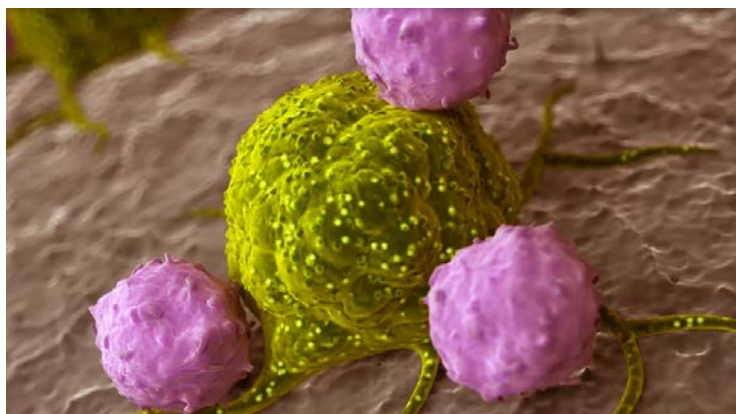


第4回がん新薬開発合同シンポジウム

がん免疫細胞療法開発への企業側の取り組み

がん免疫遺伝子治療への取り組み

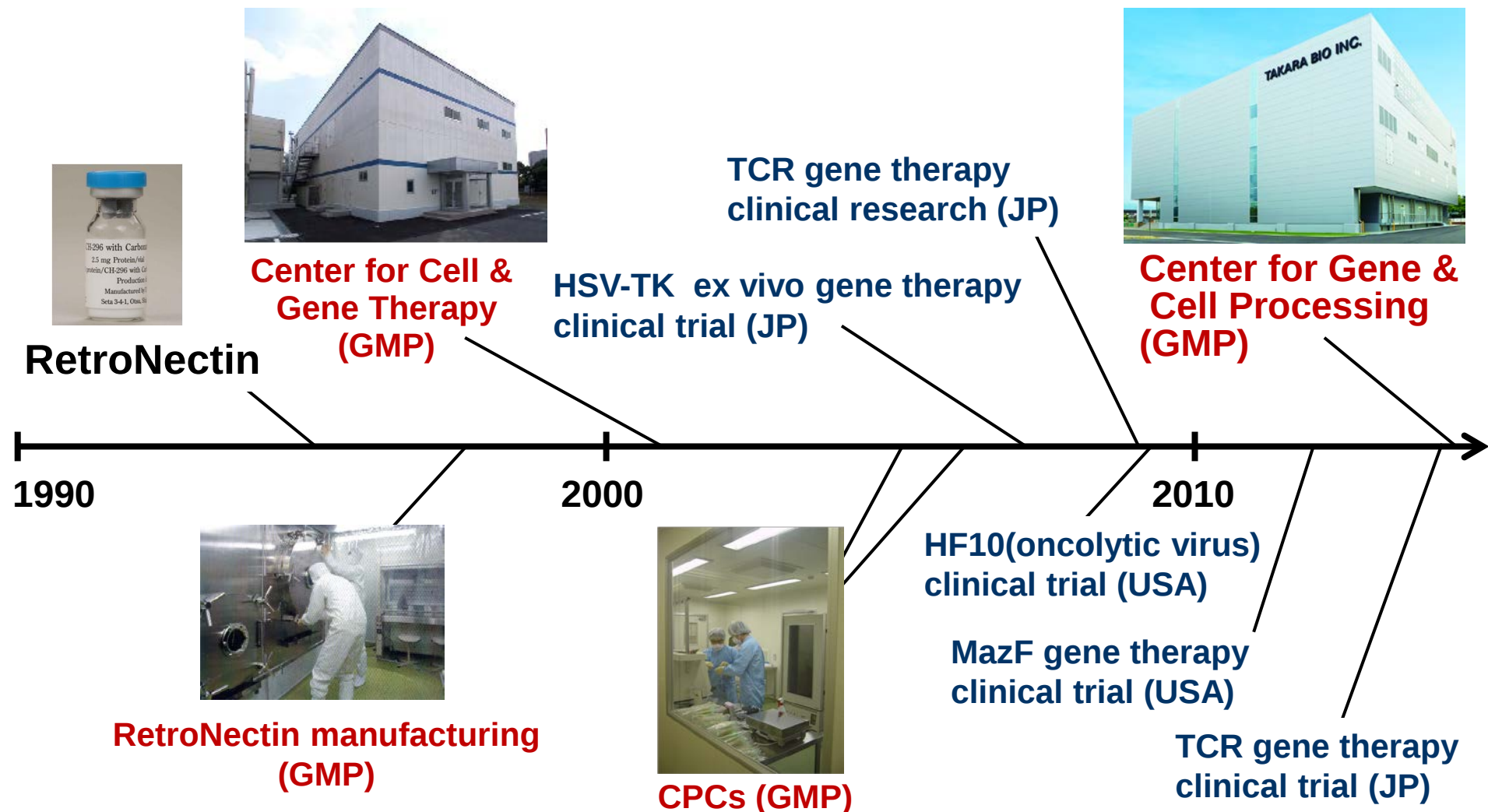


峰野純一

タカラバイオ株式会社 バイオ産業支援事業部門

that's
GOOD
science!™

Takara Bio, Gene therapy supporting history

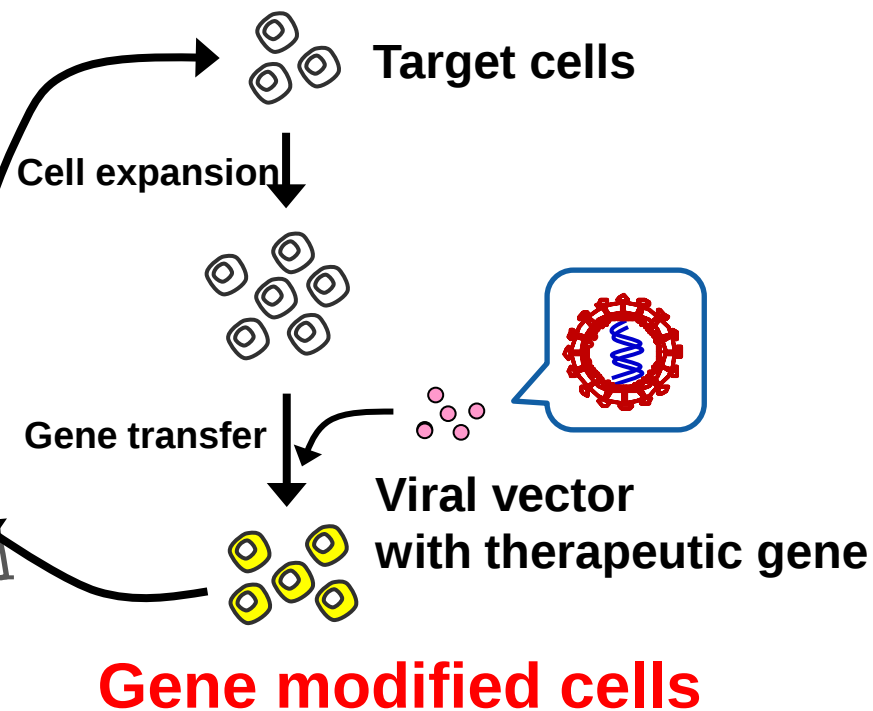
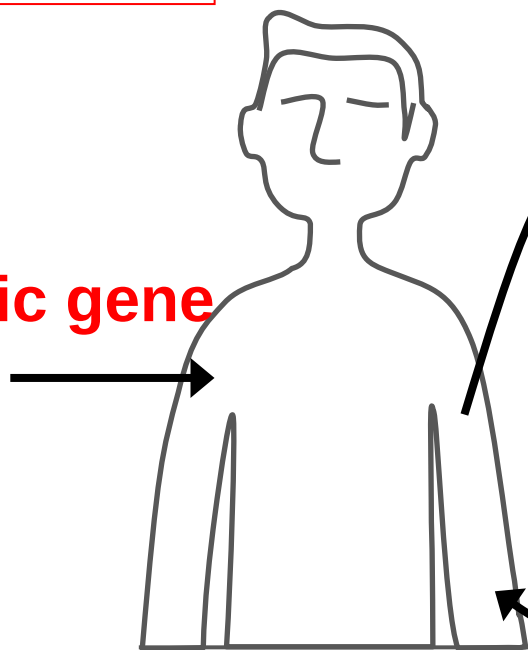
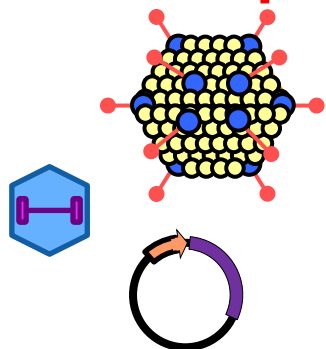


Gene Therapy

in vivo Gene Therapy

ex vivo Gene Therapy

**Viral vector
with therapeutic gene**

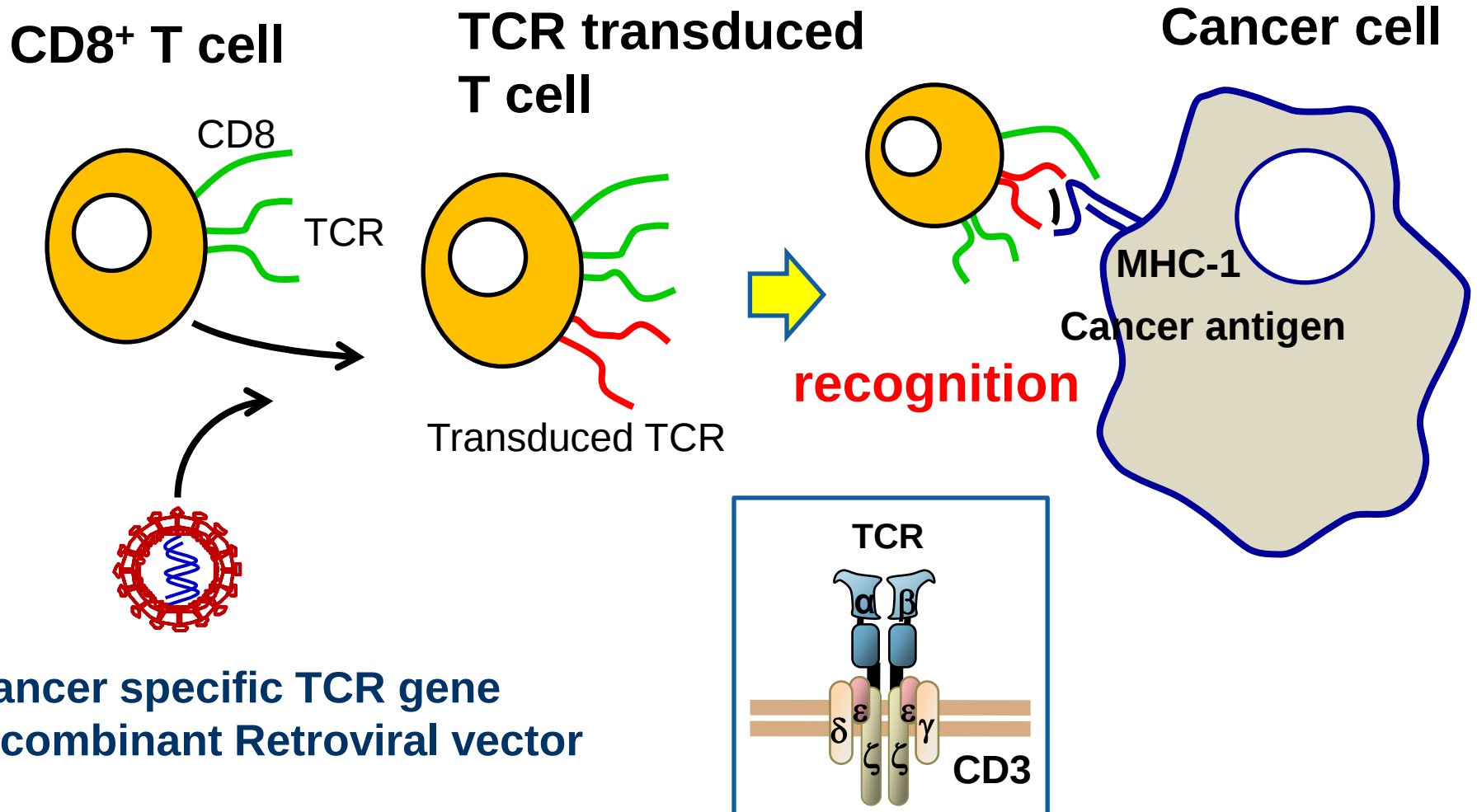


Schedule for Clinical Development of Gene Medicine of Takara Bio

	Preclinical trials	Phase I clinical trials	Phase II clinical trials	Phase III clinical trials	Commercialization
HF10 (oncolytic virus) anti-cancer therapy				Phase II clinical trials in the U.S.	FY2018
		Phase I clinical trials in Japan (commence in FY 2014)			
MAGE-A4 TCR gene therapy for cancer		Phase I clinical trials in Japan			FY2021
MazF gene therapy for AIDS		Phase I clinical trials in the U.S.			FY2022
NY-ESO-1 TCR gene therapy for cancer		Phase I clinical trials in Japan (commence in FY 2014)			

TCR Gene Therapy

TCR gene-modified CD8⁺ T cell recognition of cancer antigen



What we have been doing

- ✓ **Vector development**
- ✓ **Development of cell expansion**
- ✓ **Development of gene transfer method**
- ✓ **Closed system cell processing**
- ✓ **GMP manufacturing of MCB and vector**
- ✓ **Development of QC method and validation**
- ✓ **Construction of GMP facility**

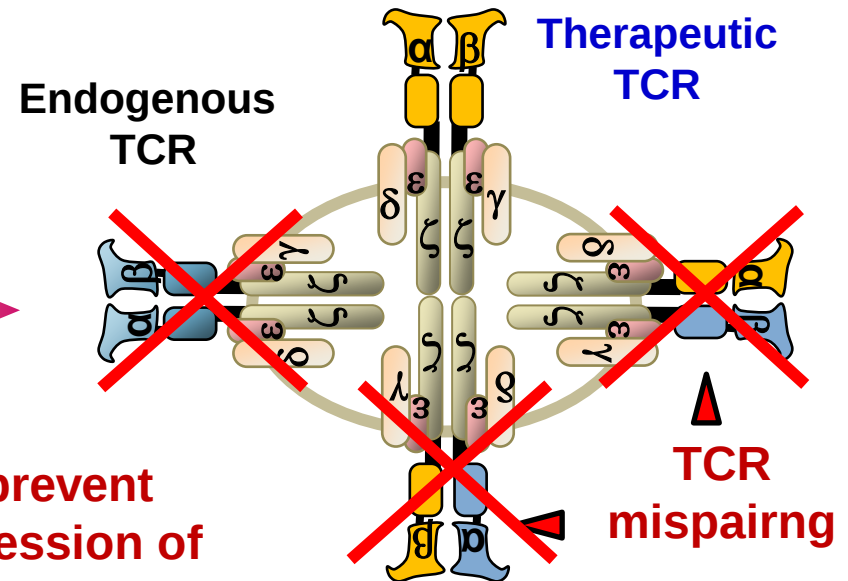
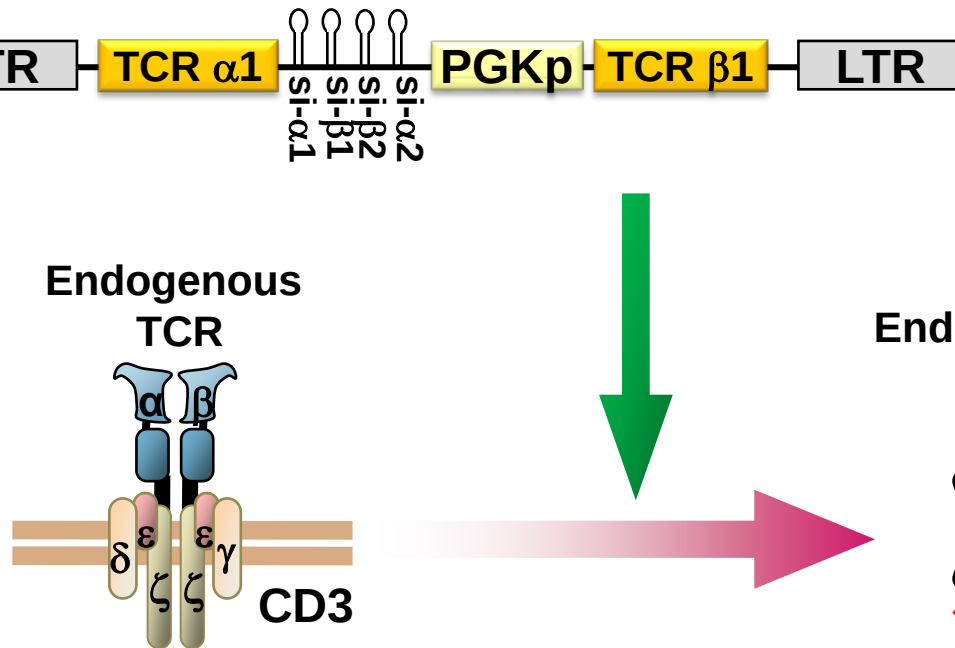
Vector development: siTCR

- silencing of endogenous TCR by siRNAs -

siTCR Gamma-retroviral Vector

siTCR vector can express 6 kinds of genes

codon-modified(optimized) TCR α : **TCR α 1**
codon-modified(optimized) TCR β : **TCR β 1**
siRNAs for TCR α : **si- α 1, si- α 2**
siRNAs for TCR β : **si- β 1, si- β 2**



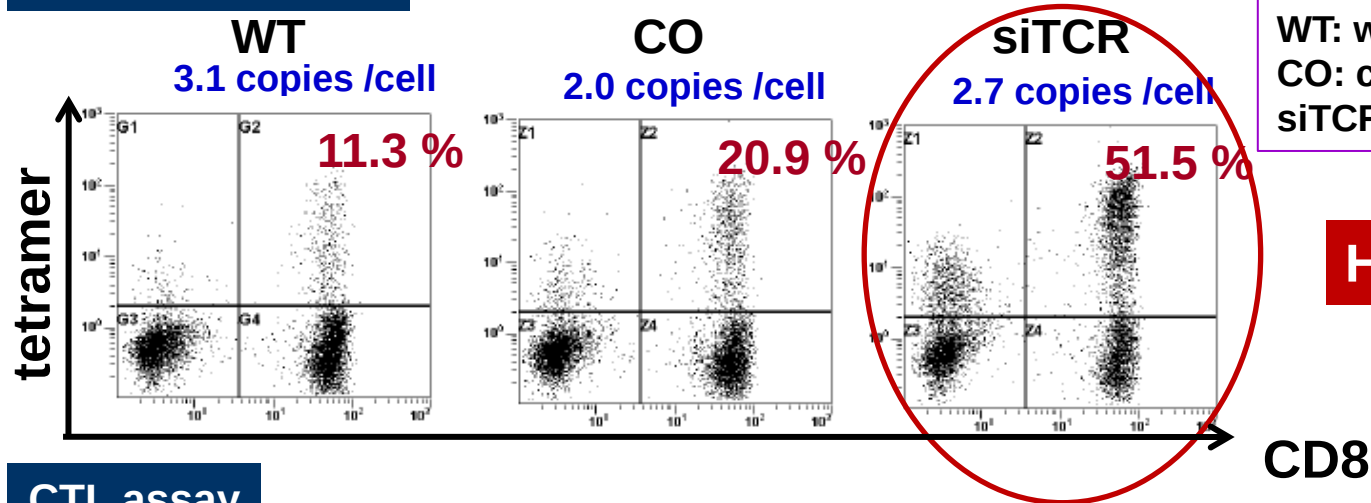
Knockdown of endogenous TCRs could prevent TCR mispairing and lead to efficient expression of the introduced TCRs

Okamoto et al., Cancer Res 2009

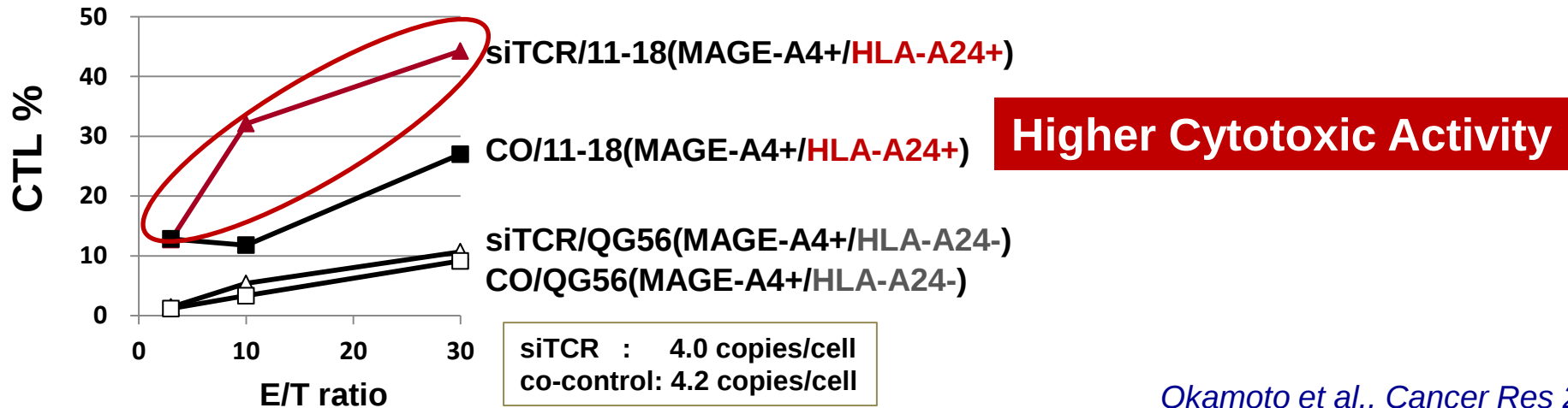
Vector development: siTCR

- Higher expression and cytotoxic activity -

Tetramer Staining



CTL assay

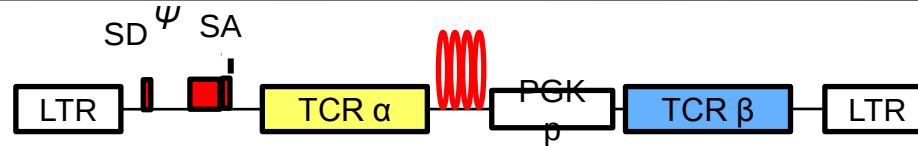


Okamoto et al., Cancer Res 2009

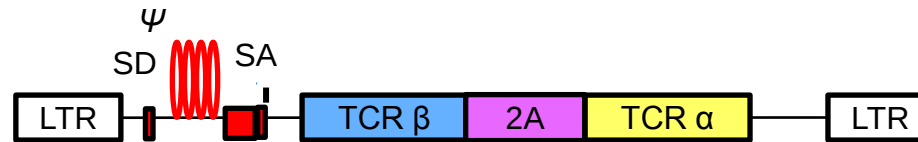
2nd Generation siTCR Vector

- Enhancement of TCR specific lysis -

1st gen. siTCR
(internal promoter)



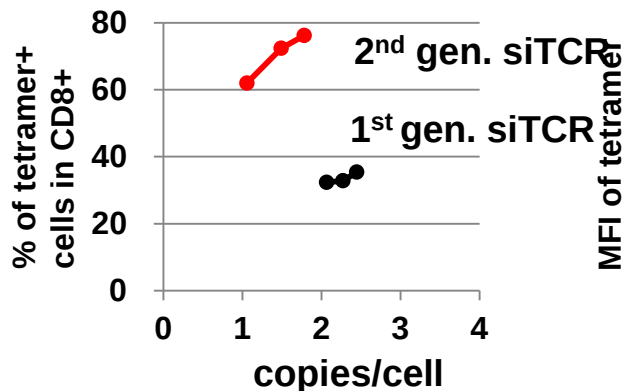
2nd gen. siTCR
(2A peptide)



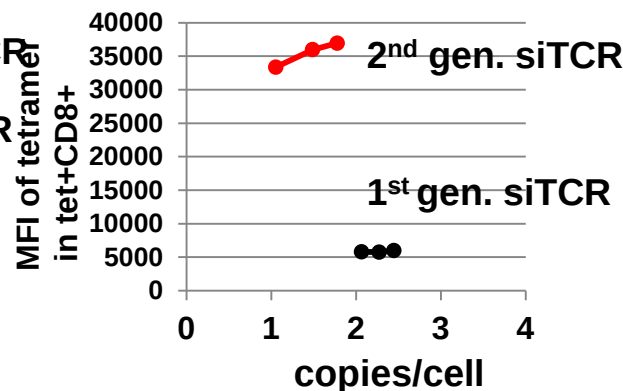
HLA-A*0201 restricted MART-1 specific TCR α β genes

Tetramer Staining

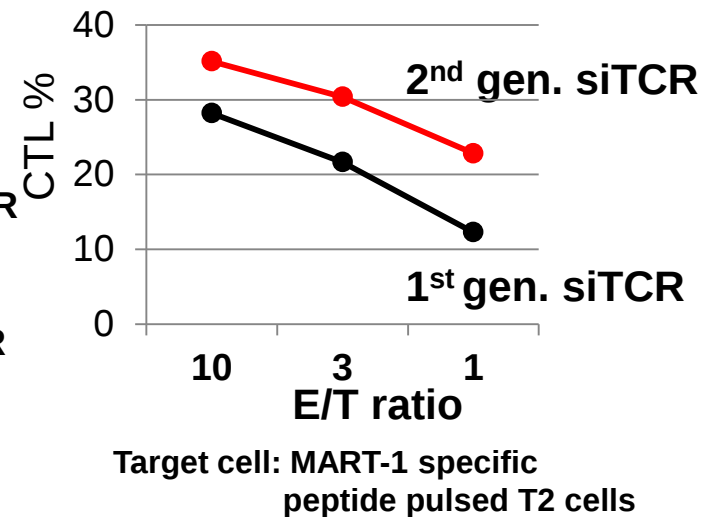
% of tetramer+



MFI of tetramer+



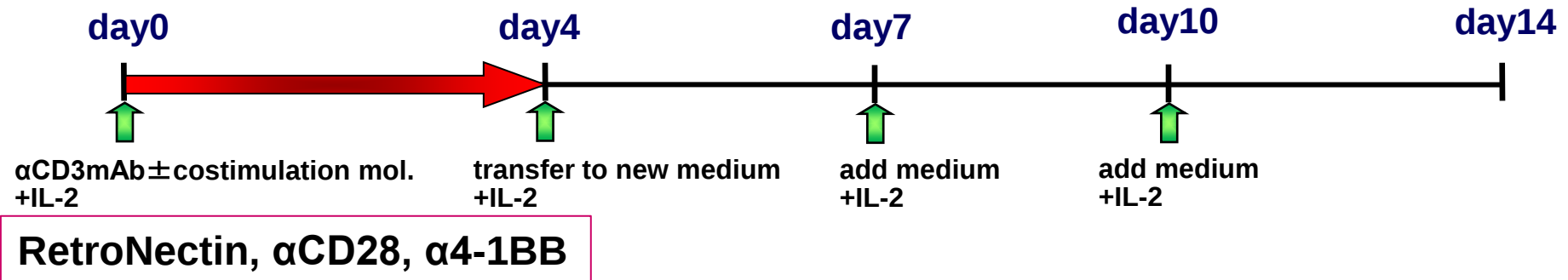
CTL assay



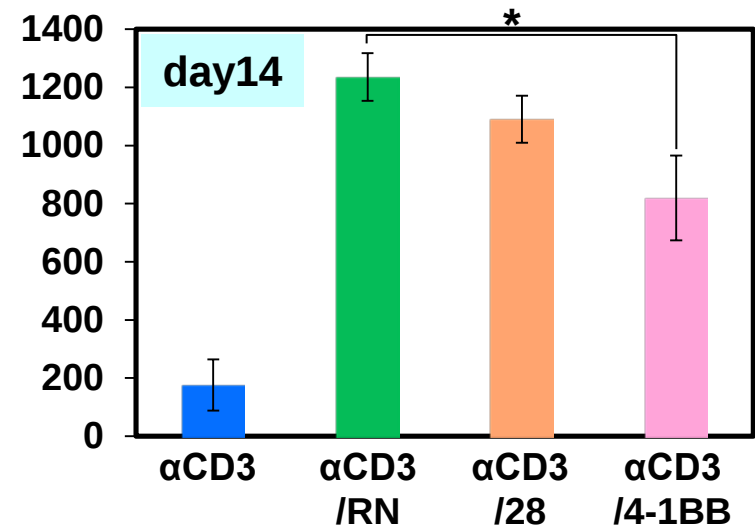
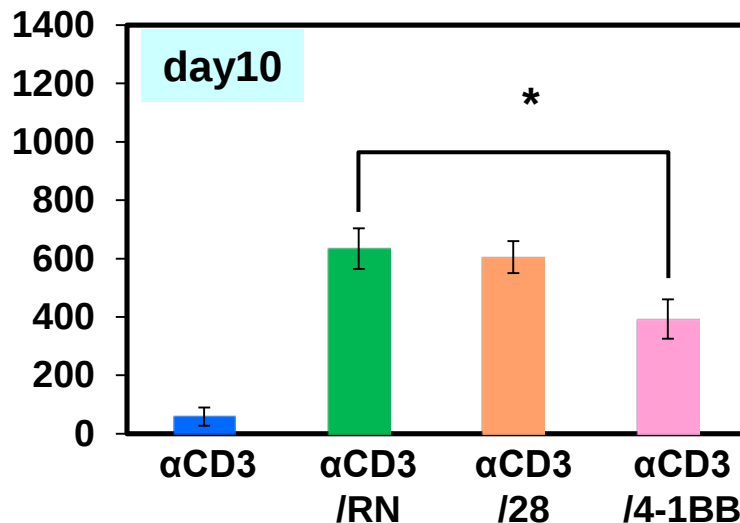
Okamoto et al., Mol Ther Nucleic Acids. 2012

Development of cell expansion

- RetroNectin Expansion Method -



Expansion fold



error bar: means $\pm$ SEM (5 donors) * p <0.05, Tukey-kramer test

Yu et al., Cancer Gene Ther 2008

Development of gene transfer method & Closed system cell processing

Stimulation

- PBMC recovery
- Stimulation by T-Expander CD3/CD28

Transduction (RBV-LTS)

Vector
pre-load

Transduction
➤ GaLV retroviral vector

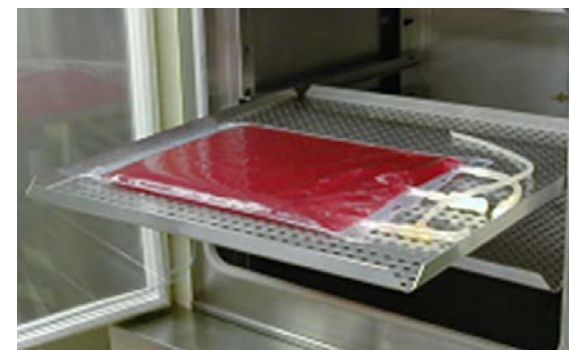
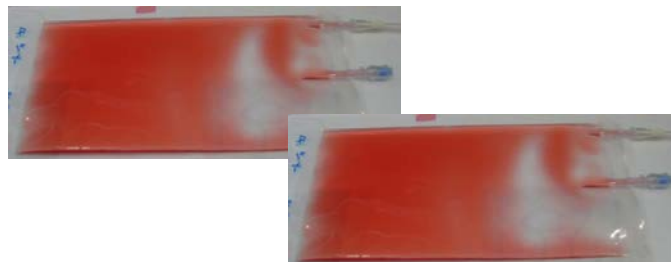
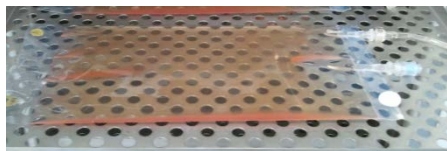
Vector
pre-load

Transduction
➤ GaLV retroviral vector

Expansion

Expansion
(CultiLife Eva)

Harvest



CultiLife Eva (640cm²)

Takara Bio to establish a new facility for the research and manufacturing of pharmaceutical products for cell and gene therapies

August 9, 2012 ---- Takara Bio announced that it has entered into an agreement with a third party to purchase its land and buildings, and the Company will build a new facility for the research and manufacturing of pharmaceutical products for cell and gene therapies. The role of the new facility will be to manufacture gene modified cells or viruses in compliance with GMP (Good Manufacturing Practice) as well as vectors for gene transduction, which include retroviral, plasmid, lentiviral, adenoviral and adeno-associated viral vectors etc. In this new facility, the Company will also conduct research and development of fundamental biotechnologies in the field of cell and gene therapy.

Takara Bio is conducting clinical trials of HSV-TK gene therapy for leukemia in Japan, MazF gene therapy for HIV and HF10 anti-cancer therapy in the U.S., and plans to commercialize these gene therapy drugs in 2017 and beyond. The Company is also developing core technologies on cell and gene therapy, and manufacturing GMP vectors for its own clinical trials or as contract services at facilities located in the Otsu and Kusatsu cities of Shiga Prefecture.

Center for Gene & Cell Processing (Kusatsu, Shiga, Japan)

Total floor space: 6,500 m²,

1st floor:

- Cell banking (e.g. *E. coli*)
- Plasmid vector manufacturing
- E. coli* culture for protein production
- QC test (sterility, Mycoplasma)
- Cell bank storage

2nd floor:

- Viral vector production
 - gamma retrovirus, lentivirus, HSV, adenovirus, AAV, HVJ, etc.

- Cell culture, Media preparation
- Protein purification
- Aseptic filling

3rd floor:

- Cell processing
- QC test (test for cells & viruses, qPCR, bio assay, etc.)



Center for Gene & Cell Processing



that's
GOOD
science!™

