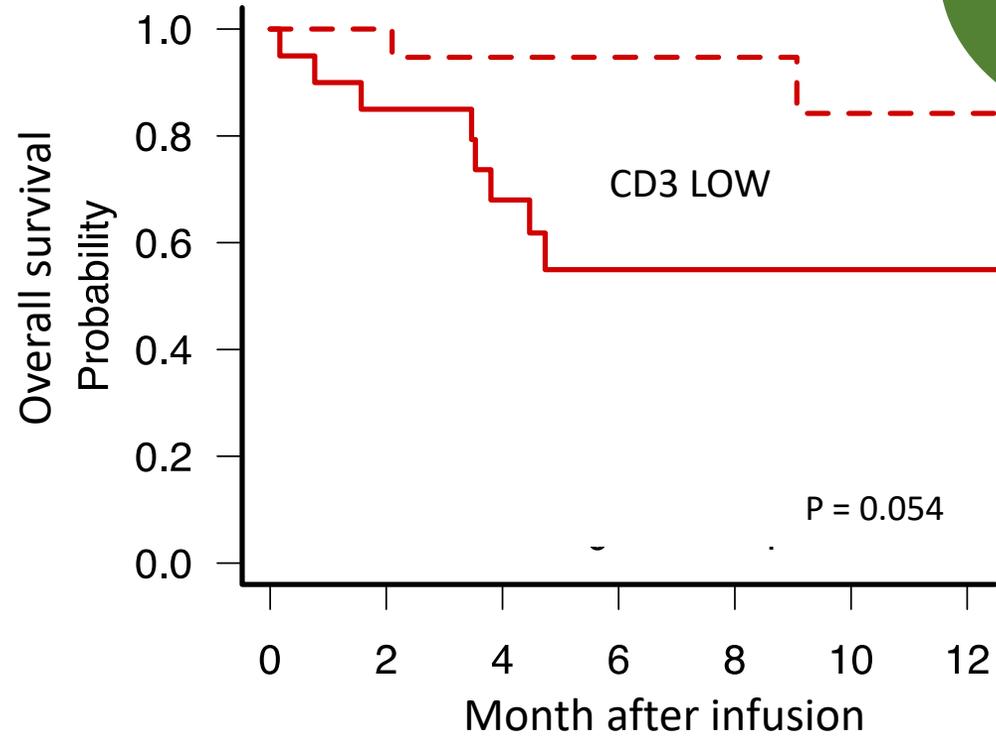
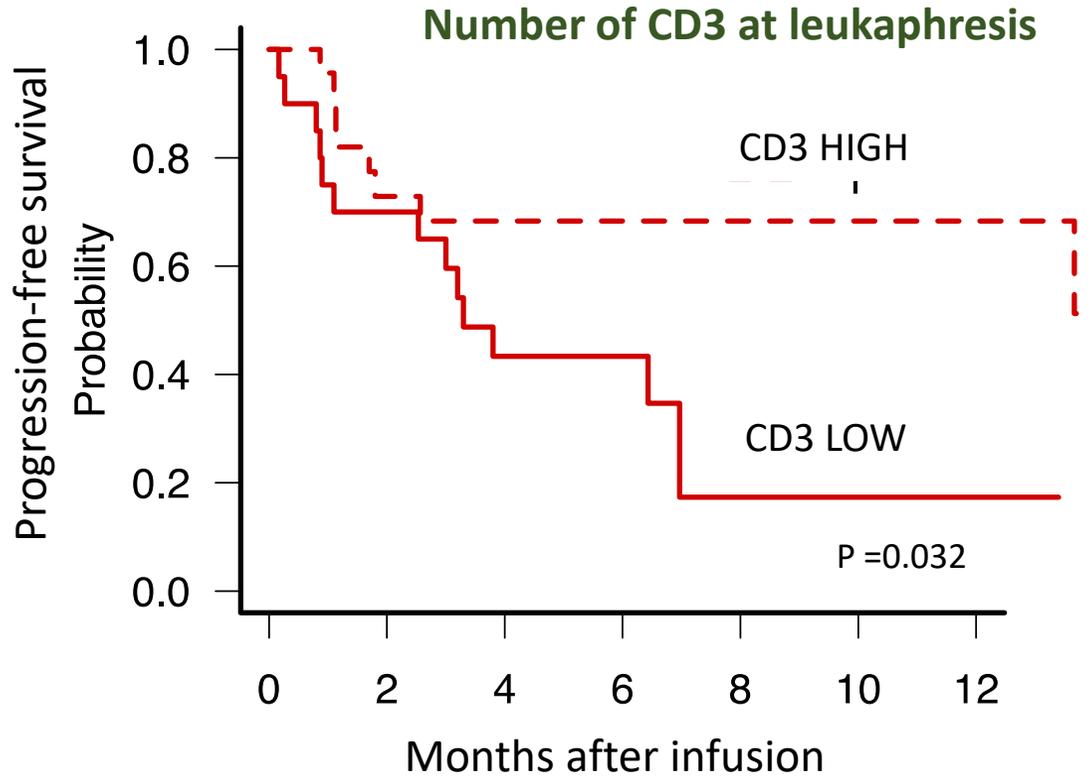
Upregulation of PAI-1 after infusion initiates disturbance in fibrinolytic system

Adverse event after infusion



Clinical Record for CAR-T Therapy in Kyoto Univ.

2019/12-2021/7 DLBCL



Number at risk :

| | | | | | | | |
|----------|----|----|----|----|----|----|----|
| | 0 | 2 | 4 | 6 | 8 | 10 | 12 |
| CD3 LOW | 20 | 14 | 8 | 6 | 1 | 1 | 1 |
| CD3 HIGH | 1 | 24 | 16 | 14 | 11 | 8 | 6 |

Number at risk

| | | | | | | | |
|----------|----|----|----|----|----|----|----|
| | 0 | 2 | 4 | 6 | 8 | 10 | 12 |
| CD3 LOW | 20 | 17 | 12 | 8 | 3 | 3 | 3 |
| CD3 HIGH | 1 | 24 | 19 | 15 | 12 | 9 | 6 |

CAR-T generated from high number of CD3 cells, retains CD3 level and shows good prognosis

Factors that can give impact to the CAR-T therapy

pre-infusion conditioning

Cyclophosphamid /Fludara **No Bendamustine**

pre-infusion conditioning by apheresis

Control timing to start therapy
Platelet, CD3, CD4/CD8 ratio

Selection of patient
Responder/non-responder

Control timing of apheresis

Selection of T cell fraction
Maintain Naïve/stem cell population

Index of therapeutic effect

**CAR T in blood : CCR7+CD45RA-,
CCR7+CD62L+,CCR7+ CD27+
CD27+,PD-1-, CD8+**

**Use of immunomodulation reagents
as post infusion conditioning**
PD-1/PD-1L LAG-3/TIM-3, IMiDs

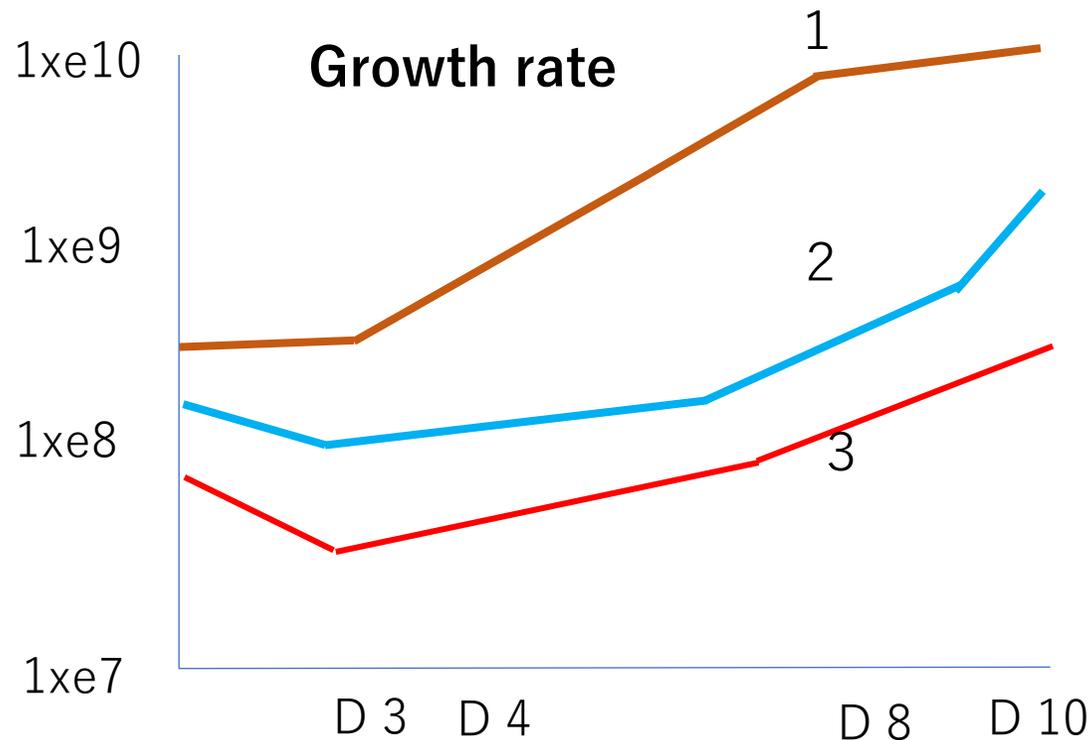
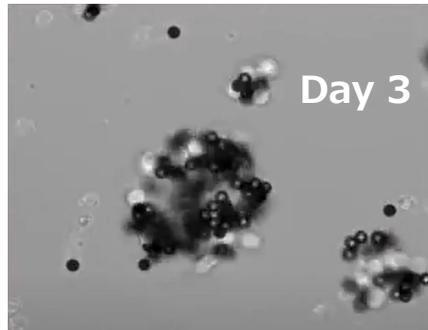
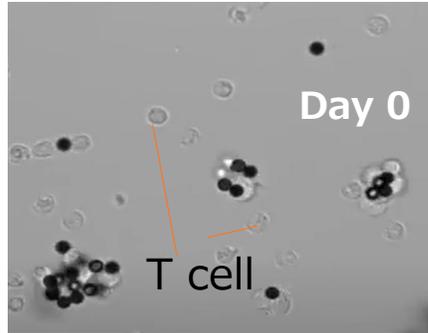
Stable supply of commercial vectors

Vector design development
Reduce off-target effect, expand application
Enhance efficacy

Allogenic application
HLA class I, TCR gene editing

Explore the CPPs for CAR T manufacturing

T cell morphology during culture



Surface molecule analysis Stem/naïve vs exhausted

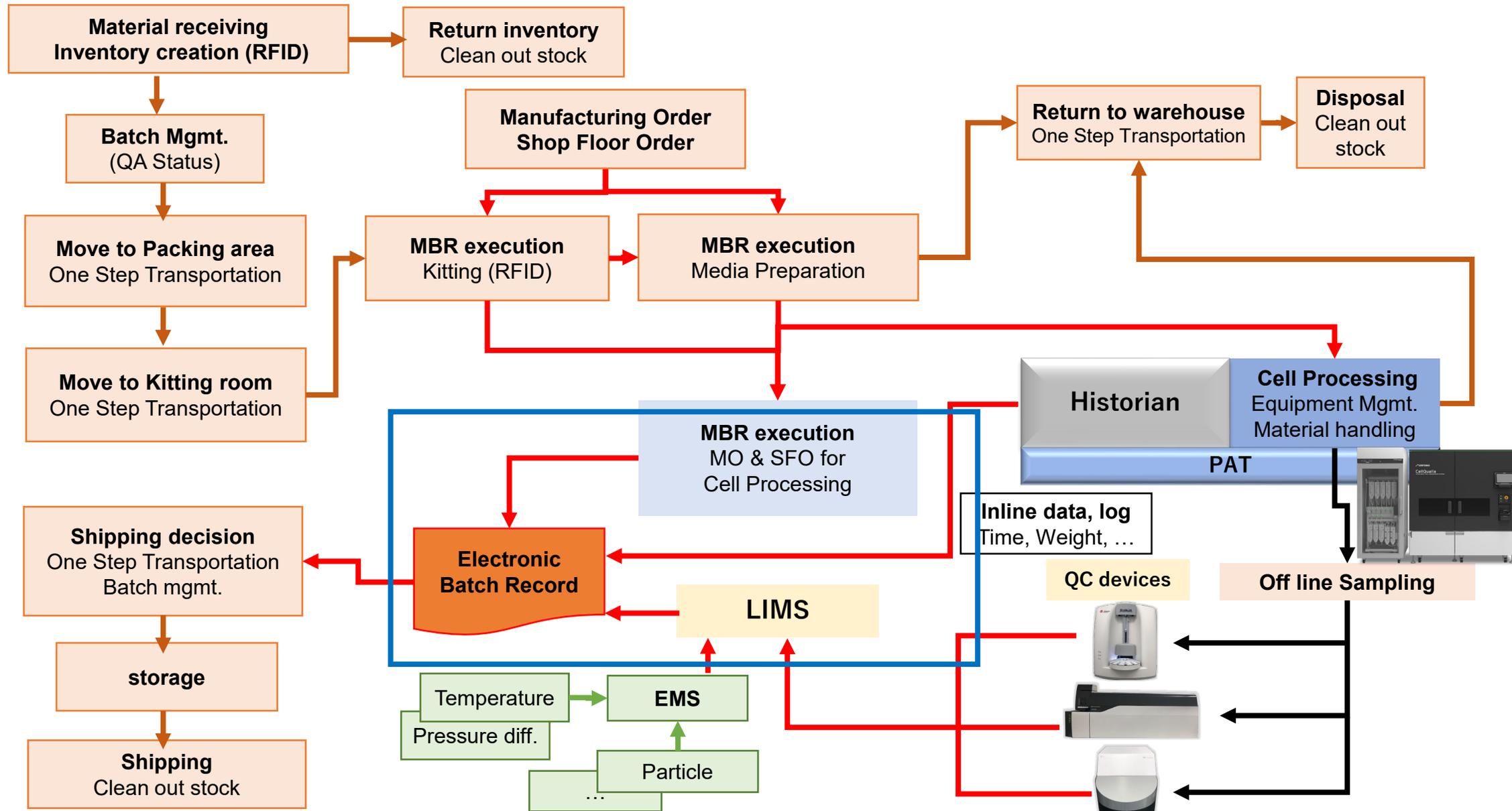
Early diff. cells ↑
CD62L, CCR7, CD27, CD45RA

Exhausted cells ↓
KLRG-1, HLA-DR, CD38, PD-1

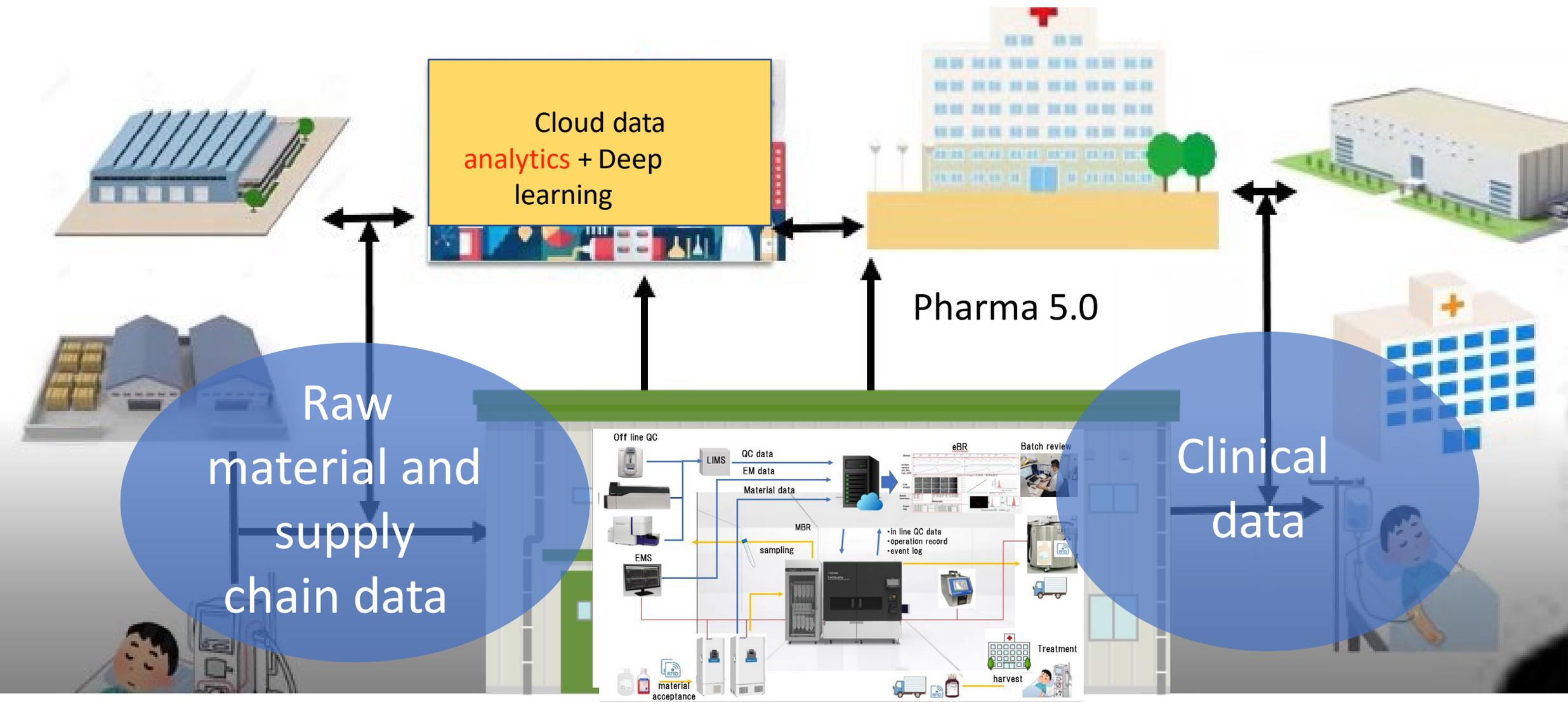
Effector EM, senescence ↓
CD45RO, CD27⁻, CD28⁻

Treg, MDSC ↓
CD25⁺ FOXP3⁺,
CD15⁺ CD14⁻ HLA-DR⁻

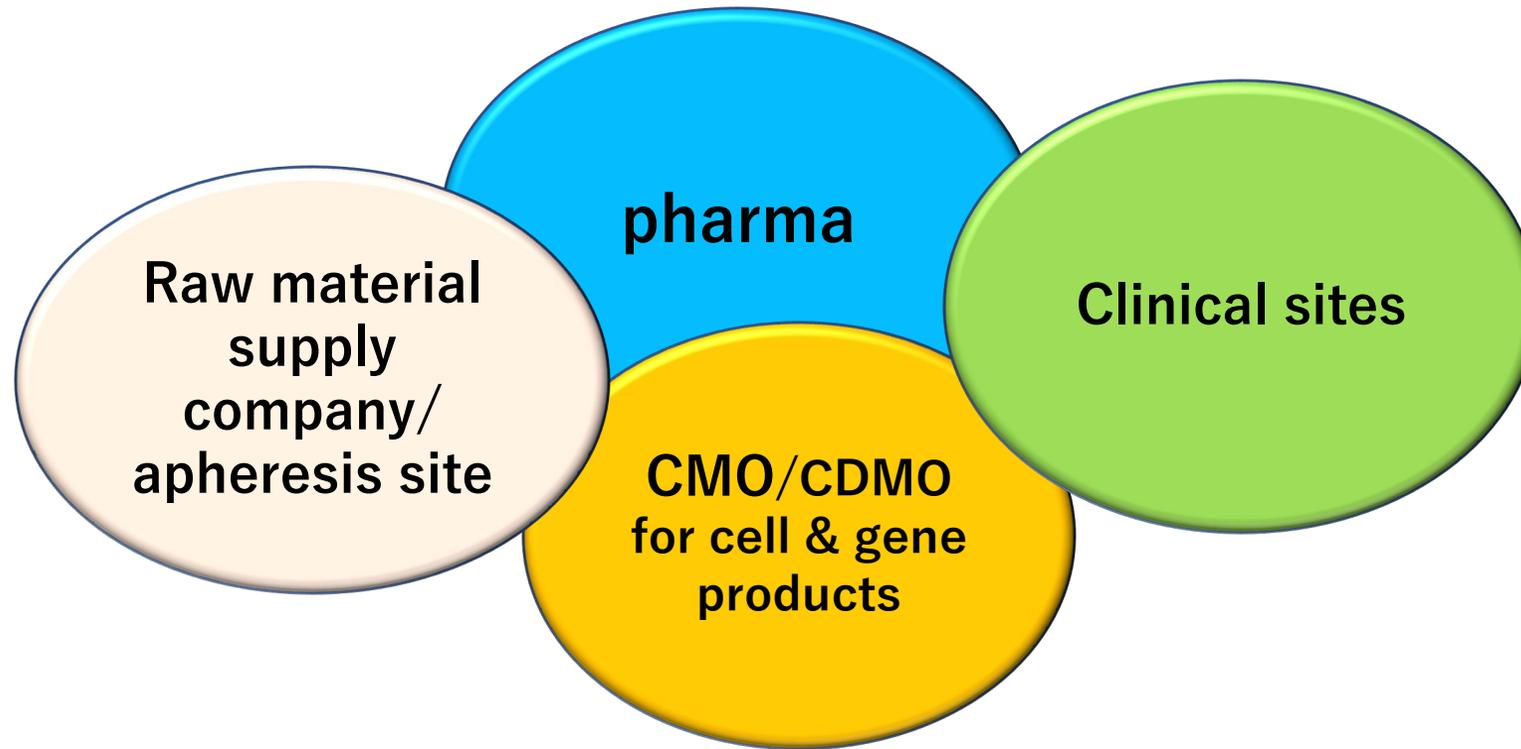
Development of cell manufacturing control system



Manufacturing information in IT format can enable linkage to manufacturing, supply chain and clinical data



For improved treatment outcomes and new drug development, the formation of an ECO system through information sharing is essential.



**Manufacturing information shall be digitized on the CMO side to combine it with supply chain and clinical information data
⇒ Need for QbD-based manufacturing**

Summary

Factors that influence the outcome of the CAR-T therapy

1. Select carefully the timing to initiate the CAR-T therapy

not to use exhausted leukapheresis to manufacture CAR-T cells successfully

no use of Bendamustine, $CD3 > 2.0 \times 10^3$, $CD4/CD8 > 1.0$, platelet $> 2.0 \times 10^5$,

2. Manufacture CAR-T cells retaining growth potential

manufacture CAR-T cells with growth potential so that they survive in patient over 6M

indication: stem cell expression of CD62L, CCR7, CD27, CD45RA

no exhausted cells KLRG-1, HLA-DR, CD38, PD-1

no effector cell CD45RO, CD27-, CD28-

no Treg, MDSC CD25+FOXP3+, CD15+CD14-HLA-DR-

Collaboration among clinical site/CMO/Pharma is indispensable to improve the cell therapy and conduct an effective **Reverse Translational Research**.

Manufacturing **data in digital format** is essential to improve supply chain control through linking with clinical data and develop new CAR-T product.

Acknowledgment

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Keio Univ. Hospital
Dept of Transfusion
Cell Therapy

Ryouji Tanozaki



FBRI
R&D Center for Cell Therapy

Takeaki Kubo
Satoru Fujimoto
Masashi Murakami





Thank you for your attention. 多謝