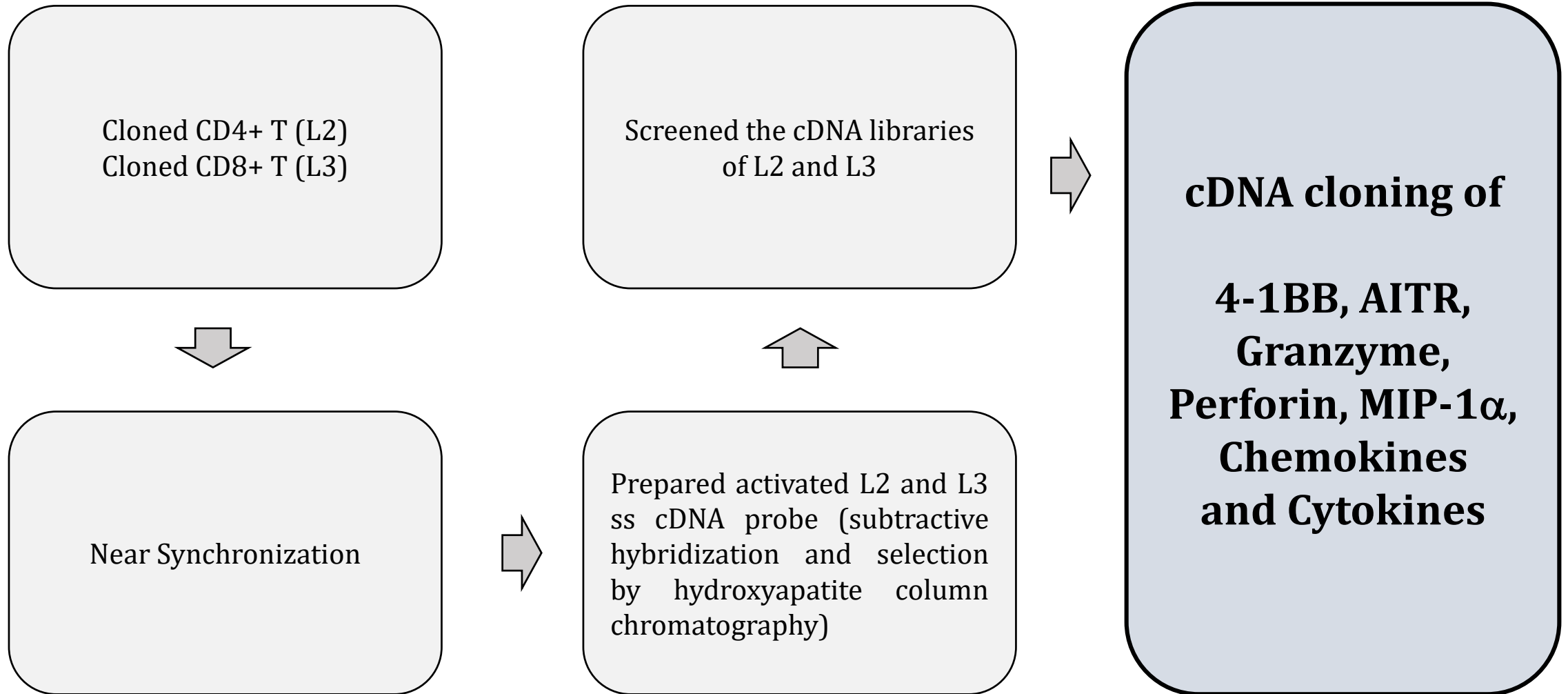


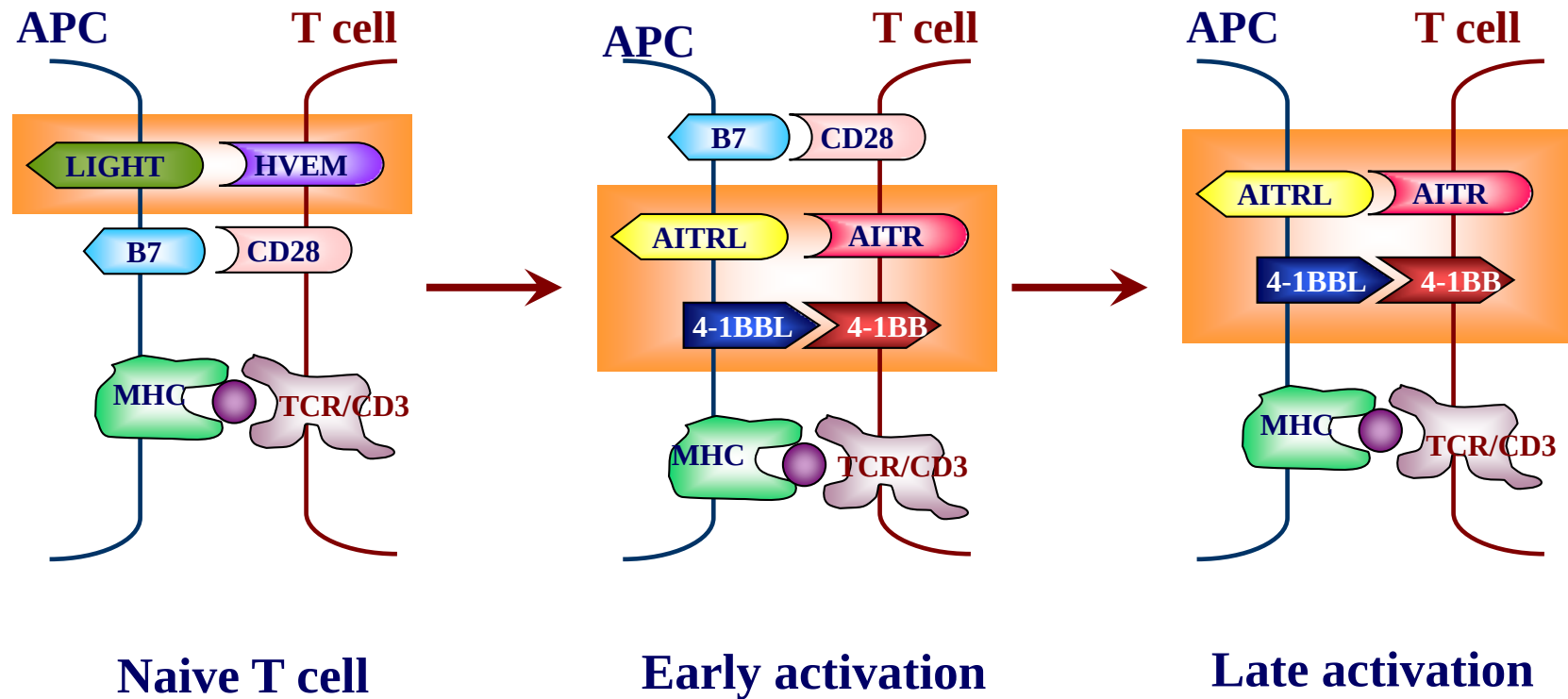
# Webinar outline

- Discovery of 4-1BB and 4-1BB immuno-biology
  - **Byoung S. Kwon, PhD** - *Eutilex*
- 4-1BB as an important prosurvival signal for CAR T cells
  - **Michael Milone, MD, PhD** - *University of Pennsylvania*
- Novel antibody-based approaches targeting the 4-1BB pathway
  - **Christian Klein, PhD** - *Roche Innovation Center Zurich*
- Translational and reverse translational research in 4-1BB co-stimulation
  - **Ignacio Melero, MD, PhD** - *CIMA, Clinica Universidad de Navarra*

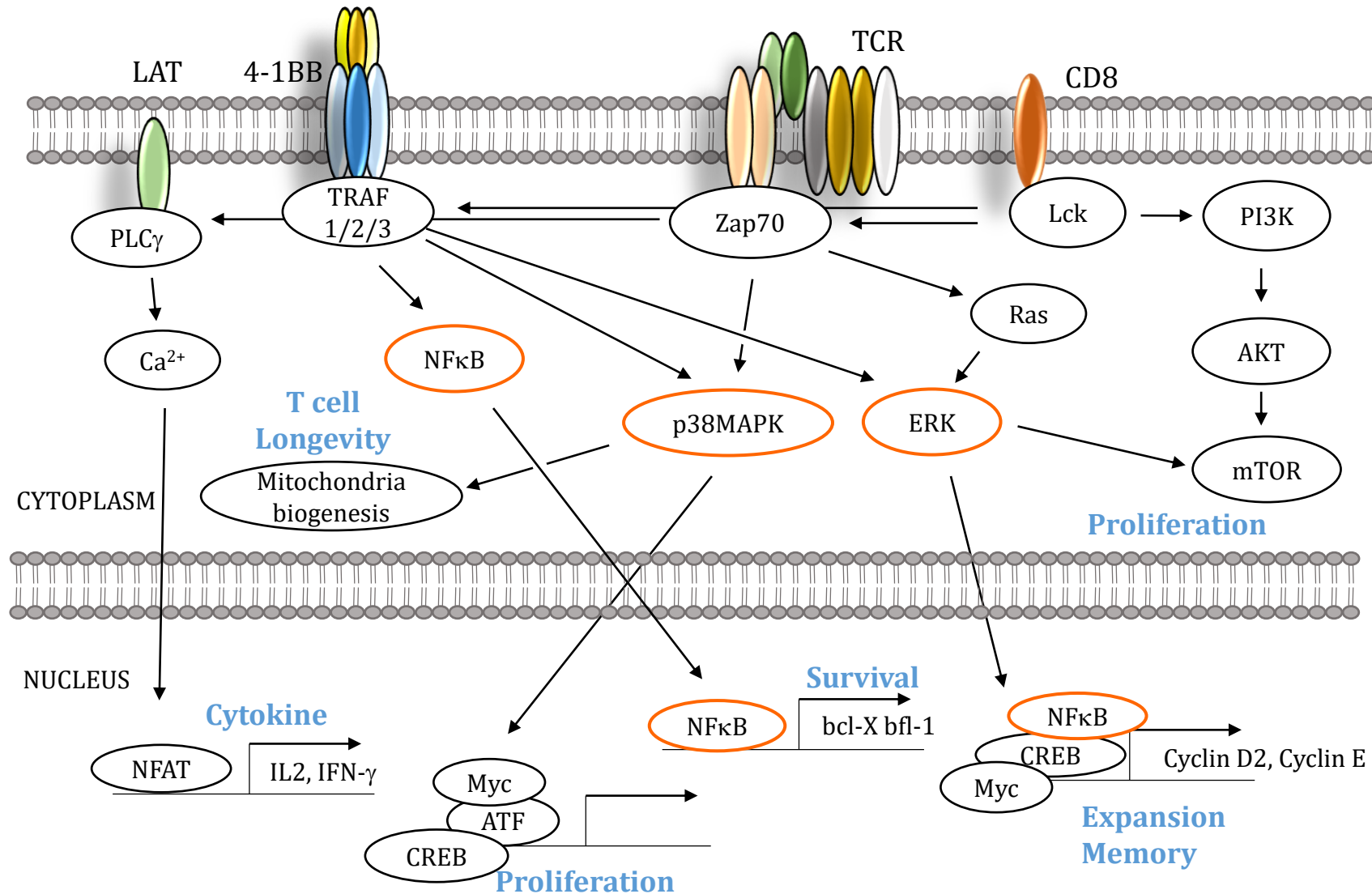
# Discovery of 4-1BB



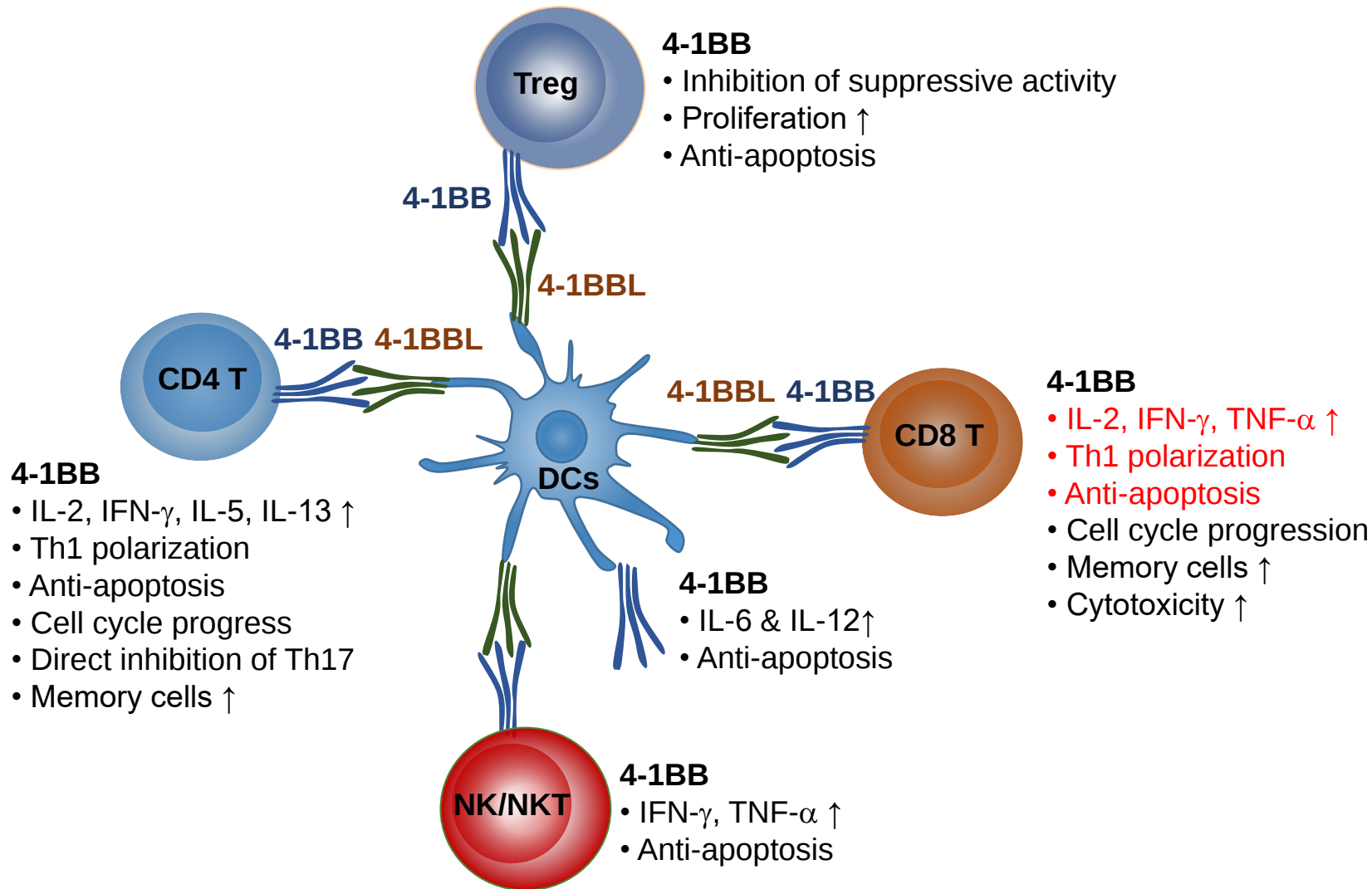
# 4-1BB-4-1BBL System in T cell Activation



# 4-1BB-mediated signaling pathway



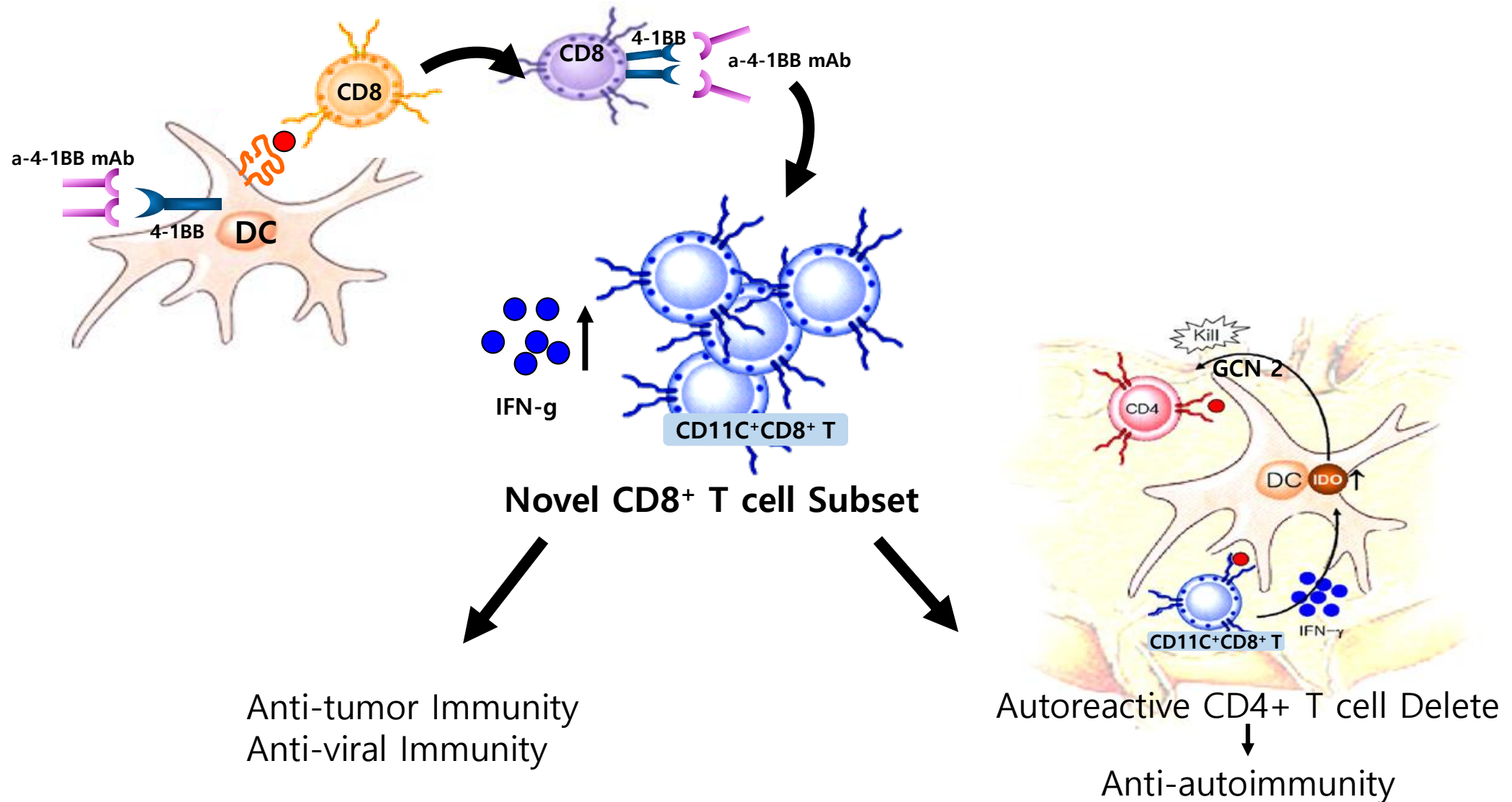
# Effect of 4-1BB stimulation on lymphocytes



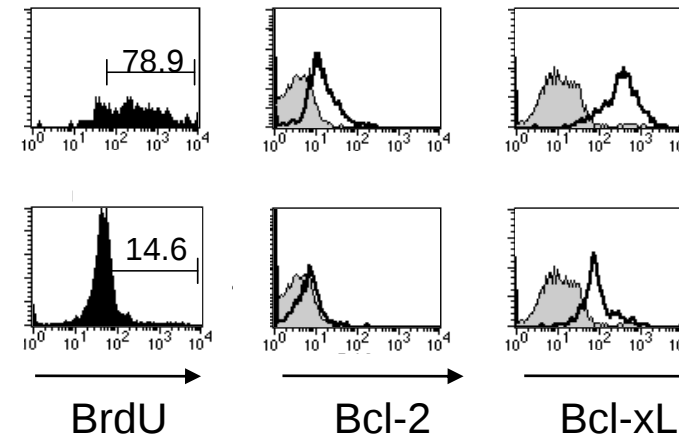
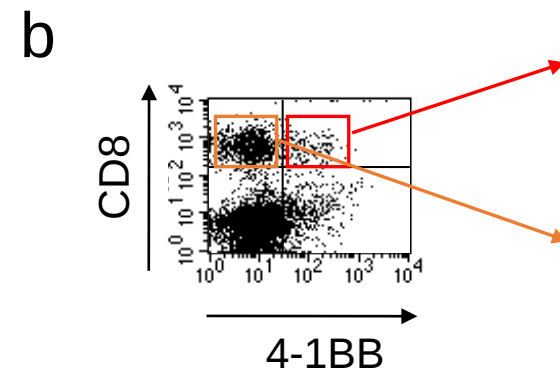
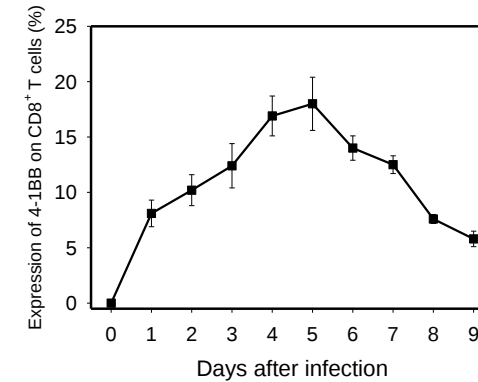
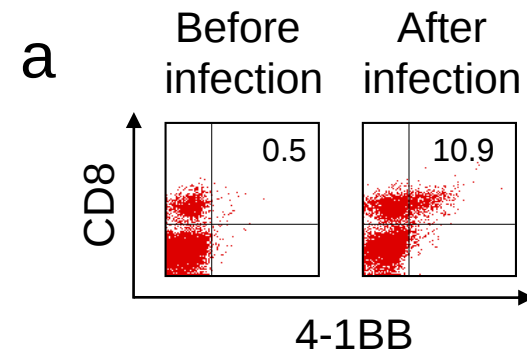
# Autoimmune diseases that are treated by anti-4-1BB in animal models

1. Rheumatoid Arthritis
2. Systemic Lupus Erythematosus
3. Sjögren Syndrome
4. Experimental Autoimmune Uveoretinitis
5. Crohn's Disease
6. Mercury-induced Autoimmune Disease
7. Allergic Asthma
8. Chronic GVHD

# Mechanism for 4-1BB-targeted Immunotherapy

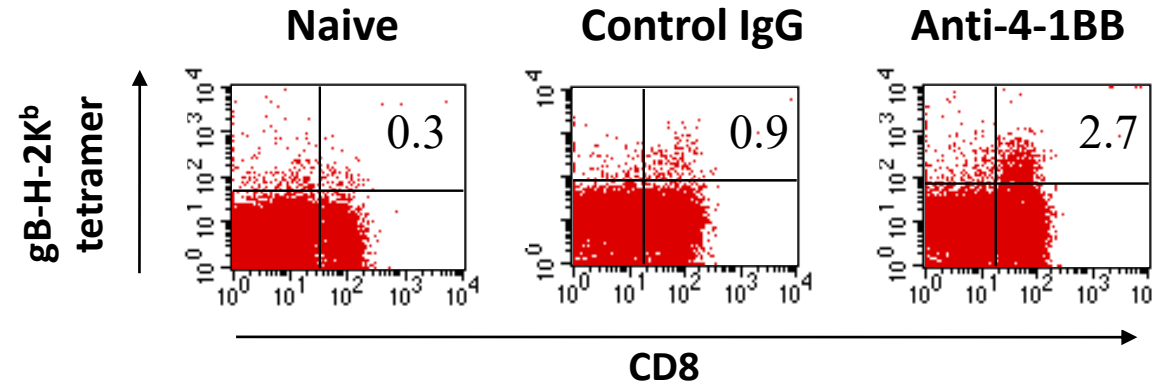


# Viral infection and 4-1BB expression

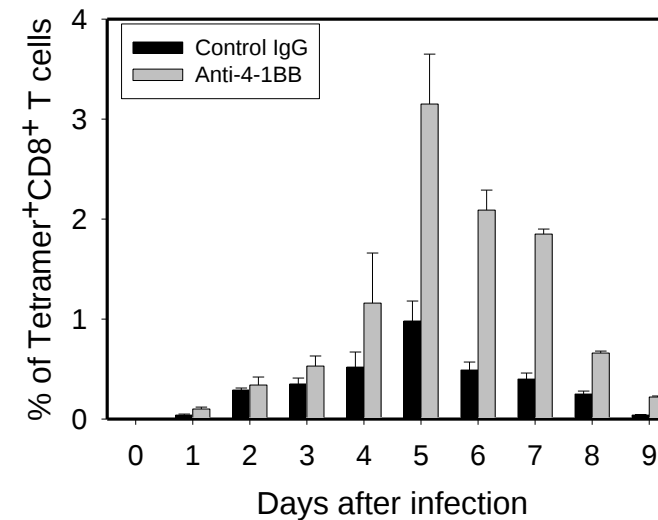


# Effects of *in vivo* stimulation of 4-1BB on HSV-1-specific CD8<sup>+</sup> T cells

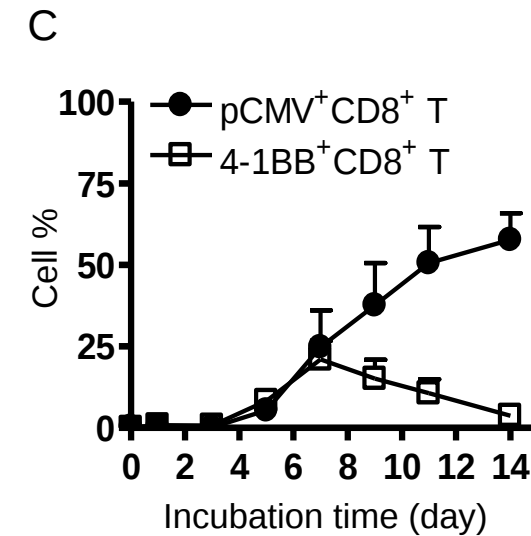
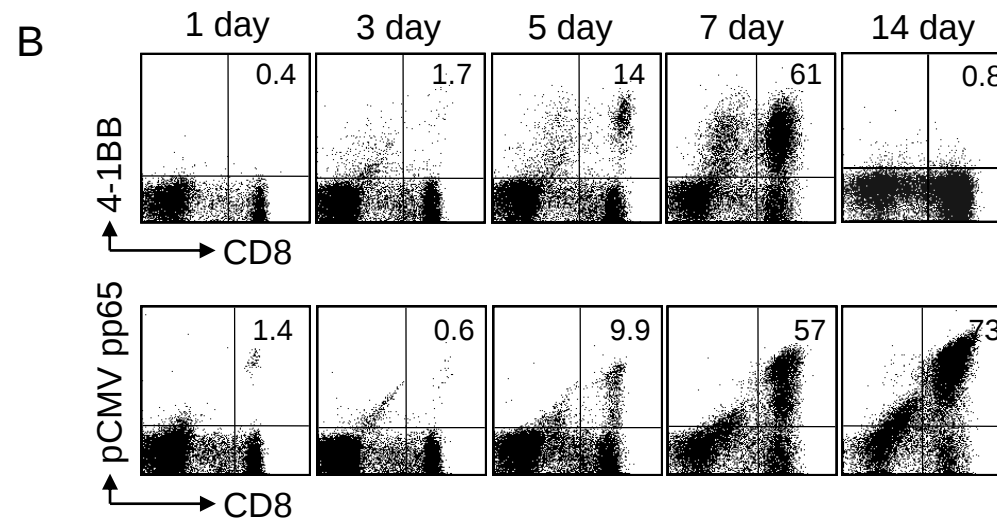
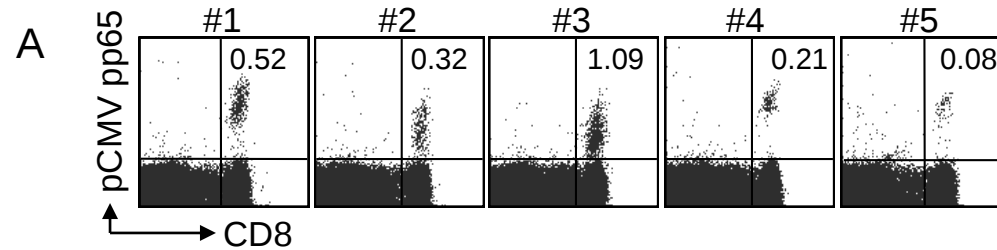
a



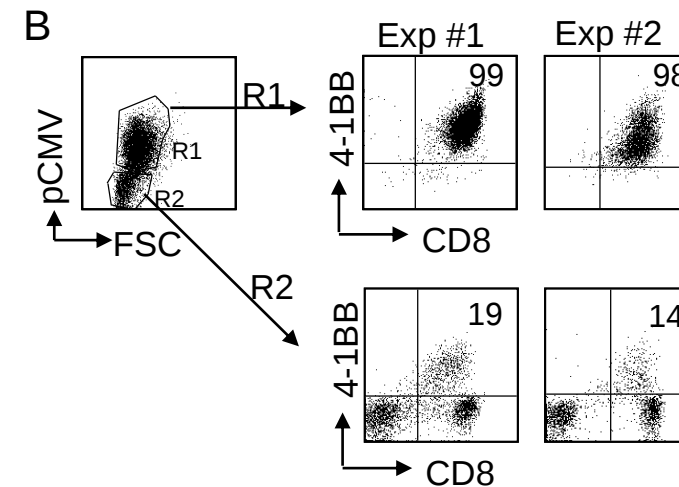
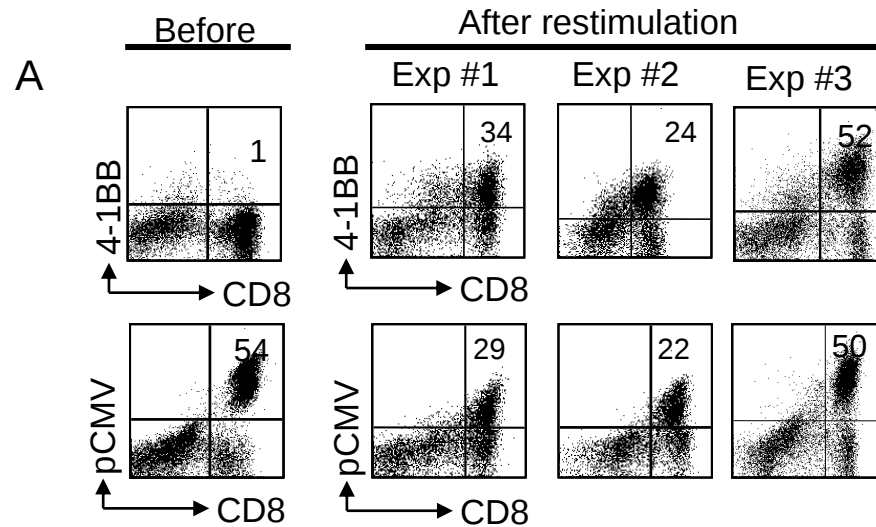
b



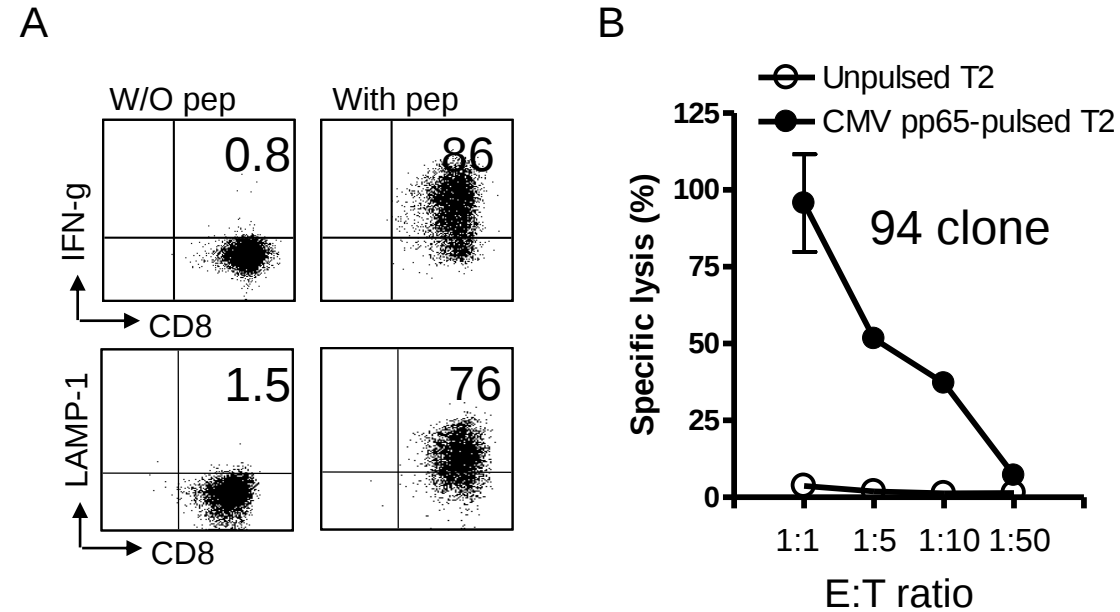
# Kinetics of 4-1BB expression on human CD8<sup>+</sup> T cells



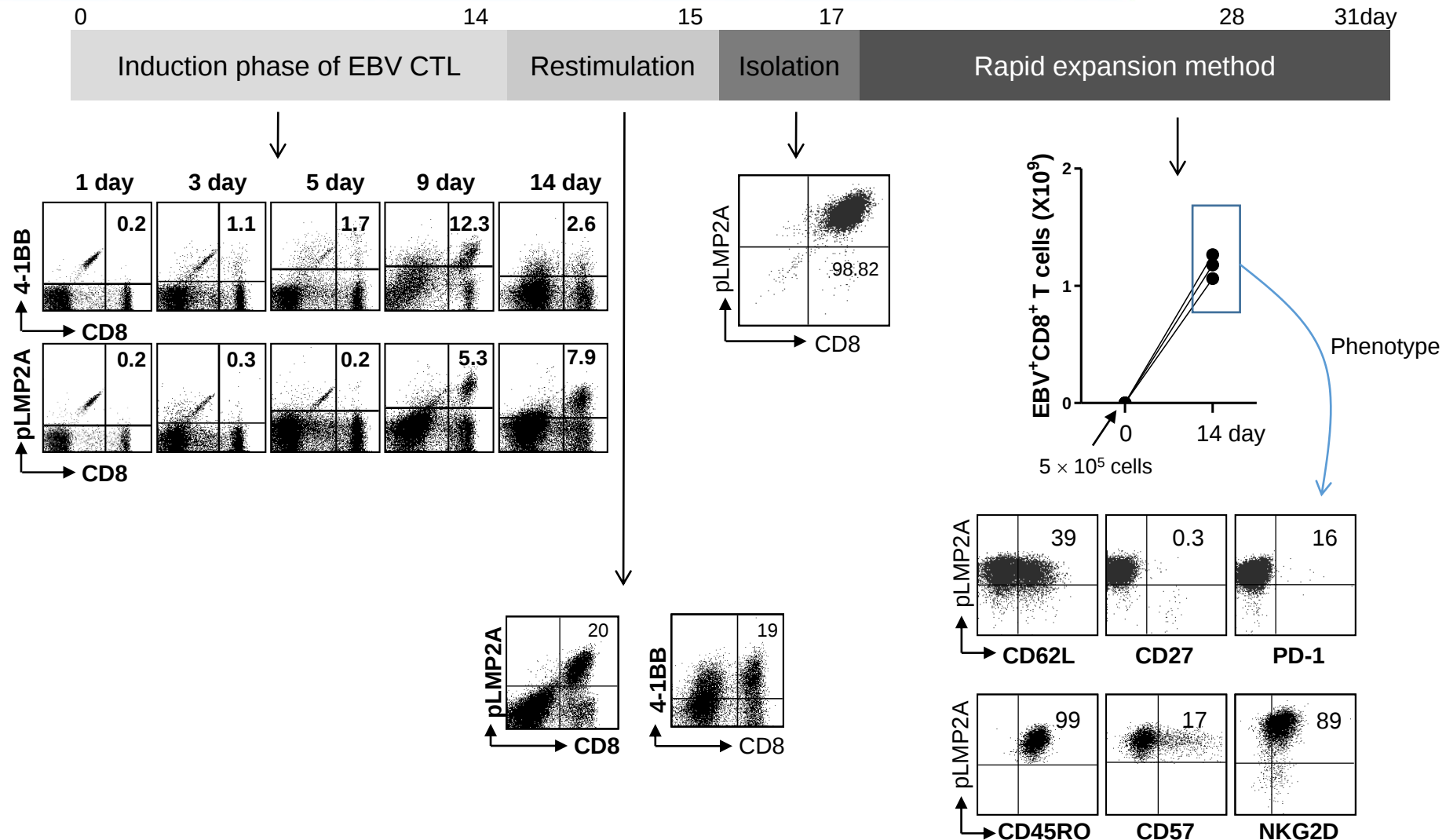
# 4-1BB expression on human CD8<sup>+</sup> T cells upon restimulation



# Target killing activity of 4-1BB-based CMV-specific CTLs



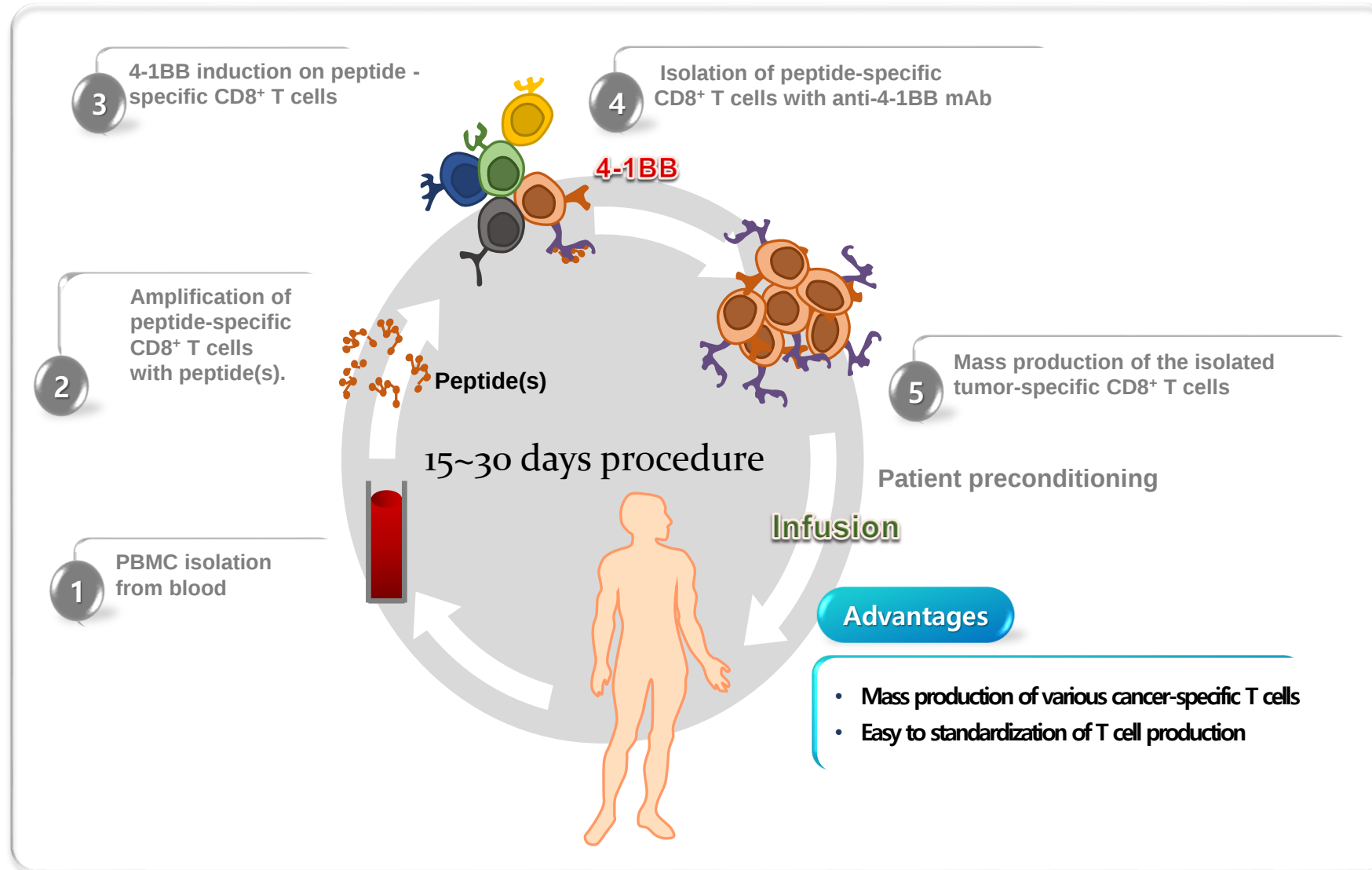
# Isolation and expansion of EBV LMP2A T cells



Isolation of antigen-specific CD8 T cells using 4-1BB

- **Name:** EBViNT Cell; EBV-induced Natural T Cell
- **Indications:** Standard therapy-failed EBV-positive Cancers; Lymphomas, Gastric Cancers, Nasopharyngeal Carcinomas

# Platform technology to produce anti-tumor CD8<sup>+</sup> T cells



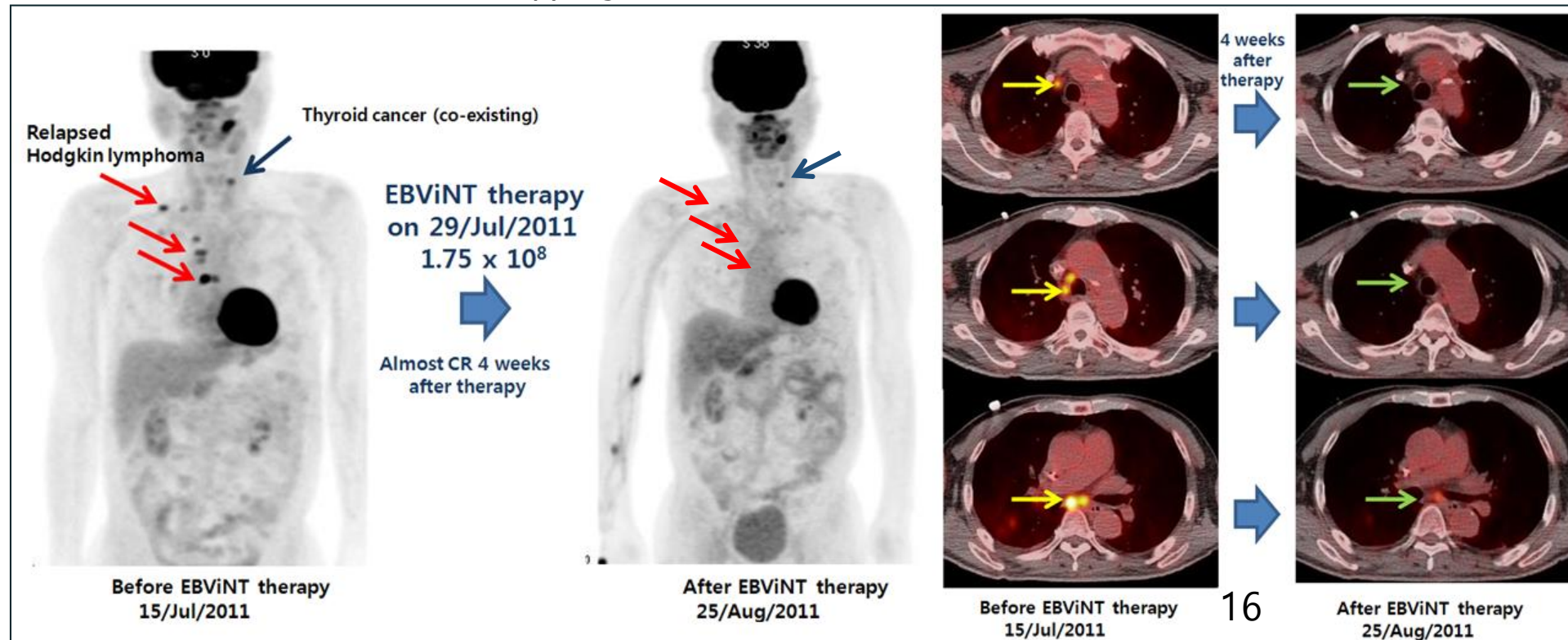
# EBViNT Cell Dose II (1/2 bag; $1.75 \times 10^8$ cells, NCC-02 HCS)

## NCC-02 HCS

- ✓ 62 year-old, male
- ✓ ECOG PS 1
- ✓ Hodgkin's disease
- ✓ Prior chemotherapy regimen: #2

## EBViNT Cells

- ✓ Lot # HCS-CT-110628
- ✓ Ag HLA-A\*24-restricted EBV/LMP2A



# EBViNT Cell Dose II (1 bag; $3.5 \times 10^8$ cells, NCC-03 KMS)

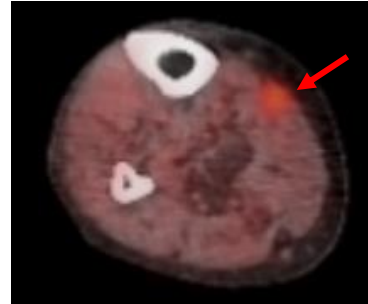
## NCC-03 KMS

- ✓ 63 year-old, female
- ✓ ECOG PS I
- ✓ Extranodal NK/T cell lymphoma
- ✓ nasal cavity: EBV-ISH negative
- ✓ Rt lower leg mass: EBV-ISH positive
- ✓ r/o renal abscess, r/o tumor
- ✓ Prior chemotherapy regimen: #1

## EBViNT Cells

- ✓ Lot # KMS-CT-110830
- ✓ Ag HLA-A\*24-restricted EBV/LMP2A

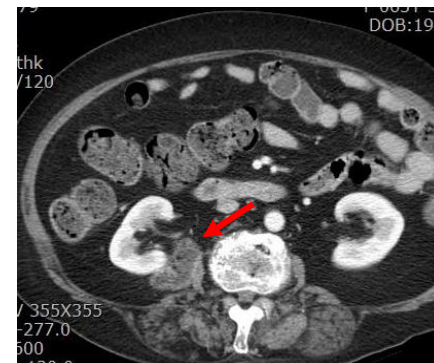
Rt lower leg lesion



4 weeks after  
EBViNT Therapy



6 months after  
EBViNT Therapy



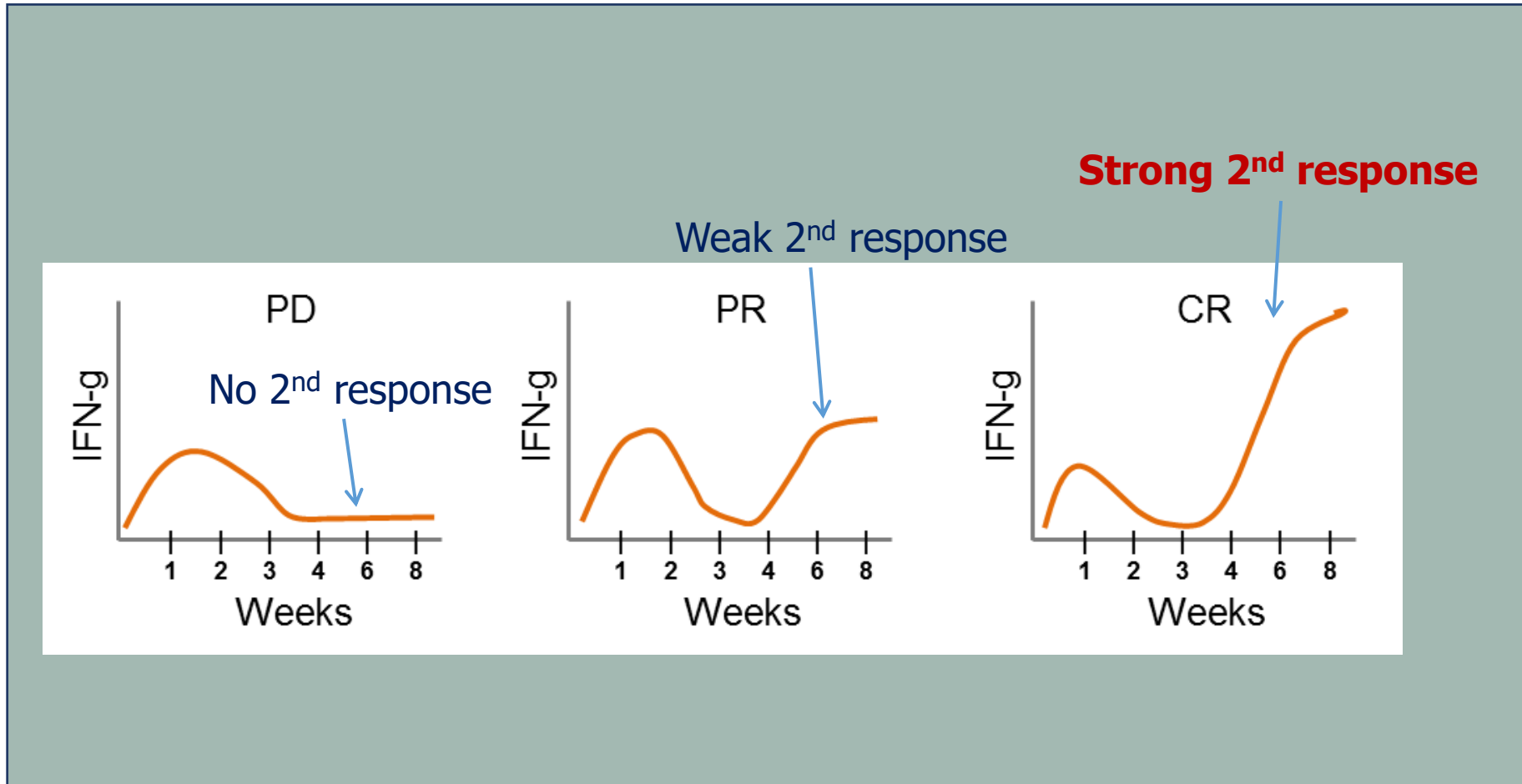
9 months after  
EBViNT Therapy



# Summary of EBViNT cell Therapy

|                  | Dose I<br>(1)       | Dose II<br>(2)             | Dose III<br>(3)   | Dose IV<br>(4)    | Dose IV<br>(5)    | Dose IV<br>(6)    | Dose IV<br>(7)    | Dose IV<br>(8)    |
|------------------|---------------------|----------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Cancer type      | NPC                 | HL                         | NKT               | NPC               | NPC               | HL/DLBCL          | NKT               | NPC               |
| Initial          | KHS                 | HCS                        | KMS               | SME               | PHA               | JBR               | HPS               | PJH               |
| Sex & age        | F/63                | M/61                       | F/63              | M/47              | M/39              | F/66              | F/69              | M/50              |
| Prior treatments | Chemo<br>× 4        | Chemo<br>Radio<br>Auto-BMT | Chemo<br>Radio    | Chemo<br>× 2      | Chemo<br>Radio    | Chemo<br>× 3      | Chemo             | Chemo             |
| Infused T cell # | $0.875 \times 10^8$ | $1.75 \times 10^8$         | $3.5 \times 10^8$ | $7.0 \times 10^8$ | $7.0 \times 10^8$ | $7.0 \times 10^8$ | $7.0 \times 10^8$ | $7.0 \times 10^8$ |
| Infusion date    | 2011.06.30          | 2011.07.28                 | 2011.09.30        | 2011.12.22        | 2013.07.11        | 2012.11.08        | 2012.12.28        | 2013.09.12        |
| Toxicity         | None                | None                       | None              | None              | None              | None              | None              | None              |
| Response         | PD                  | PR                         | CR                | PD                | PD                | PR                | CR                | PD                |

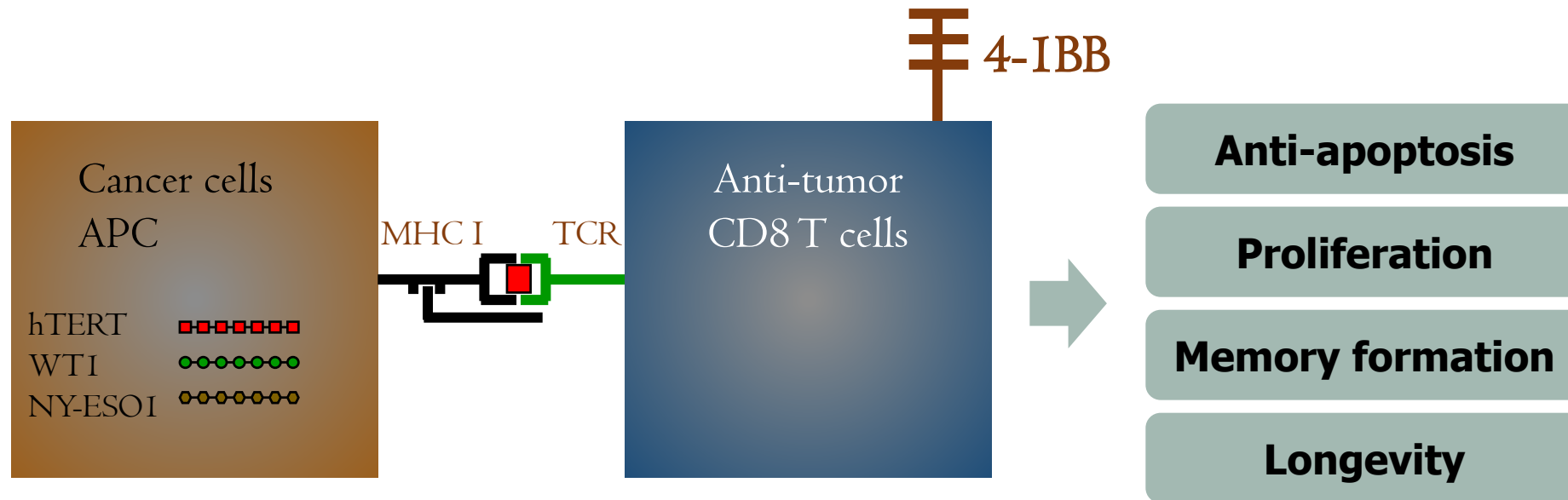
# Strong 2<sup>nd</sup> immune responses are found in the patient with complete regression



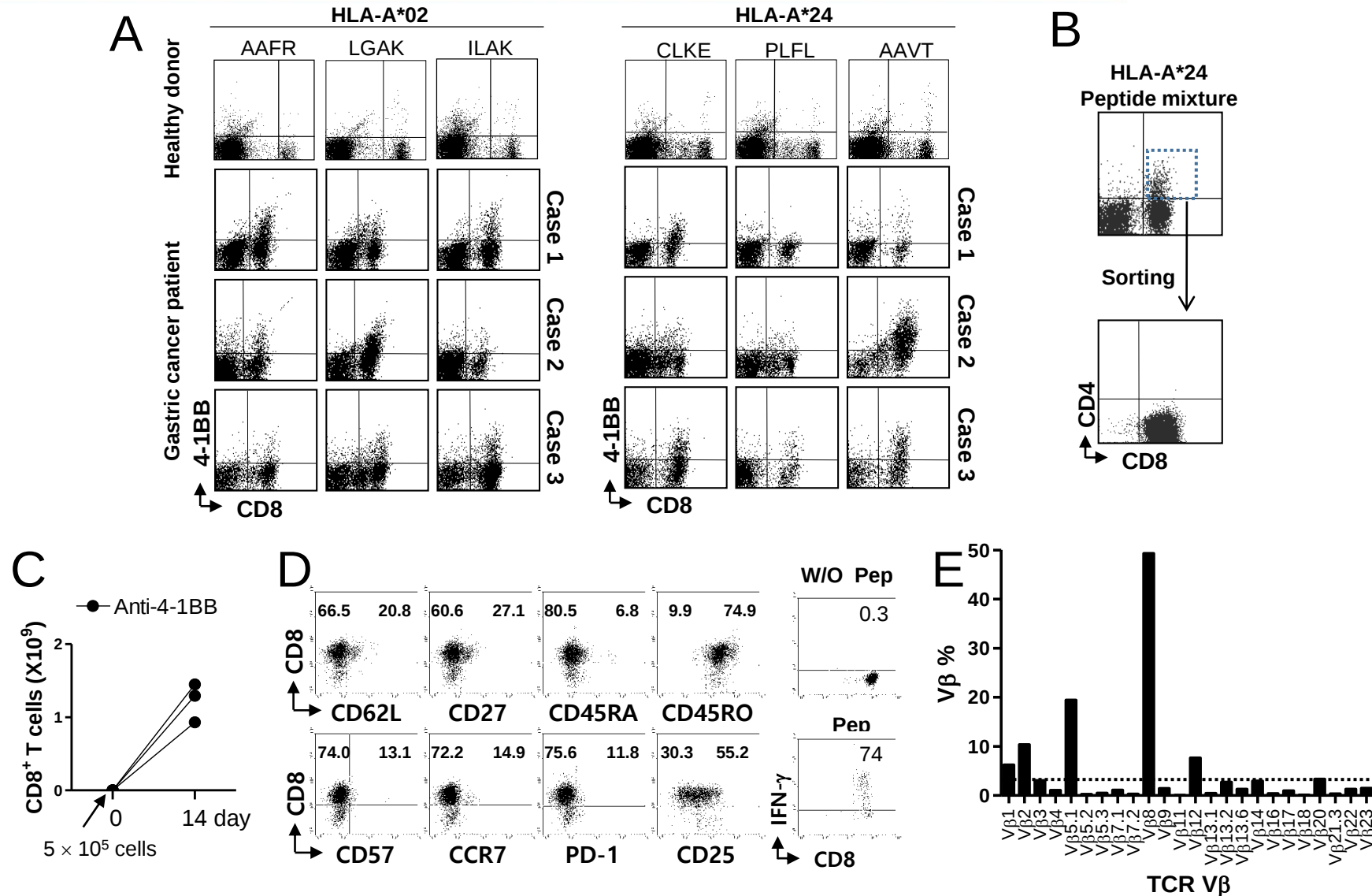
## Benefits

- **Complete durable regression can be induced**
- **No/minimum toxicity**
- **Epitope Spreading at a full dose of CTL**
- **High rate of manufacturing success (17/18)**

# 4-1BB-mediated Selection of Ag-specific T cells

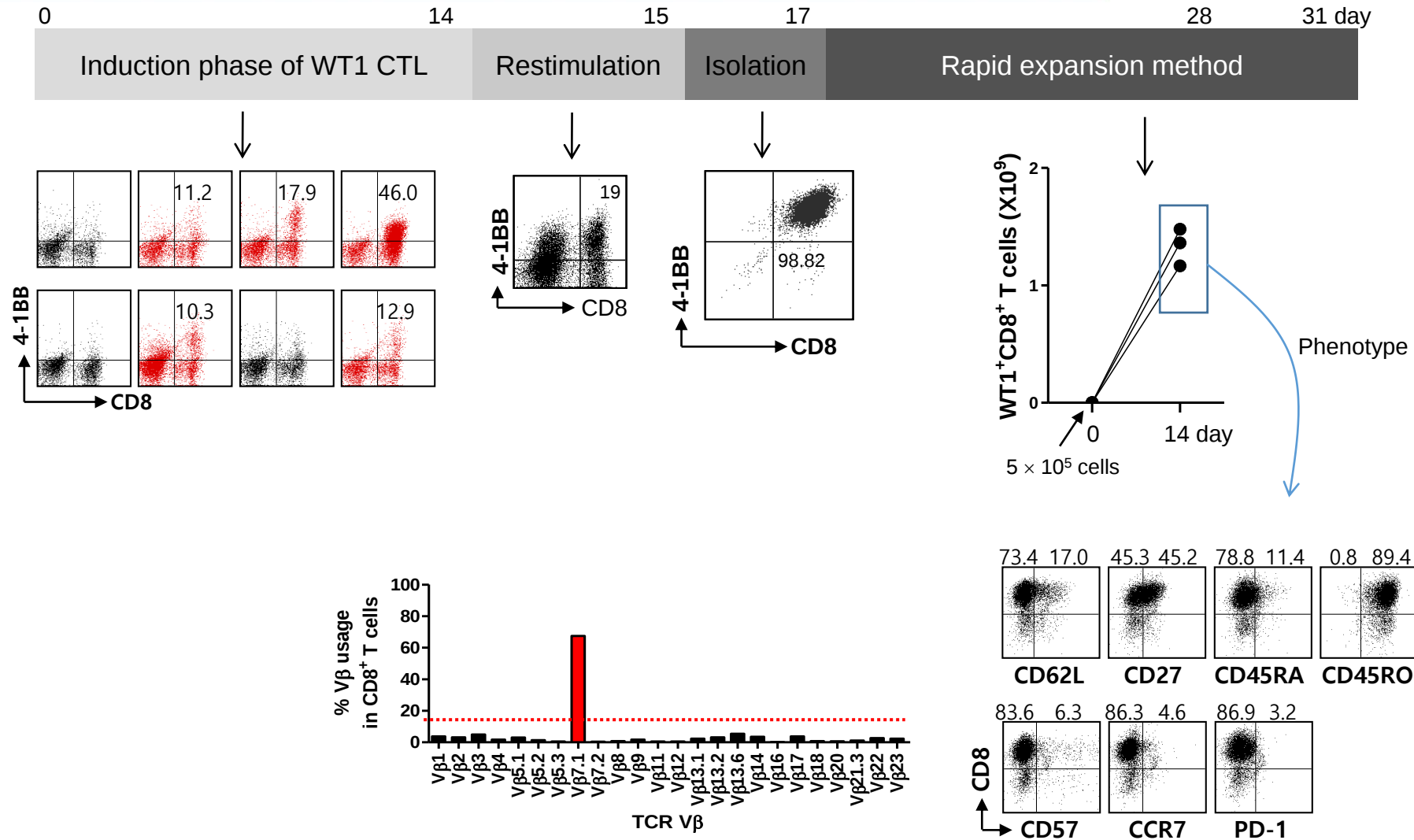


# Isolation and expansion of hTERT-specific CD8<sup>+</sup> T cells



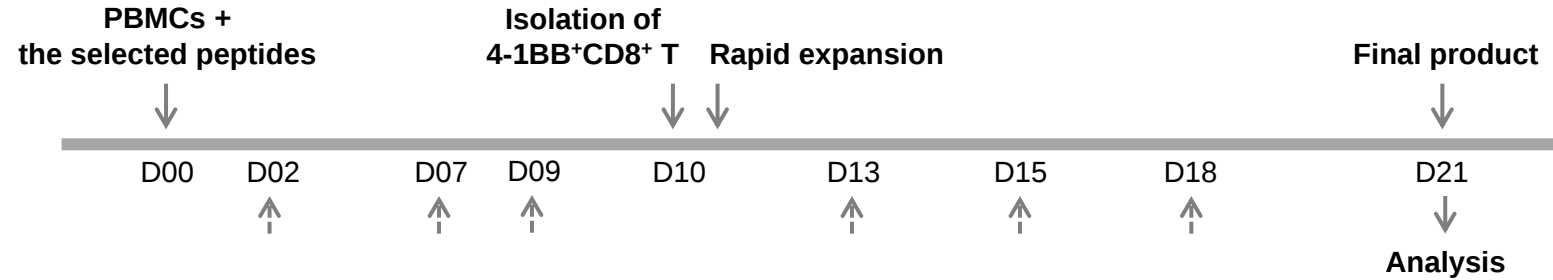
Isolation of self-tumor antigen-specific CD8 T cells

# Isolation and expansion of WT1-specific CD8<sup>+</sup> T cells

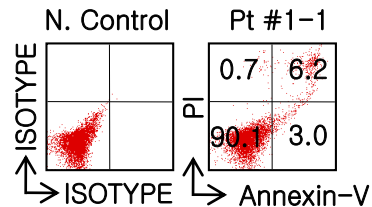


Isolation of antigen-specific CD8 T cells using 4-1BB

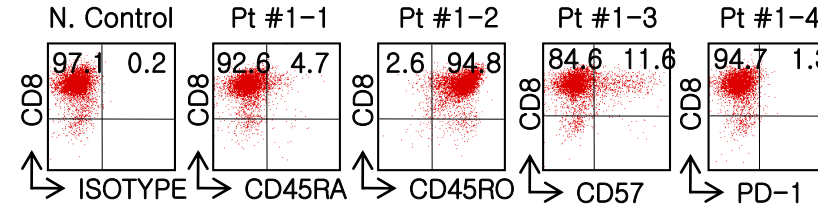
# Isolation and expansion of Neoantigen-specific CD8<sup>+</sup> T Cell



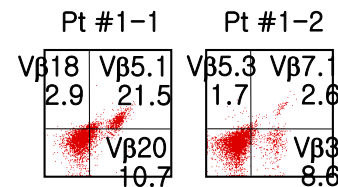
## A. Viability: 90.1%



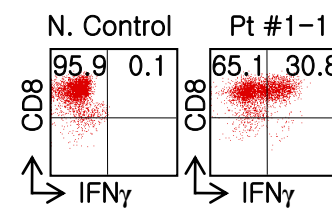
## B. Phenotypes: CD8<sup>+</sup>(97%)CD45RA<sup>-</sup>CD45RO<sup>+</sup>CD57<sup>-</sup>PD-1<sup>-</sup>



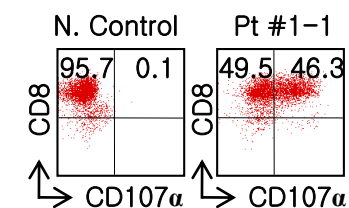
## C. Major Vβ TCR types: Vβ5.1 & Vβ20



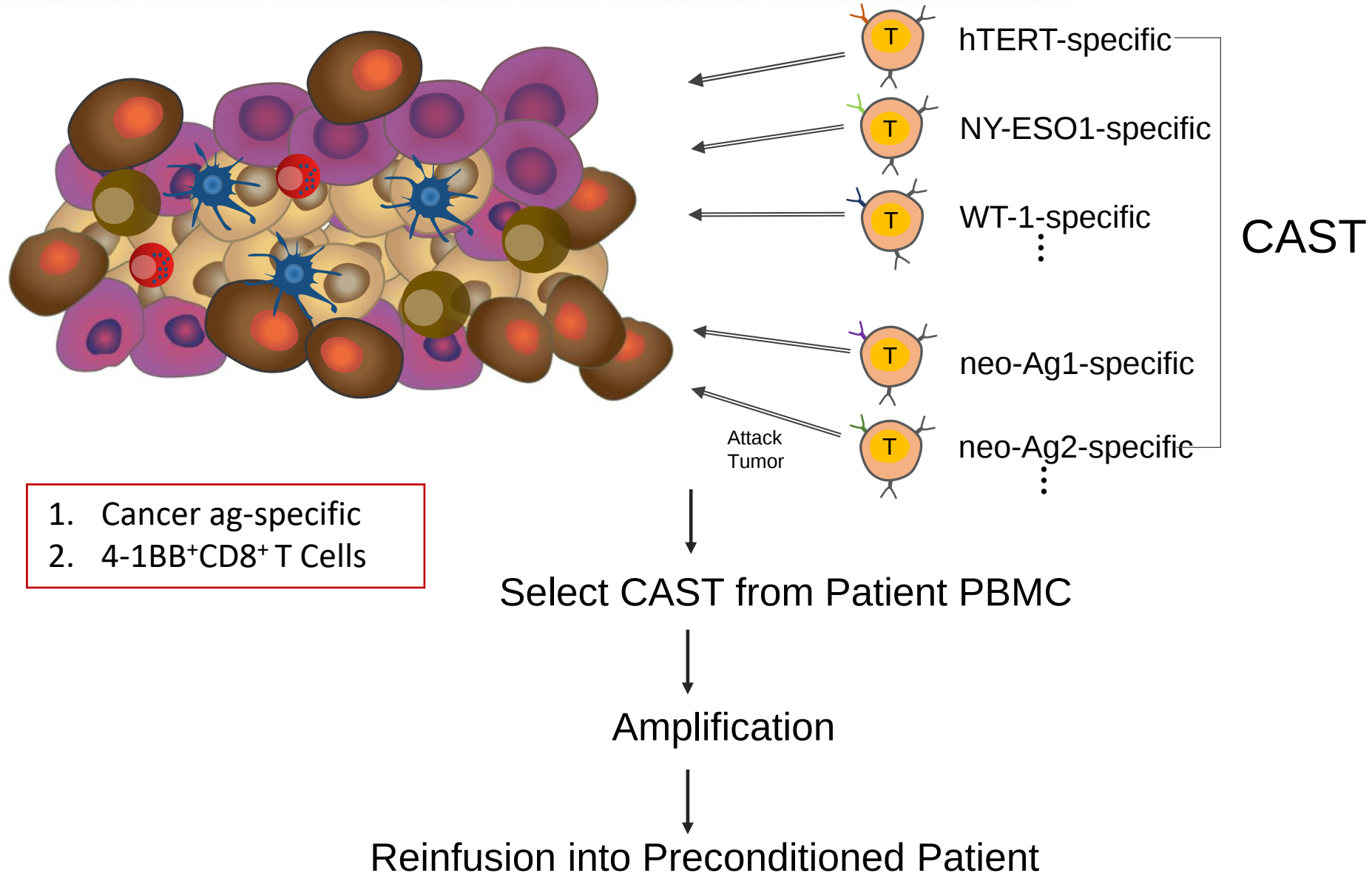
## D. IFN-γ production



## F. Cytotoxicity



# CAST (cancer antigen-specific T cell) Therapy



# CAST Therapy: NSCLC

➤ CYB, M/51

- Disease: **NSCLC**

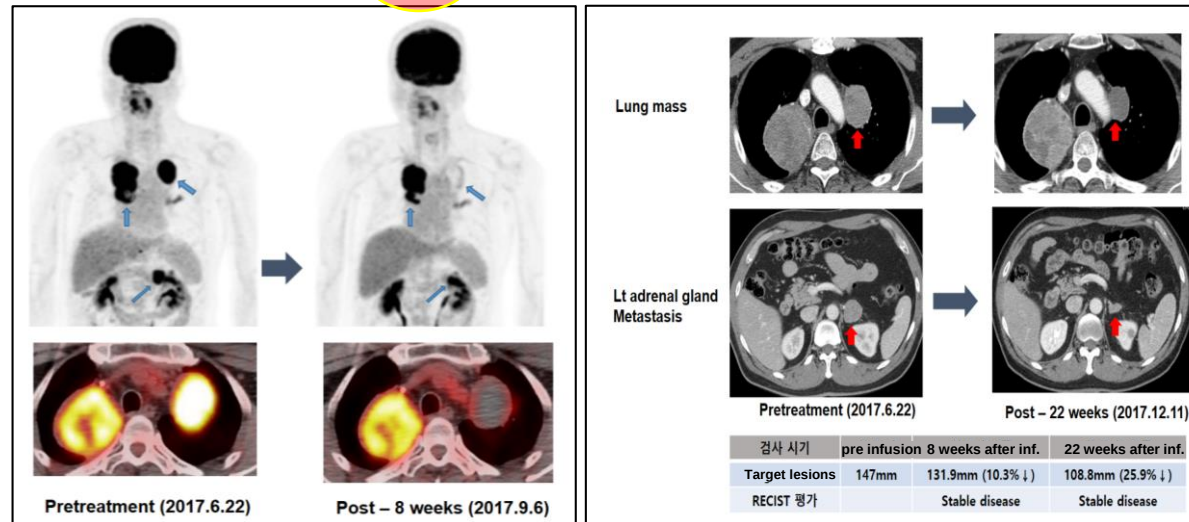
- Initial stage: IV

- Previous treatments: DP 1 LINE, IP→Icb 2 LINE, Alimta 3 LINE, TCb 4 LINE, GN 5 LINE

- Infused cell #:  $1.4 \times 10^9$  cells in 100ml bag as single infusion

- **Clinical response: tumor shrinkage 25.9% 22 weeks after infusion. Stable disease**

- **Adverse Drug Reactions: None**

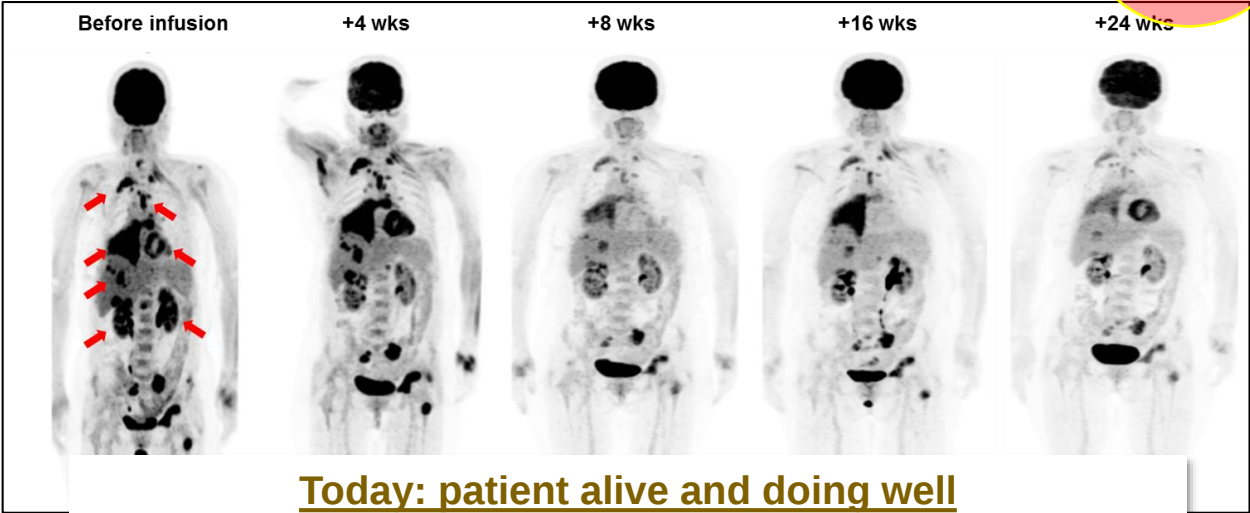


# CAST Therapy: Breast Cancer

| Patient no. | Gender/ age | Diagnosis     | Initial stage | Previous treatments | Involved sites before TERTiNT cell therapy |
|-------------|-------------|---------------|---------------|---------------------|--------------------------------------------|
| C714-08     | F/61        | Breast Cancer | IV            | Chemo + Radiation   | Lymph nodes, liver, bone                   |

B. Clinical response of TERTiNT Cell

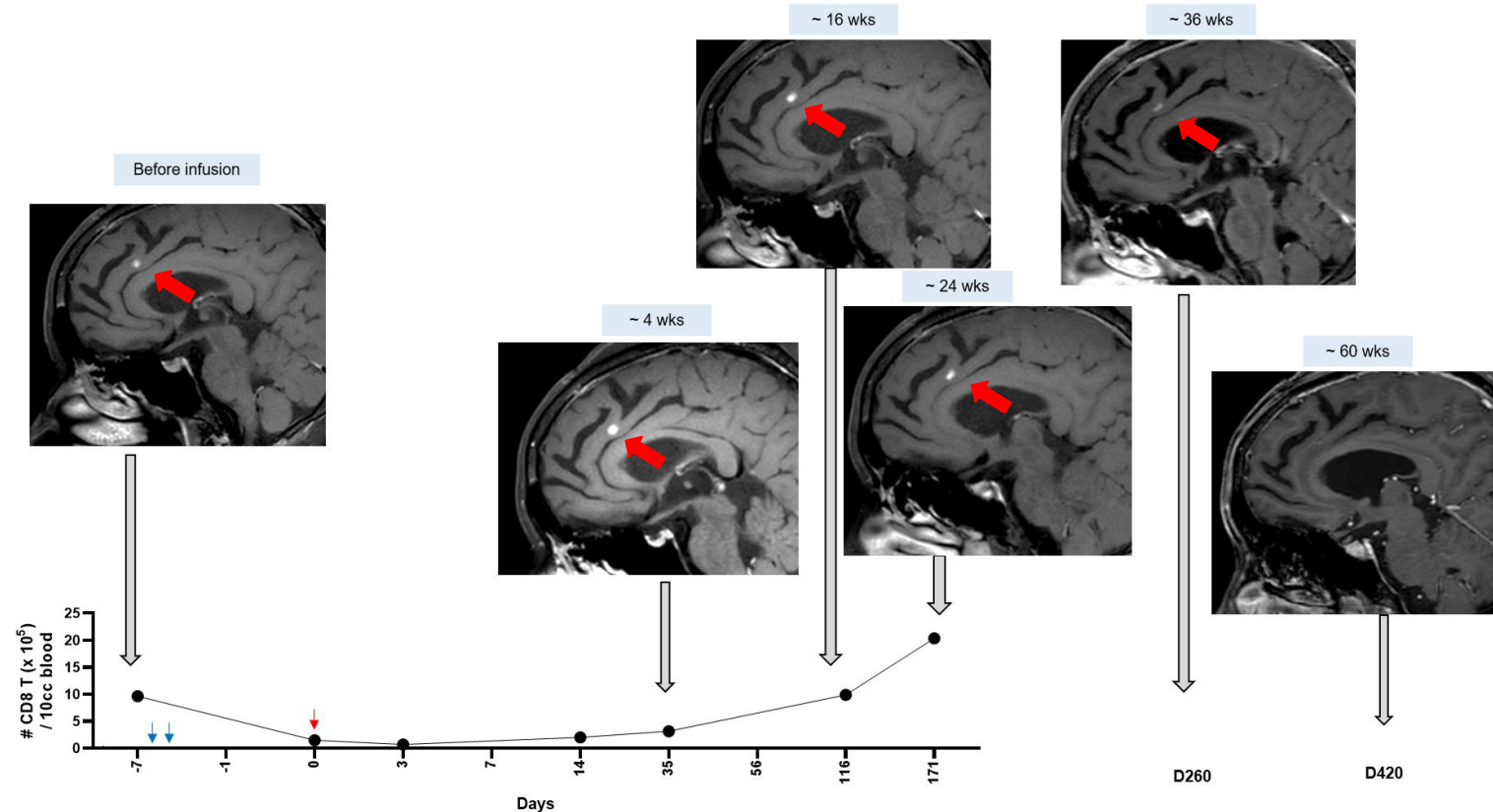
| Patient no. | Infused cell no. (x10 <sup>8</sup> /m <sup>2</sup> ) | Lympho depletion          | IL-2 | 4 wk | 8 wk | 16 wk | 24 wk | Last status | Toxicity (Any grade) |
|-------------|------------------------------------------------------|---------------------------|------|------|------|-------|-------|-------------|----------------------|
| C714-08     | 4.0                                                  | 1g/m <sup>2</sup> Cytosan | -    | SD   | SD   | SD    | SD    | Alive       | None                 |



Today: patient alive and doing well  
Transformation from terminal patient to treatable patient

# CAST Therapy: Anaplastic Oligodendroglioma

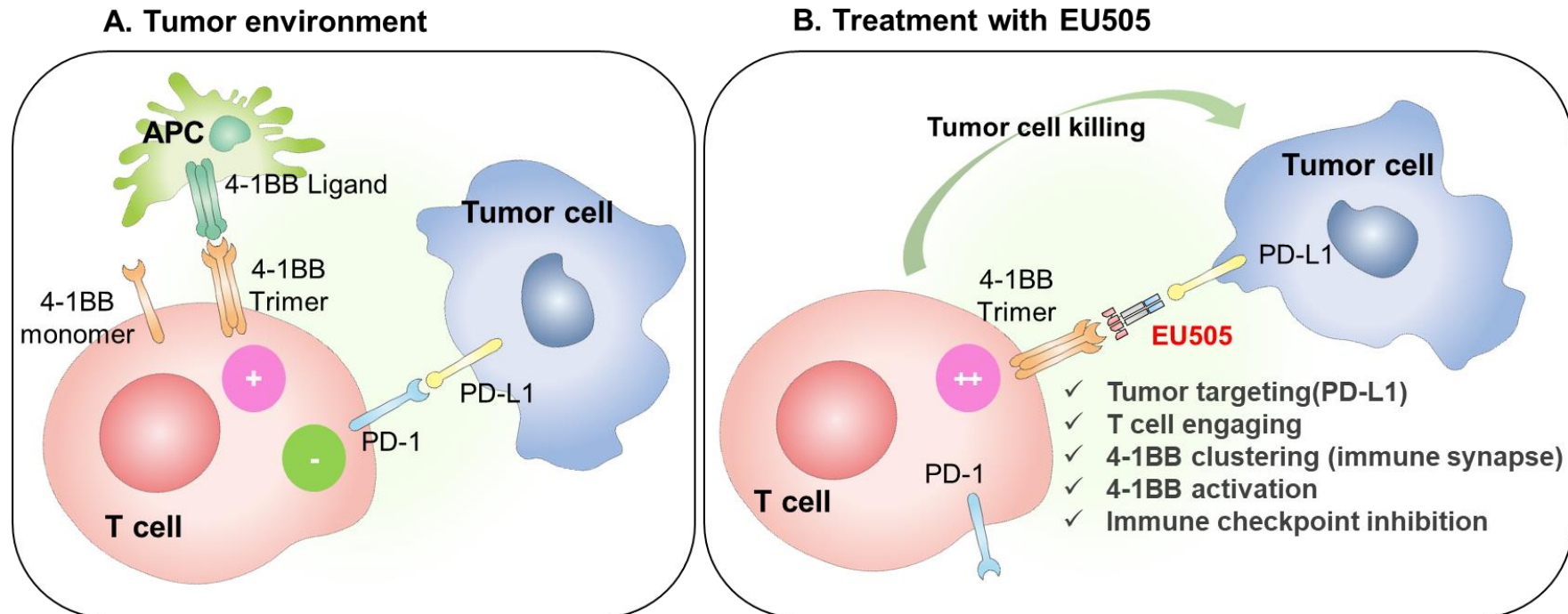
Previous treatment: Craniotomy → RT → Vincristine → Craniotomy



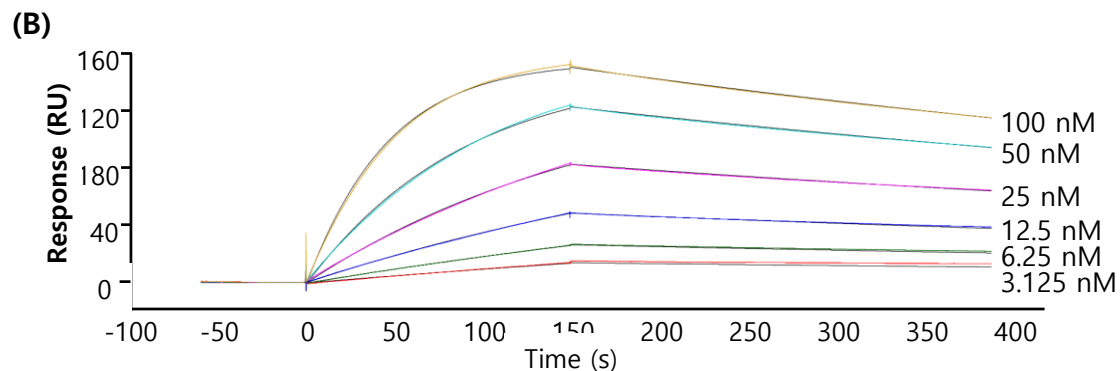
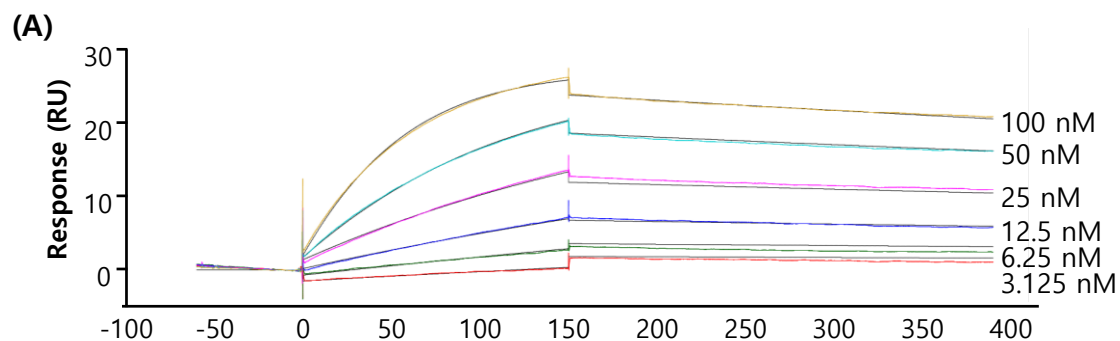
- Dose/Stage:  $4.0 \times 10^8/\text{m}^2$  (Stage IV- 3)
- Adverse Event: None
- Radiologic response : SD by 24 weeks, PR at 36 weeks, **CR at 60 weeks**

# Anti-4-1BB X PD-1ECD, bispecific anti-cancer therapeutics

EU505 stimulates 4-1BB and blocks PD-1



# EU505 binds to 4-1BB and PD-L1 at a high affinity



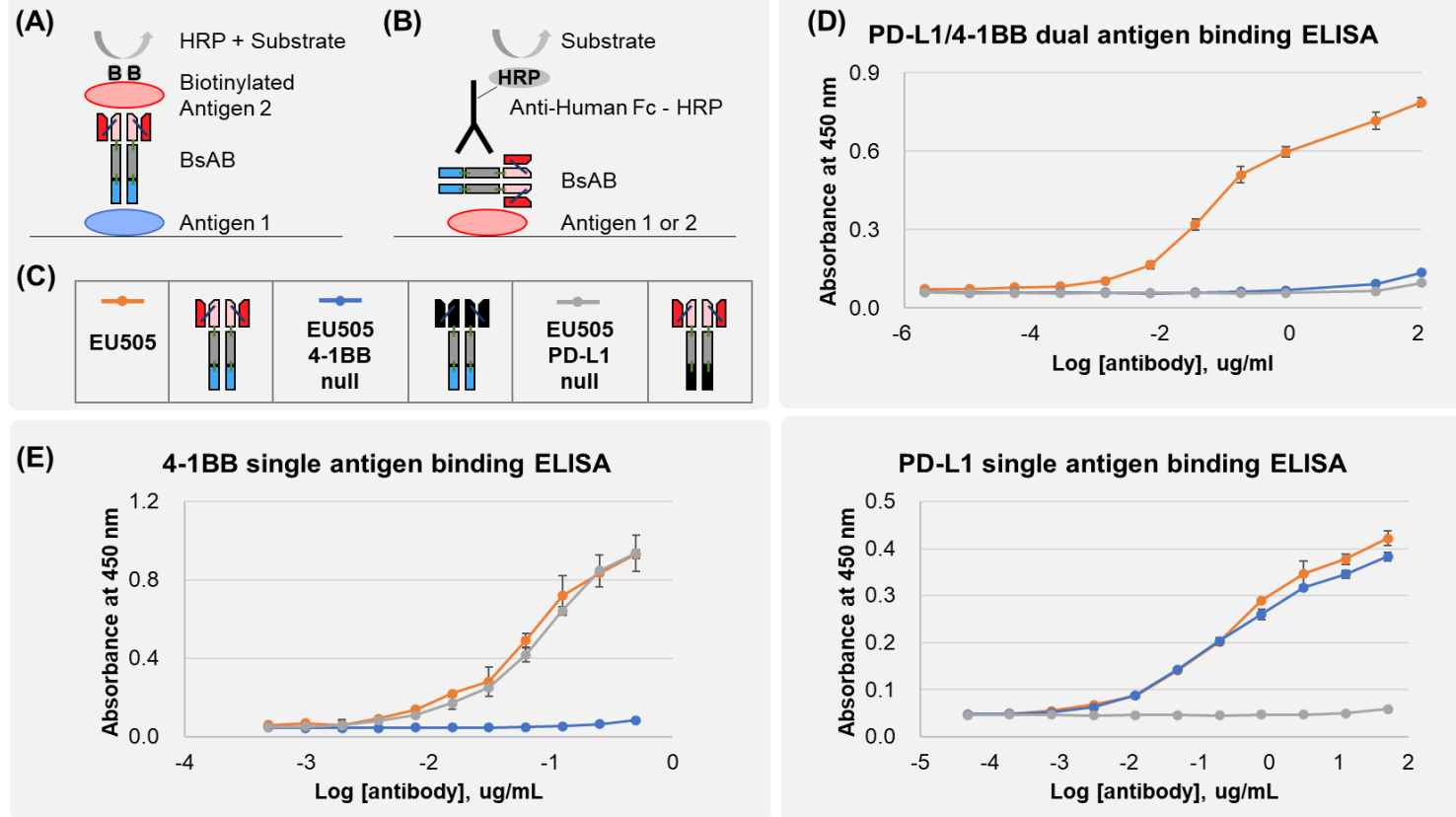
(C)

| PD-L1 binding |          |         |
|---------------|----------|---------|
| Ka (1/Ms)     | Kd (1/s) | KD (nM) |
| 2.27E+05      | 1.15E-03 | 5.07    |
| 4-1BB Binding |          |         |
| Ka (1/Ms)     | Kd (1/s) | KD (nM) |
| 2.08E+05      | 6.34E-04 | 3.05    |

# Single and Dual binding of EU505

## Dose-dependent bindings of EU505 to 4-1BB and PD-L1

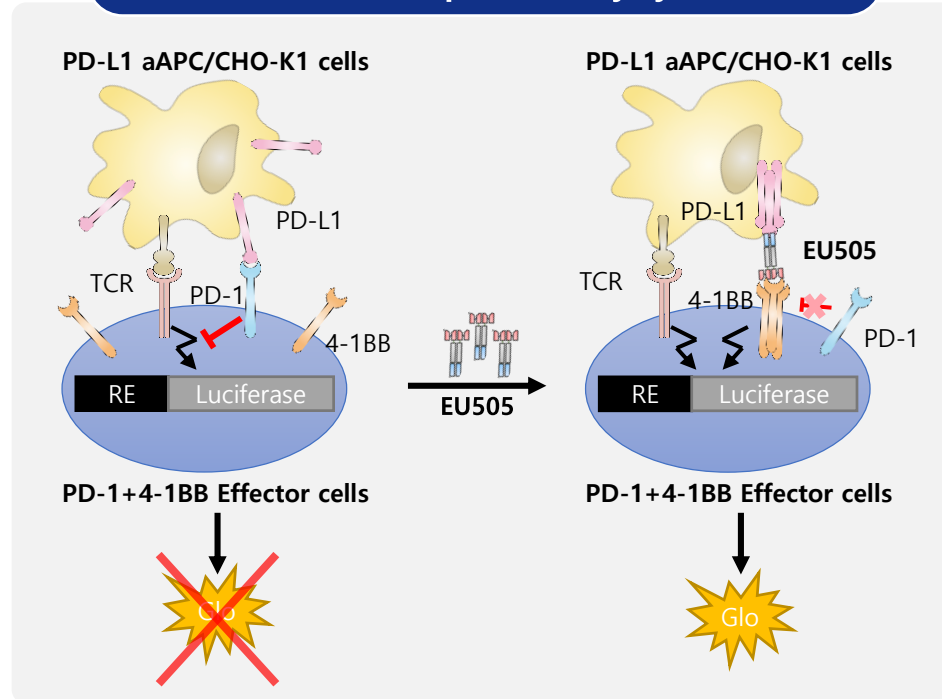
### Dual antigen binding ELISA and single antigen binding ELISA



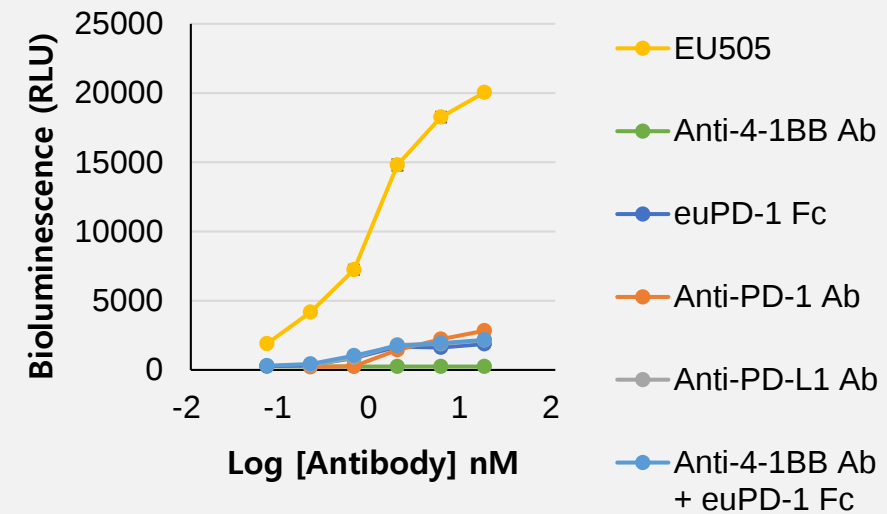
# Synergistic effect of 4-1BB activation and PD-L1 Blockade

EU505 showed a synergistic effect of stimulating 4-1BB and blocking PD-L1

## 4-1BB/PD-1 reporter assay system

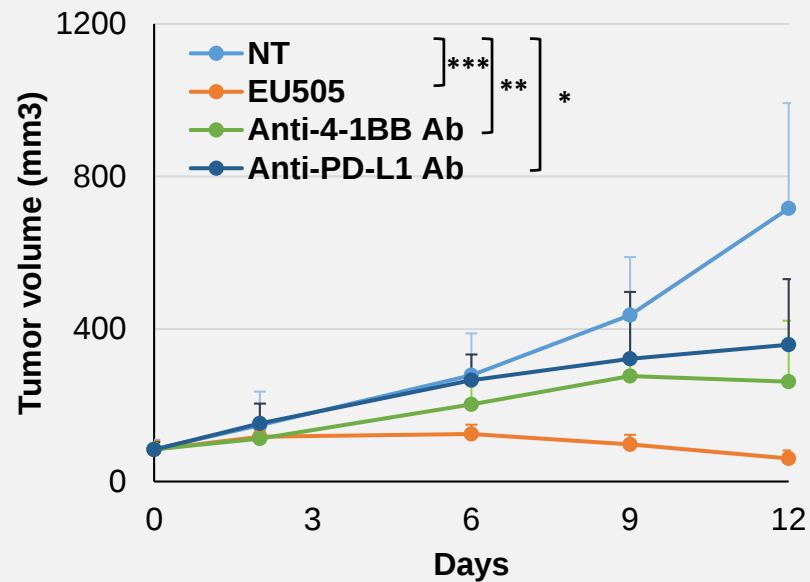


## PD-1/PD-L1 blockade effect and 4-1BB-agonist activity



# Anti-cancer activities in human 4-1BB KI mice

EU505 showed anti-cancer effects and no signs of liver toxicity



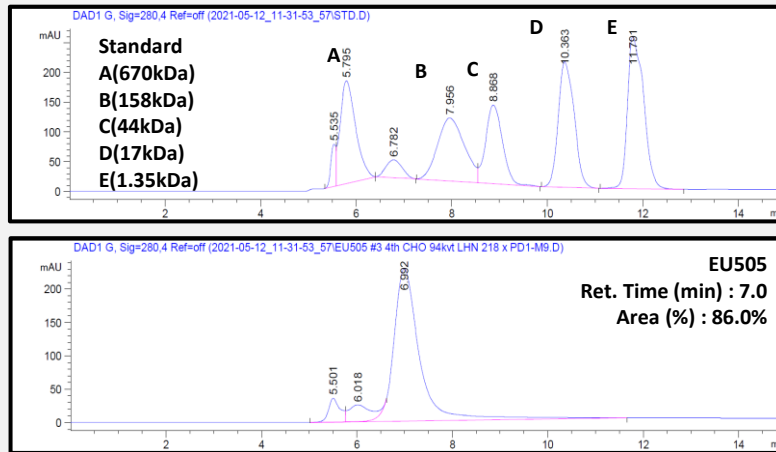
Inhibit tumor growth

|               | ALT (U/L)    | AST (U/L)     | BUN (mg/dL)  |
|---------------|--------------|---------------|--------------|
| Normal range  | 17 - 77      | 54 - 298      | 8 - 33       |
| NT            | 26.27 ± 1.67 | 97.40 ± 31.32 | 24.61 ± 1.60 |
| EU505         | 40.67 ± 8.00 | 59.00 ± 6.63  | 23.21 ± 1.70 |
| Anti-4-1BB Ab | 34.67 ± 6.93 | 69.50 ± 13.40 | 28.72 ± 2.51 |
| Anti-PD-L1 Ab | 28.37 ± 9.24 | 69.50 ± 19.42 | 31.27 ± 8.56 |

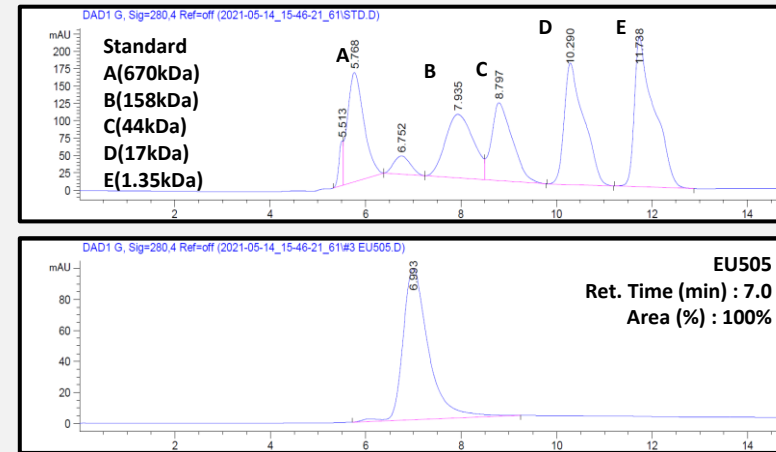
No liver toxicity

✓ 100% purity by two column chromatography

1<sup>st</sup> purification: Protein A column



2<sup>nd</sup> purification: Size exclusion



1. Discovery, signaling and immuno-biology of 4-IBB were reviewed.
2. EBV-specific 4-IBBCTLs showed a therapeutic effect against NK/T, Hodgkin and non-Hodgkin lymphomas.
3. Self/tumor Ag-specific 4-IBBCTL showed a therapeutic effect against lung, brain and breast cancers.
4. Anti-4-IBB X PD-IECD may produce synergistic activities of 4-IBB activation and PD-LI blockade against cancers.

# Acknowledgement

## ◎ Eutilex

- Team Antibody Therapy
- Team Bispecific Antibody
- Team antibody engineering
- Team CMC



## ◎ Samsung Biologics

## ◎ National Cancer Center

- Research Institute



## ◎ National Cancer Center Korea Onco-Innovation Unit (NOIU)