uBrigene *in vivo* CAR T platform (uVivo CAR LVV）

A novel Cocal-G LVV based *in-vivo* CART

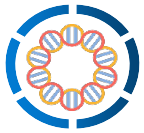
uBrigene Global Footprint & Infrastructure

Established in 2015, uBriGene is committed to advancing and implementing cutting-edge CGT technologies and offering comprehensive solutions for the CGT industry.



uBriGene's Global Manufacturing Platforms





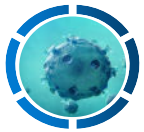
Plasmids

- Plasmid construction
- Strain banking
- Plasmid PD & manufacturing



Cell therapy

- CAR-T
- CAR-NK
- MSC, iPSC, cell banking



Viral Vectors

- Adenovirus
- AAV
- LVV
- OV



Gene Editing

- IVT-sgRNA
- Nuclease
- RNP PD



RNA-LNP

- mRNA
- circRNA, saRNA
- sgRNA



QC release testing

- Purity
- Safety
- Identity
- Potency

Representative Gene Therapy Products

No.	AAV Serotype	Indication	Route of Administration	IND Approval Date	Country	Clinical Stage
1	AAV2	Treatment of inherited retinal dystrophy (IRD) associated with biallelic RPE65 mutations	Subretinal injection	Apr 2022	China	Phase III
2	AAV8	Hemophilia caused by congenital coagulation factor IX deficiency	Intravenous injection	Apr 2022	China	Phase I
3	AAV9	Crystalline retinopathy	Subretinal cavity injection	Nov 2022	China	Phase III
4	AAV9	Amyotrophic lateral sclerosis (ALS)	Intrathecal injection	May 2024	China	Phase I
5	AAV9	Amyotrophic lateral sclerosis (ALS)	Intrathecal injection	May 2025	USA	Phase I
6	AAV2	Treatment of neovascular (wet) age-related macular degeneration (AMD); Diabetic macular edema	Subretinal injection	Aug 2024	China	Phase I
7	AAV8	Neovascular (wet) age-related macular degeneration (nAMD)	Intravitreal injection	Nov 2024	USA	Phase I
8	AAV2	Neovascular (wet) age-related macular degeneration (nAMD)	Intravitreal injection	Oct 2024	China	Phase I
9	AAV2	Neovascular (wet) age-related macular degeneration (nAMD)	Intravitreal injection	Jul 2024	USA	Phase I
10	AAV2	X-linked retinoschisis	Intravitreal injection	Dec 2024	USA	Phase I
11	AAV8	Wilson’s Disease	Intravenous injection	Nov 2025	USA	Phase I

Representative Cell Therapy Products

No.	Product Type	Indication	Route of Administration	IND Approval Date	Country	Clinical Stage
1	Drug-device combination product	Acute-on-chronic liver failure caused by hepatitis B virus, etc.	Extracorporeal dialysis	Nov 2022	China	Phase I/II
2	TIL	Advanced solid tumors	Intravenous infusion	Apr 2024	China	Phase 1
3	CAR-T	CD30-positive tumors (e.g., Hodgkin lymphoma, anaplastic large cell lymphoma)	Intravenous infusion	Jul 2024	China	Phase I
4	CAR-T	CD30-positive tumors (e.g., Hodgkin lymphoma, anaplastic large cell lymphoma)	Intravenous infusion	Aug 2024	USA	Phase 1
5	CAR-T	Waldenstrom macroglobulinemia	Intravenous infusion	2024	USA	Phase 1/II
6	CAR-T/HSV	Glioblastoma	Intratumor injection	2024	USA	IND
7	TIL	Solid tumor	Intravenous injection	Dec 2025	USA	Phase I

Representative CGT Products for IIT

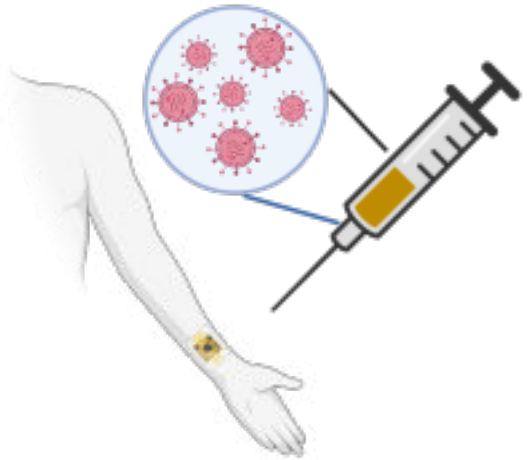
No.	Product Type	Indication	Route of Administration	Date of Approvement by Ethic Committee
1	AAV2	Inherited retinal dystrophy (IRD) associated with biallelic RPE65 mutations	Subretinal injection	Jun 2021
2	AAV9	Crystalline retinopathy (BCD)	Subretinal cavity injection	Apr 2022
3	AAV9	Glutaric acidemia type I	Lateral ventricle injection	2022
4	AAV8	Hemophilia caused by congenital coagulation factor IX deficiency	Intravenous injection	Jun 2022
5	AAV9	Amyotrophic lateral sclerosis (ALS)	Intrathecal injection	2023
6	HSC	X-linked severe combined immunodeficiency	Intravenous infusion	Oct 2023
7	Oncolytic virus	Advanced malignant solid tumors	Intravenous injection	Dec 2023
8	AAV9	Type 2 SMA (late-onset, ambulatory impairment)	Intrathecal injection	Sep 2024
9	AAV9	Rett syndrome	-	2024
10	AAV2	Inherited retinal disease associated with RDH12 gene variants	Intravitreal injection	Sep 2024
11	AAV2	X-linked retinoschisis	Intravitreal injection	2024

Representative CGT Products for IIT

No.	Product Type	Indication	Route of Administration	Date of Approvement by Ethic Committee
12	AAV8	Wilson's disease	Intravenous injection	Sep 2024
13	AAV9	Duchenne muscular dystrophy (DMD)	Intravenous injection	2024
14	AAV9	Duchenne muscular dystrophy	Intravenous injection	Sep 2024
15	AAV9	Post-spinal cord injury pain	Intracranial injection	Jun 2025
16	AAV2	Primary open-angle glaucoma	Anterior chamber injection	Mar 2025
17	AAV6	HER2-positive breast cancer with brain metastasis	-	Jun 2025
18	AAV9	Brain disorders	-	-
19	Oncolytic virus	Advanced malignant solid tumors	Intravenous injection	May 2025 (Australia)
20	AAV9	Refractory temporal lobe epilepsy (TLE) and frontal lobe epilepsy	Intracranial injection	Not started
21	Dual AAV8	Wet age-related macular degeneration (nAMD)	Subretinal injection	Not started

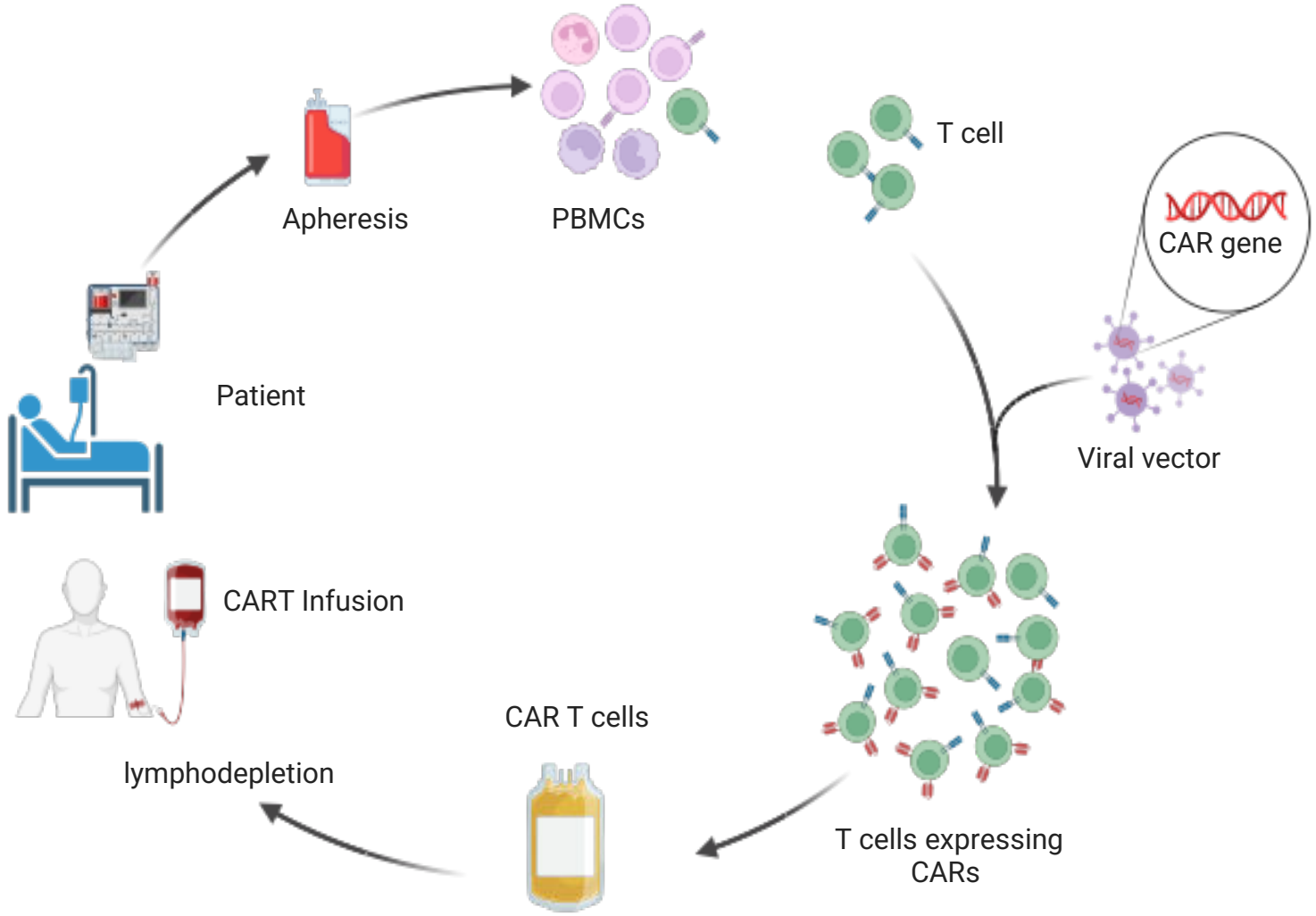
Ex vivo versus *in vivo* CAR Therapy

In vivo production of CAR T cells



- No apheresis
- No waiting time
- No lymphodepletion
- Off the shelf
- Cost effective

Ex-vivo production of CAR T cells



Major Considerations on Lentivirus for in vivo CAR

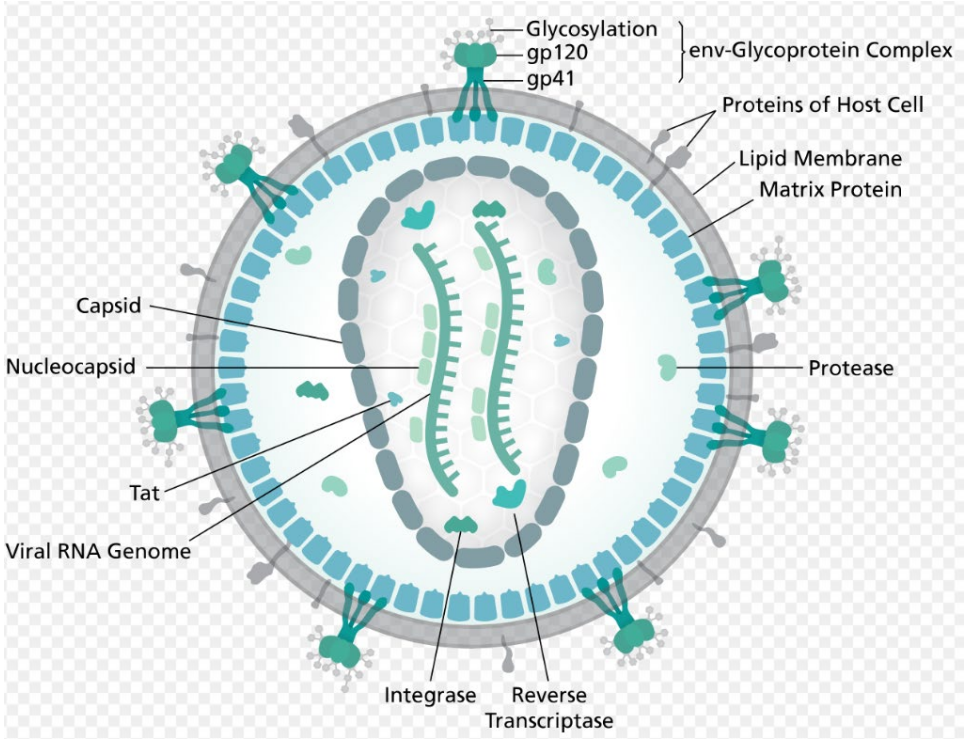
Safety

- Insertional mutagenesis
- RCL
- Immunogenicity
- Transgene expression control
- Biodistribution
- Off-target effects
  - ❖ Genomic disruption: Permanent integration into the host genome, especially into germline cells leading to inheritable genetic modification

Potency

- Host immune rejection on lentivirus
- Target cell specific transduction
- Potential epitope masking
  - ❖ Non-targeting cells are transduced by lentivirus
    - ○ Tumor cells
    - ○ Other off-targeting cell transductions
  - ❖ CAR expression on Lentivirus envelope
- Ratio of defective lentivirus

Lentivirus and its Envelope Protein



https://en.wikipedia.org/wiki/HIV

HIV-based: The core vector is built on the HIV backbone

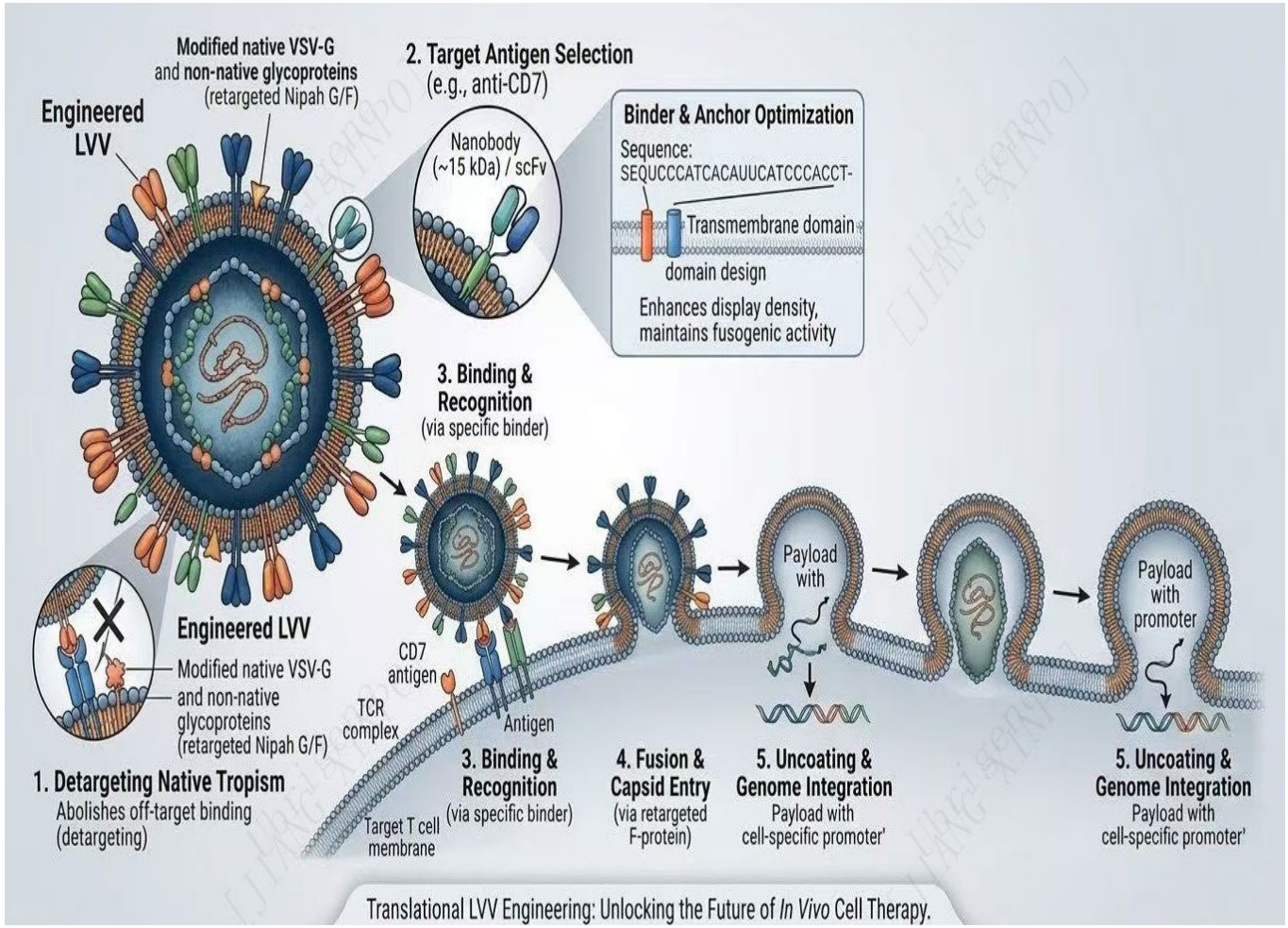
- Two copies of the (positive sense) ssRNA genome
- The enzymes: reverse transcriptase, integrase and protease
- Some minor proteins, and the major core protein

Replication-deficient: Splitting viral genome across multiple plasmids prevent the virus from replicating in the target cell

VSV-G pseudotyped: Replaced the HIV-1 Env glycoprotein with the envelope glycoprotein G of the vesicular stomatitis virus

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Envelope and Vector Design Strategies for *in vivo* LVV



Envelope glycoprotein engineering

- Detargeting native tropism to eliminate off-target transduction
- Optimization of membrane-anchoring motif and fusogenic domains

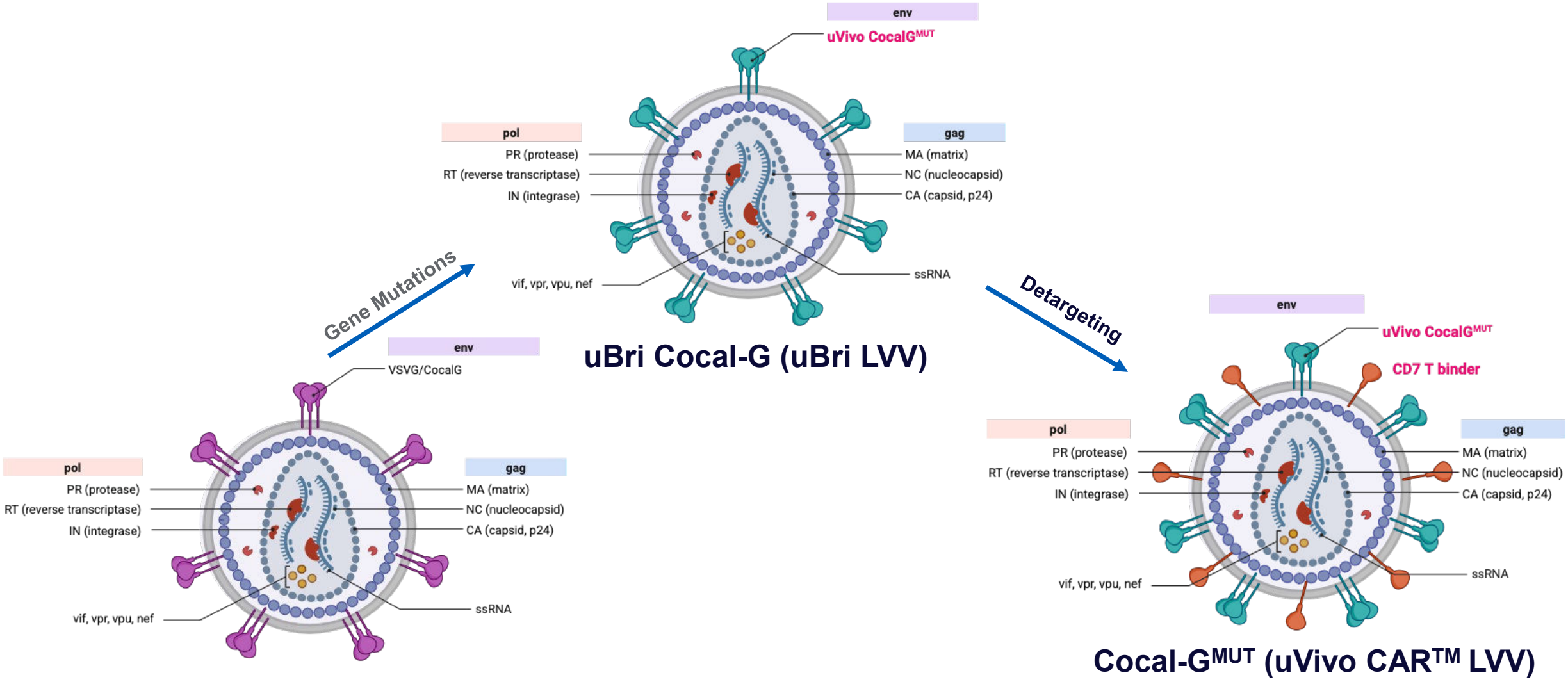
Targeting binder selection and expression

- Surface expression of high-affinity binders (ScFv, or SdAb nanobody, etc.) tailored for targeting cells
- Maintaining efficient viral infection kinetics in vivo

Vector sequence and genome optimization

- Codon-optimized payload and promotor selection for potent and safe in vivo transduction

Extensive LVV Engineering for Higher Transduction Efficicency or *in vivo* Transduction Specificity



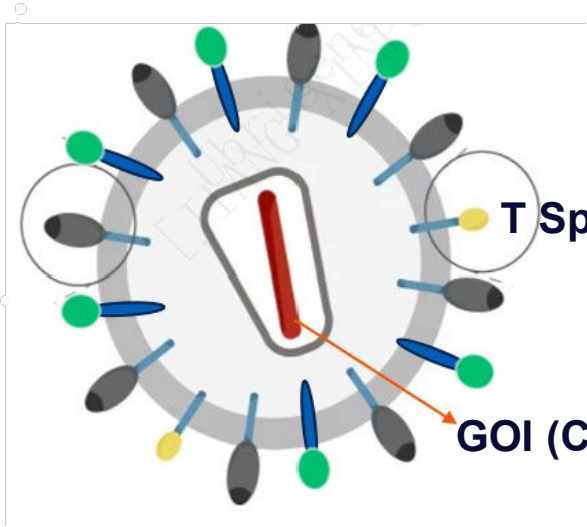
uBri Cocal-G (uBri LVV)

Wild Type Cocal G

Cocal-G^{MUT} (uVivo CAR^{TM} LVV)

uBrigene LVV Platform for in vivo CAR (uVivo CAR™ LVV)

Fusogen: Detargeted engineered uBri Cocal-G^{MUT}



uBri Cocal-G^{MUT}（Patent 2026102314491 pending)

- More resistance to serum inactivation comparing to VSV-G
- Re-engineered from uBrigene engineered high titer Cocal-G (uBri Cocal-G, Patent 2025115114075)
- uBri Cocal-G^{MUT} to abolish its native tropism while keeping its fusogenic capability

Patented membrane anchoring motif

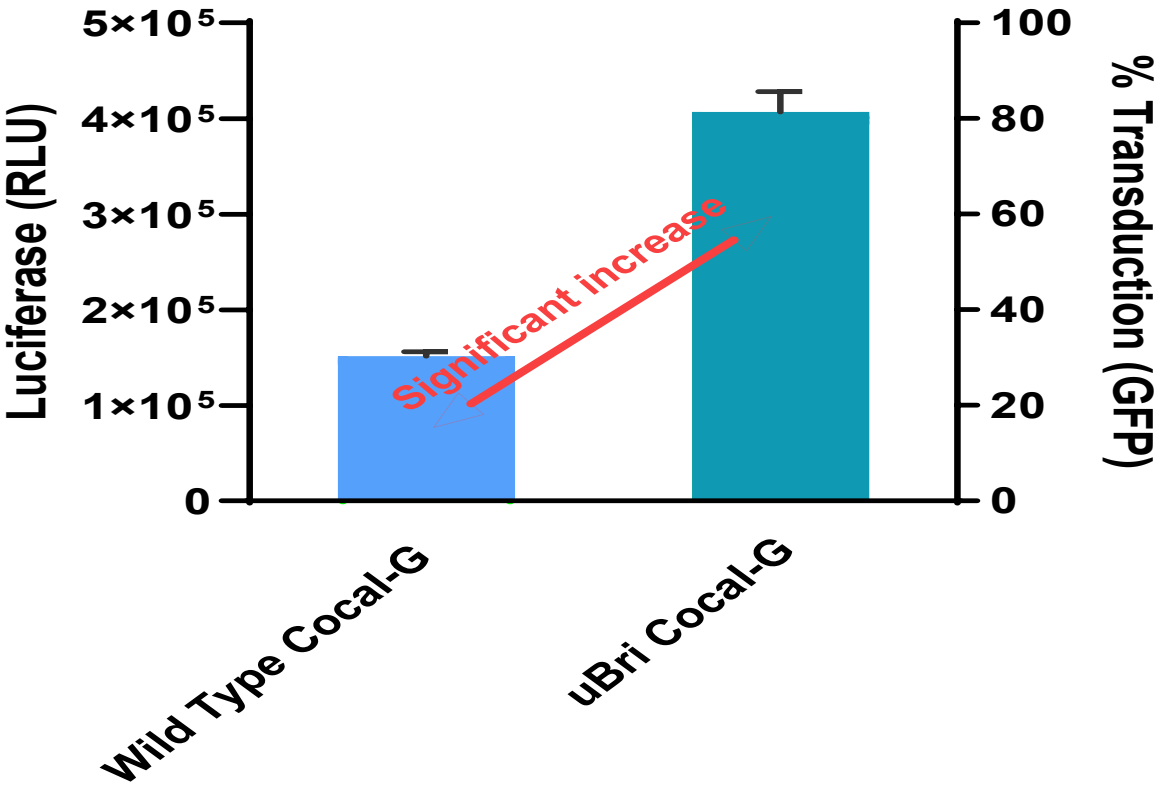
- Maximize fusion with T cells (Patent 2026102314491 pending)

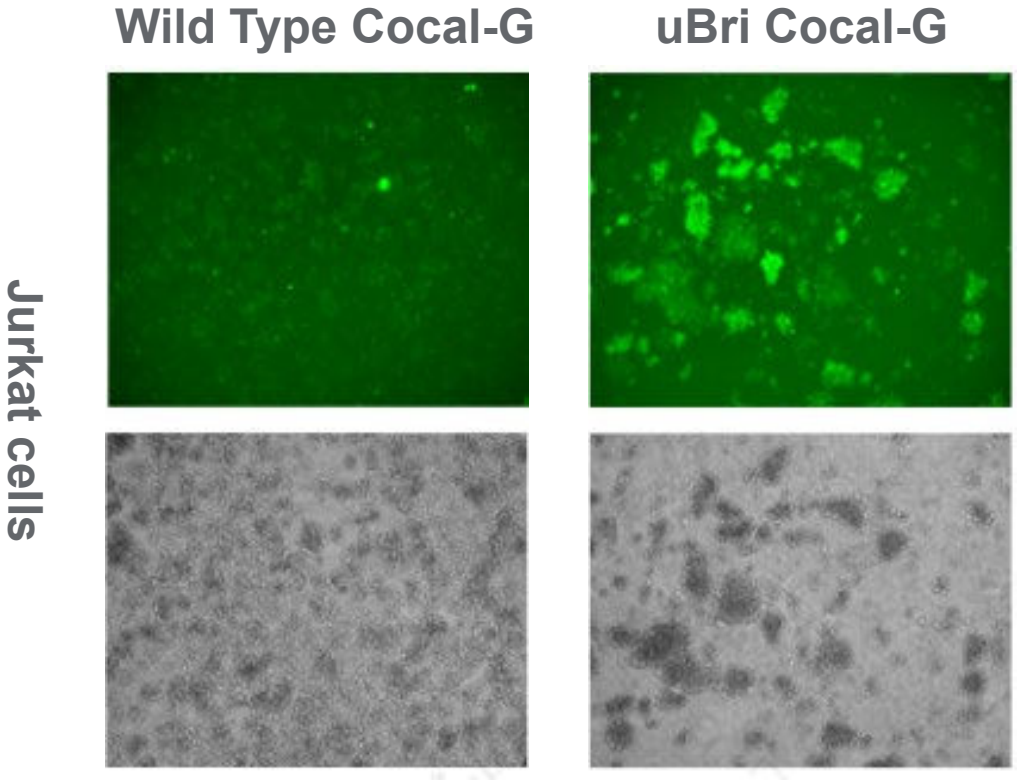
Membrane anchored cell specific binder

- Anti-CD7 nanobody (patented, collaborated with **Senlang Bio**) for T cell specific transduction

High functional Cocal-G^{MUT} titer based on optimized molecular design between Cocal-G^{MUT} and membrane anchored binder

High Titer LVV from Engineered uBri Cocal-G (uBri LVV)

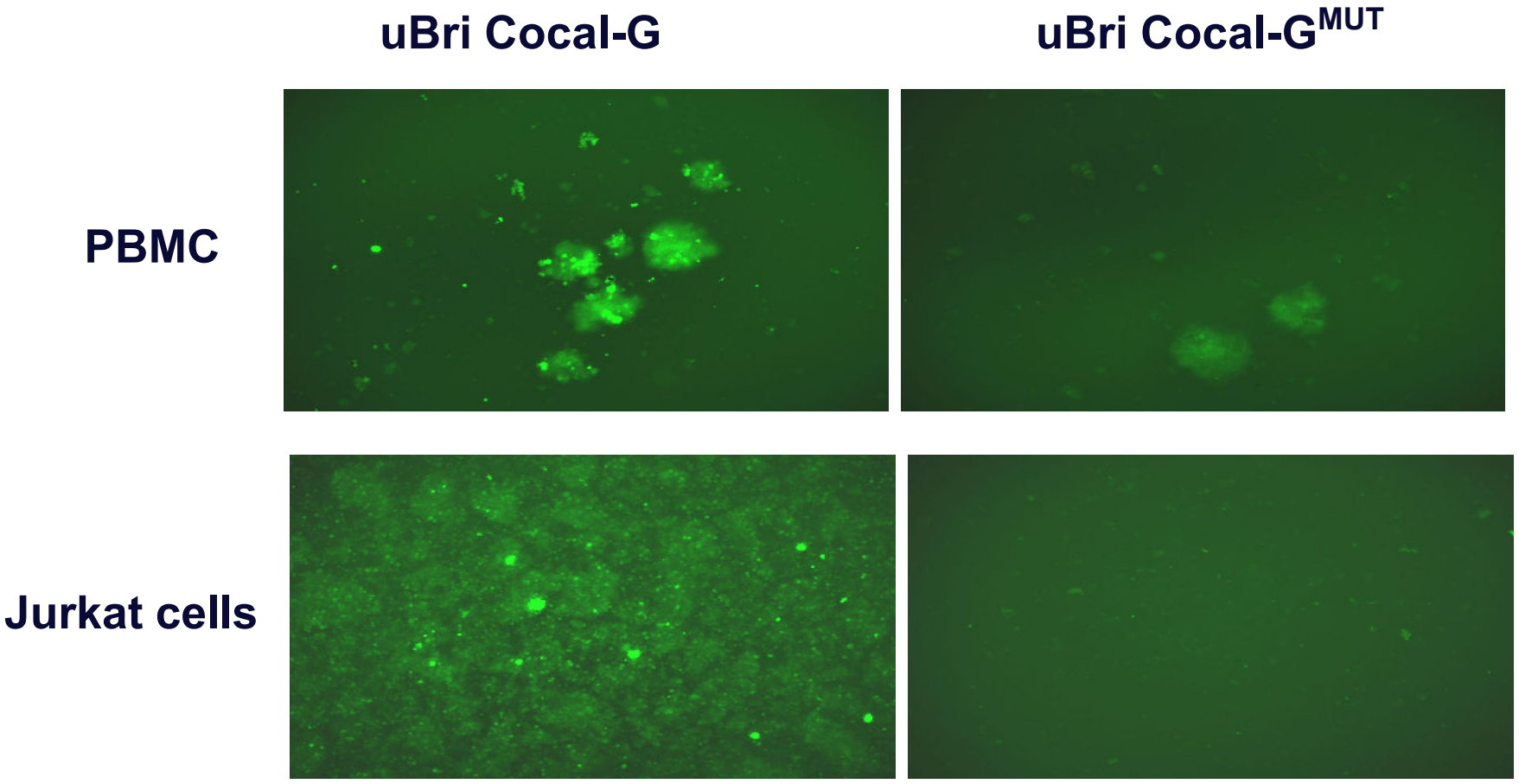




uBri-Cocal-G

- Engineered Cocal-G from its wild type
- Maintaining native tropism of wild type Cocal-G
- Generating higher titer LVV comparing to that of wild type

Abolishing Native Tropism: Engineering uBri Cocal-G^{MUT} (uVivo CAR^{TM} LVV) from the uBri Cocal-G (uBri LVV) Backbone

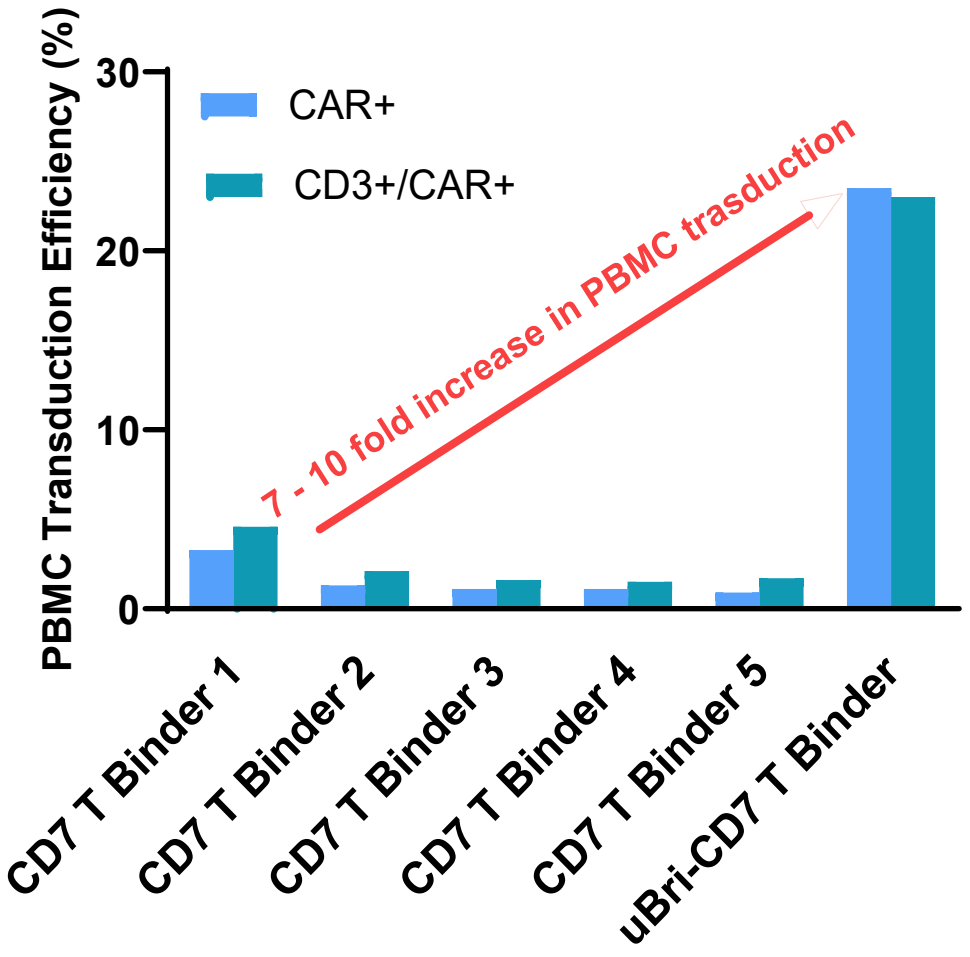


Patented T Cell Specific Binder Optimization

Anti-CD 7 nanobody specifically binding to T cells

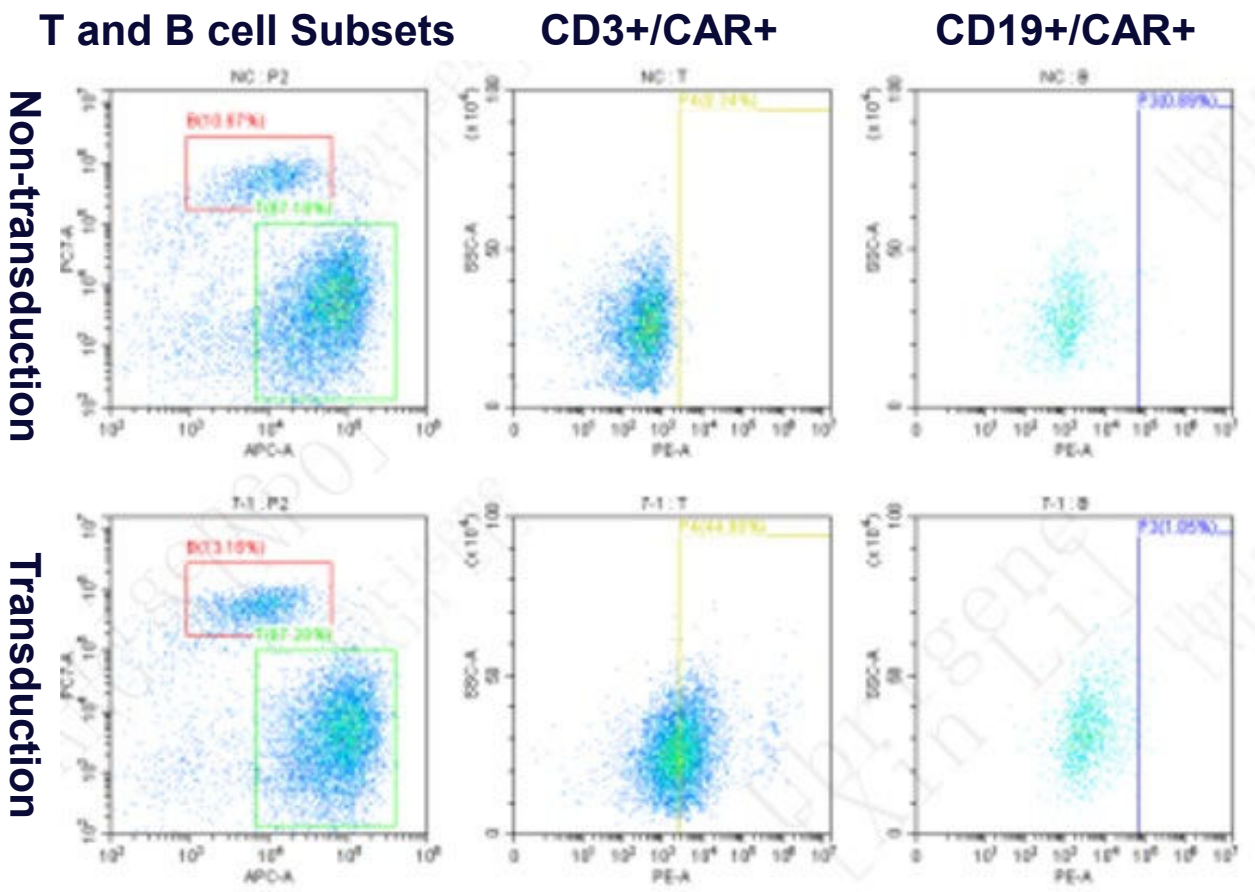
- Collaborated with Senlang Bio
- No synthetic linker required comparing to ScFv
- No misfolding derived from single domain nanobody
- Higher lentiviral envelope density due to its small molecular weight (15 KDa)

uBri-CD7 T binder significantly increased PBMC transduction after molecular optimization on membrane anchored anti-CD 7 nanobody



Specific T Cell Transduction by uVivo CAR™

Group	Mock-transduction (%)	Transduction (%)
CD3+	67.18	67.20
CD19+	10.67	13.16
CD3+/CAR+	0.34	**44.98**
CD19+/CAR+	0.89	1.05



uVivo CAR™ Transduction in non-activated T cells

Cell Specific Binder

NC

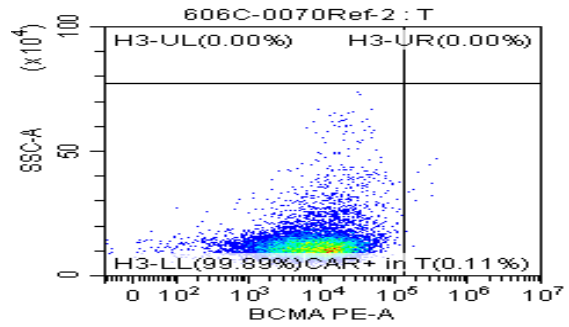
Days after Transduction

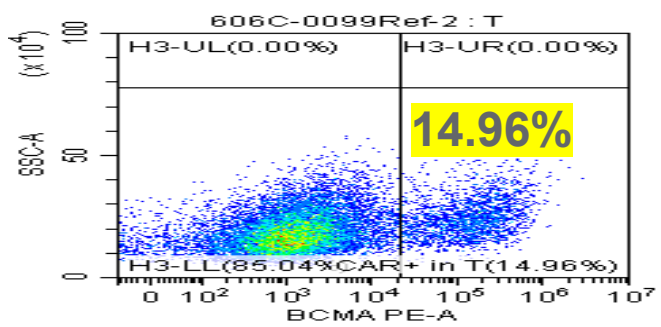
Day 3

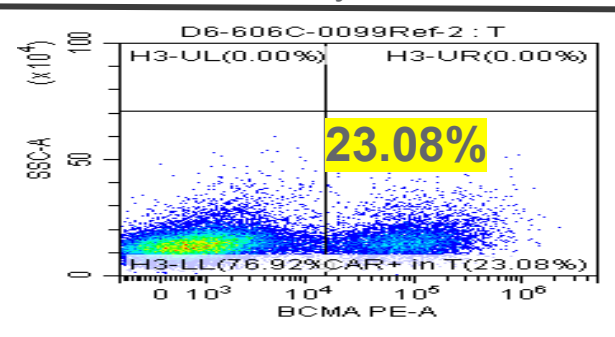
Day 6

Anti-CD3 ScFv

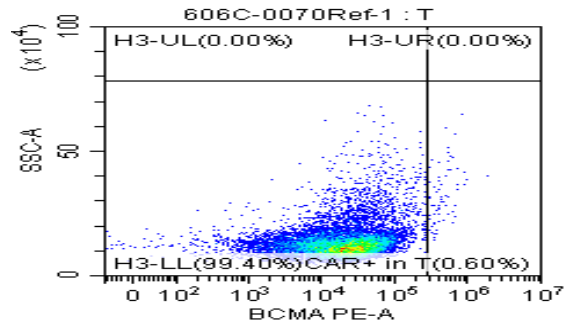
MOI 0.5

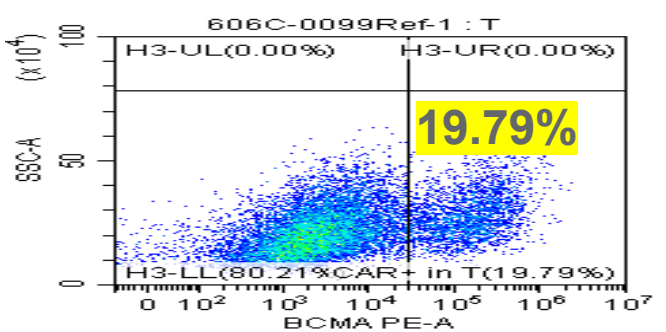


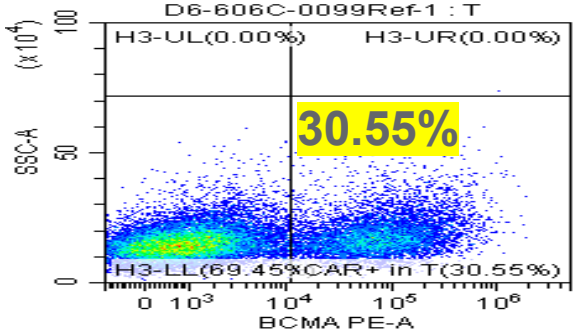




MOI 1

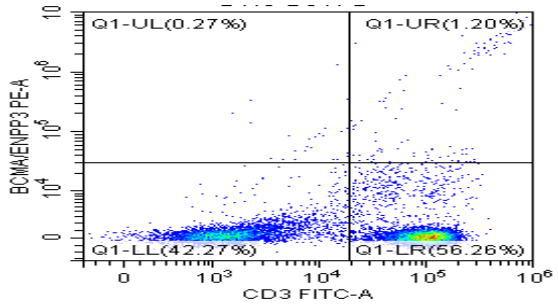


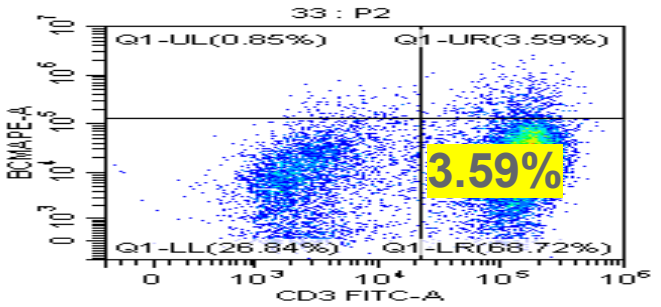


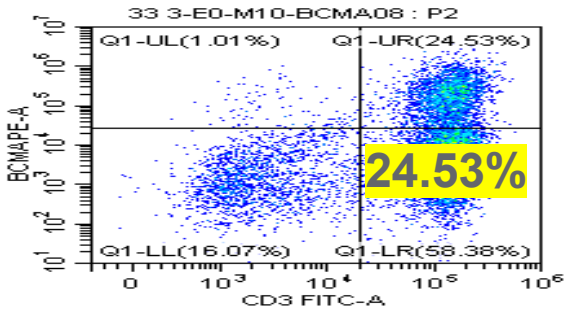


Anti-CD7 SdAb

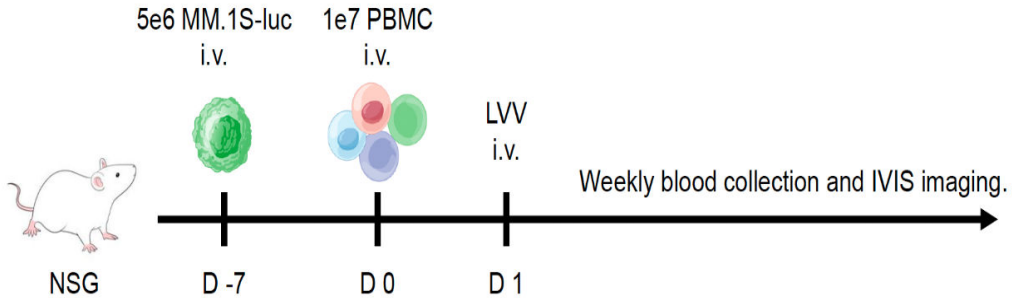
MOI 5







Anti-tumor Efficacy in Myeloid Mouse Model by uVivo CAR™ (CD7 BCMA LVV）



Control：PBS

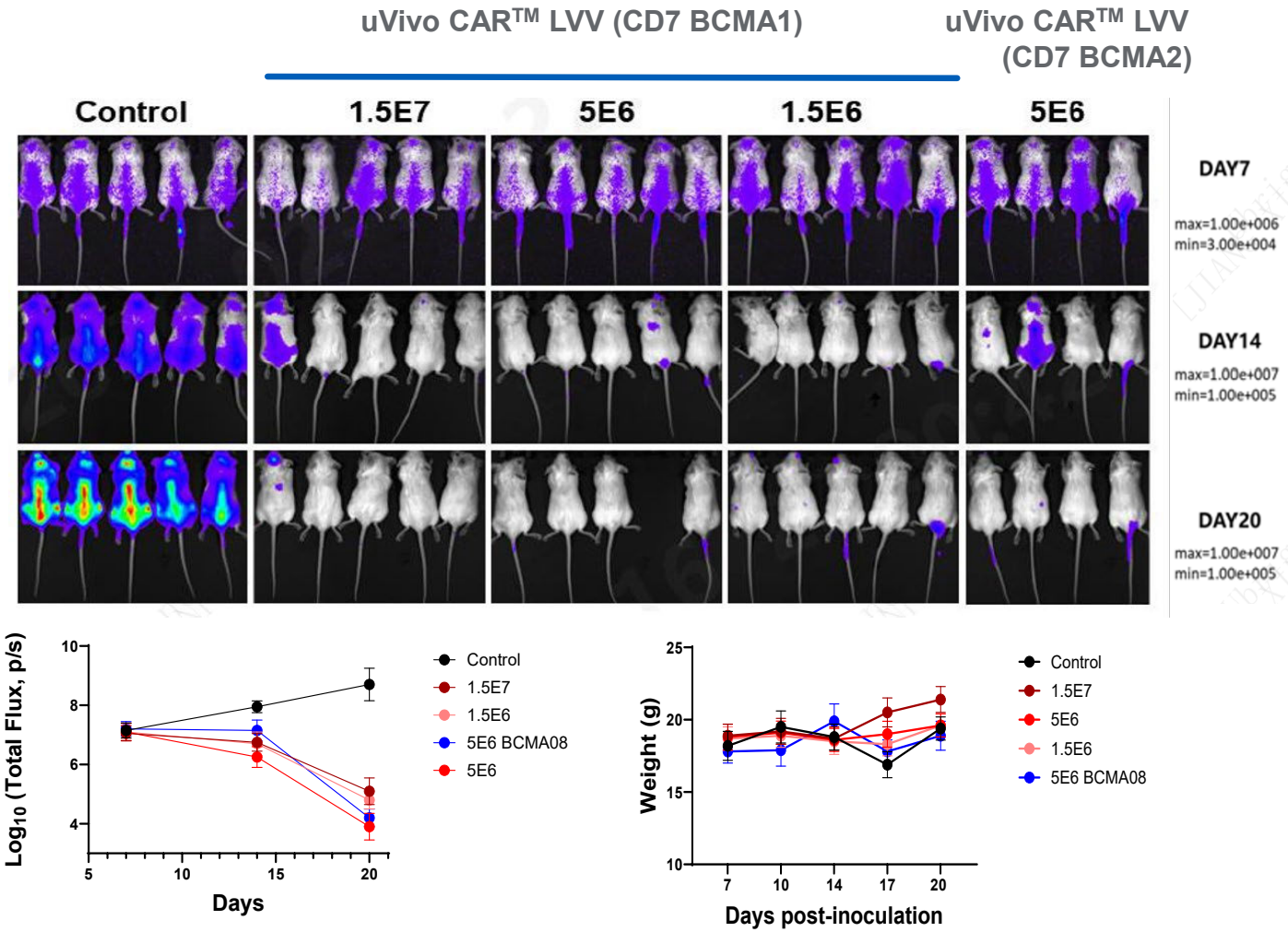
Study groups：

uVivo CAR™ LVV (CD7 BCMA1)

- 1.5 x 10^6 TU
- 5 x 10^6 TU
- 1.5 x 10^7 TU

uVivo CAR™ LVV (CD7 BCAM2)

- 5 x 10^6 TU



Data from Senlang Bio

Off-target *in vivo* Safety Profile

Animal No.	DNA Organ Source	VCN (Copies/Cell)
0349V85E63#	Lung	0.0004
	Muscle	0.0836
	Brain	0.0038
	Small Inrestine	0.0586
	Spleen	0.0019
	Liver	0.0011
	Kidney	0.0020
	Heart	0.0050
	Stomach	0.0144
	Femur	0.1424
Cell Reference Standard		7.8650
Reference Standard Recovery Rate: 95.1%		

Releasing Specification of GMP Lentivirus

Table 1
Common quality control items of vectors for the gene modification of CAR-T cells.

Category	Assays of retroviral/lentiviral vectors	Assays of transposon plasmid vectors
Identity	RT-PCR sequencing	Restriction enzyme mapping PCR Sequencing
Potency/concentration	Infectious/transducing titer Particle number (P24 ELISA) Particle to infectious/transducing titer ratio Transgene expression Bioactivity/functionality	Concentration Transgene expression Bioactivity/functionality
Purity	Host cell protein Host cell DNA Residual reagents (antibiotics, BSA, benzonase, etc.)	A_{260}/A_{280} HPLC purity (including ratio of supercoiled DNA) Host cell protein Host cell DNA Bacterial RNA Antibiotics
Safety	Sterility Bacterial endotoxin RCR/RCL Adventitious virus Mycoplasma	Sterility Bacterial endotoxin
Physiochemical characteristics	Appearance pH Osmolality Particle size distribution	Appearance pH Osmolality

RT-PCR: reverse transcription polymerase chain reaction; BSA: bovine serum albumin; HPLC: high performance liquid chromatography; RCR: replication-competent retrovirus; RCL: replication-competent lentivirus.

Yong hong Li,Yan Huo,Lei Yu, and Junzhi Wang

Lentiviral impurities

- Non-functional vector (incomplete, immature, broken, empty, and insufficient envelope proteins，etc.)
- Free vector envelope/component
- Viral aggregation

Potency Test

- Infectious titer
- Multiplicity of Infection (MOI)
- % CAR expression
- Physical Titer by p24
- In vitro CAR potent assay
  - ➢ Tumor killing
  - ➢ Cytokine releasing
- ➢ CAR T cell proliferation
- Tropism analysis: In vitro target cell specific transduction
- Expression analysis on envelope protein
  - ➢ Expression level
  - ➢ Ratio of different envelope proteins
- Analysis on CAR expression on lentiviral envelope

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