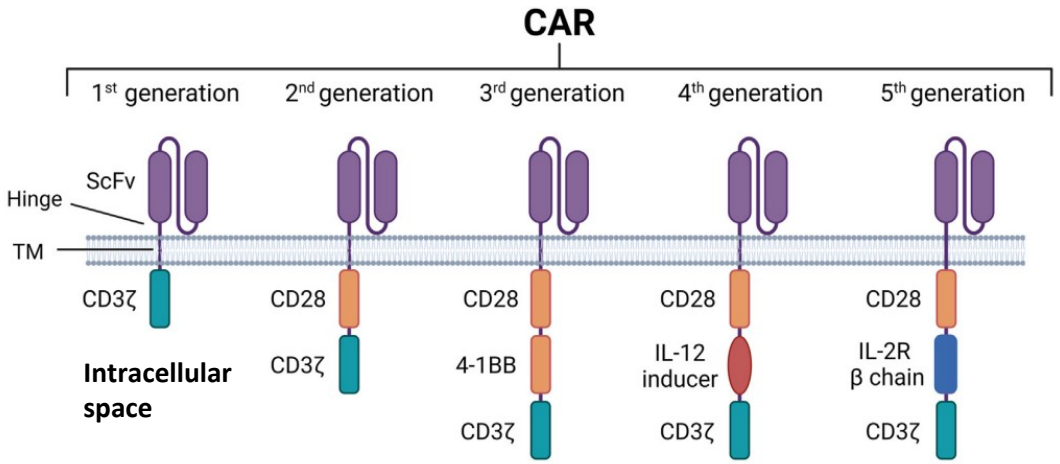
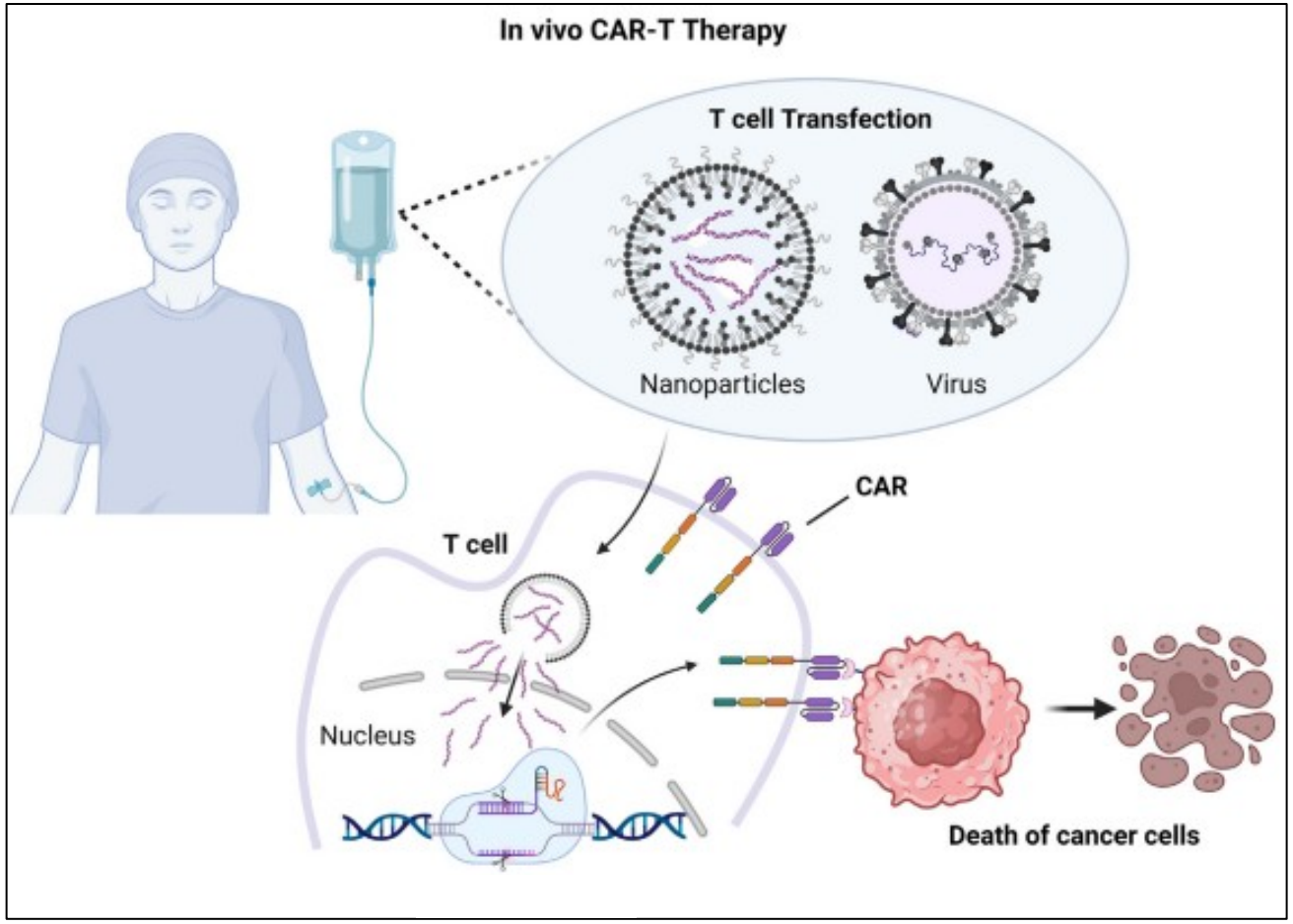
in vivo CAR T preclinical evaluation platform

2026.06

In Vivo CAR Therapy for Cancer Treatment



Extracellular Domain	Hinge Region & Transmembrane Domain	Intracellular Domain
➢ Derived from monoclonal Abs as scFVs, can include modified ligands/peptides to enhance specificity; ➢ Responsible for recognizing and binding to cancer cells	➢ Provide flexibility & stability; ➢ Ensure proper receptor function and integration into T-cell membranes	➢ Tigger T-cell activation and proliferation; ➢ Incorporate T-cell receptors (e.g. CD3ʒ, CD28, 4-1BB, IL-2 signaling) for enhanced cytotoxicity and persistence



Advancements and challenges in developing in vivo CAR T cell therapies for cancer treatment Bui, Thuy Anh et al. eBioMedicine.

In Vivo CAR-T Platform Capability

Binder Screen & Molecular Design

Phage Display & Lead Discovery

- Screening for **CAR (scFv/VHH)** or **Binder (scFv/VHH/DARPin)** platforms
- Efficient **Phage Hits** screening and identification
- Lead **scFv/VHH/DARPin** generation & characterization

CAR Engineering & Validation

- Standard CAR or multi-target CAR designs
- **"Truck" CAR** optimization (e.g., RARC, cytokines)
- High-throughput **reporter cell** activity screening
- Stringent **tonic signaling** assessment

Delivery System Evaluation

Non-viral: LNP-RNA Platform

- **mRNA Sequence Design:** MFE and CAI optimization
- In-house **mRNA/circRNA** generation & characterization
- Integrated **LNP-mRNA / Ab-LNP-mRNA** generation and characterization

Viral: Lentiviral Vector Platform

Translational Preclinical Studies

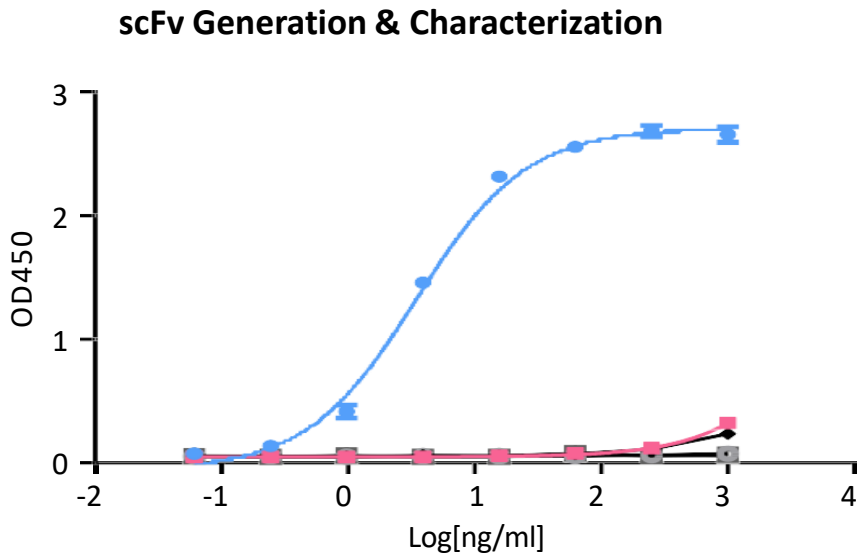
In Vitro Characterization

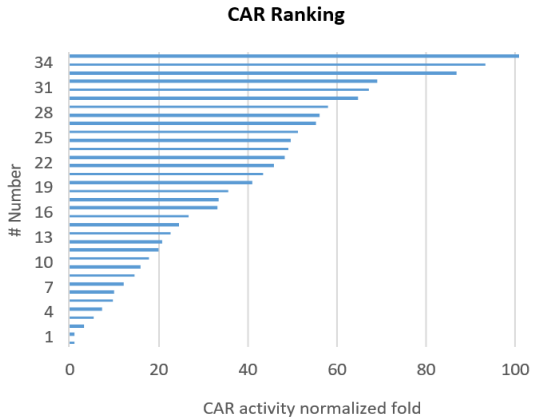
- **CAR Expression:** Resting vs. pre-activated T cells
- T cell activation and proliferation kinetics
- **Subset Profiling:** T, NK, B, monocytes subset specificities
- Cytokine secretion & killing efficiency vs. target cells

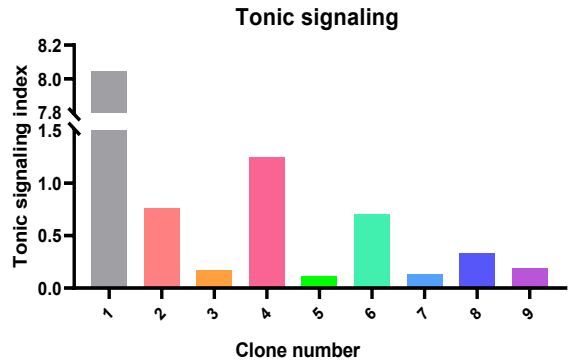
In Vivo Proof-of-Concept

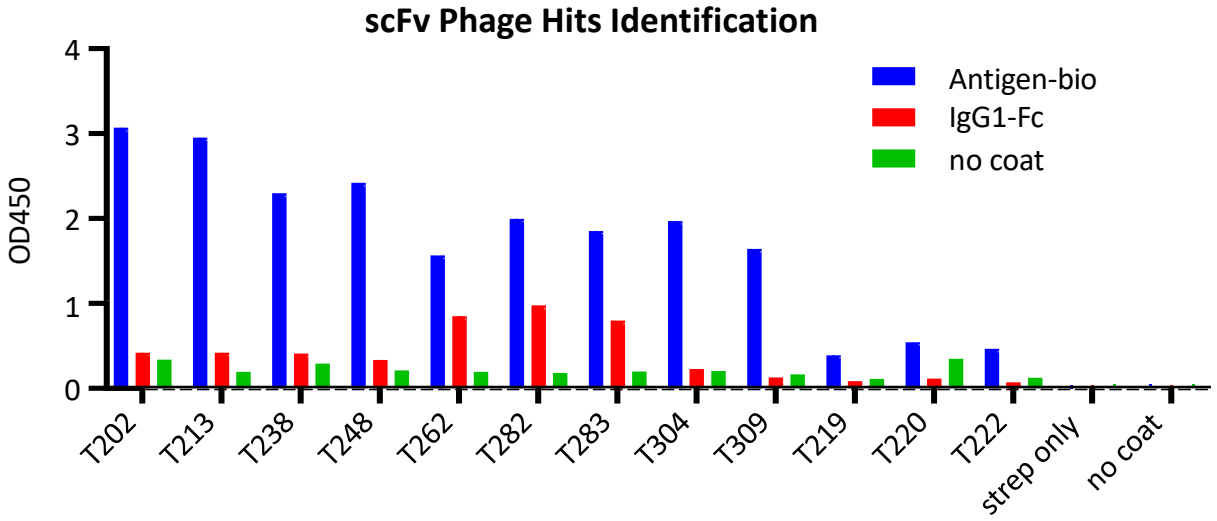
- **Efficacy:** Targeted tumor cell depletion kinetics
- **Kinetics:** Target CAR-T cell distribution and persistence
- **Safety:** Early safety evaluation and tox screening

Binder Screen and CAR Molecular Design Capability





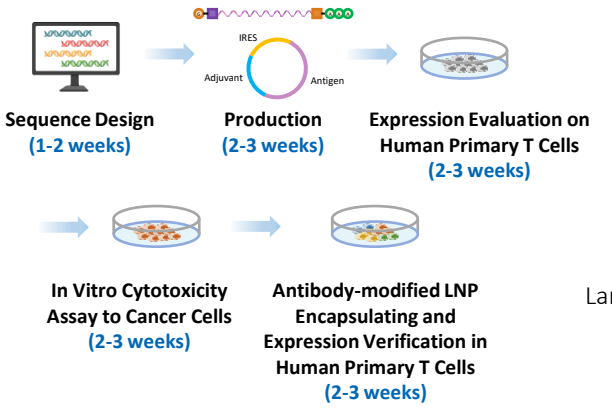


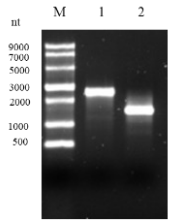


- High-throughput binder (scFv/VHH/DARPin) and CAR (scFv/VHH) discovery
- Variety of ready-to-go CAR functional screening assays available to identify front-running CAR candidates for further development and preclinical evaluation

LNP-RNA Delivery System Production And Evaluation

Linear & Circular CAR mRNA Production and Analysis Workflow



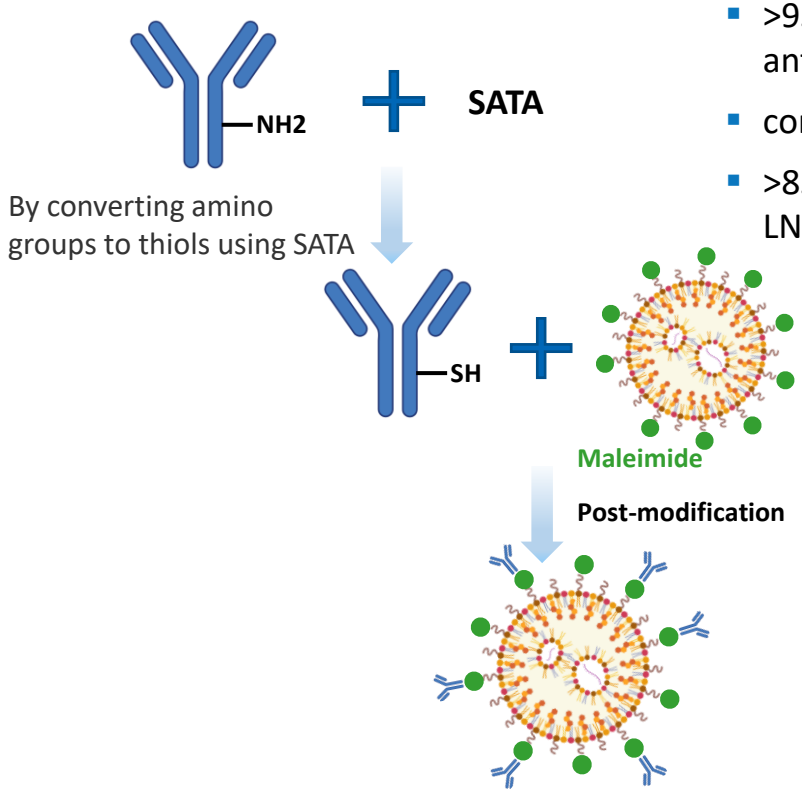


mRNA electrophoresis

Lane M: ssRNA ladder (NEB N0362S)
Lane 1: mRNA A (2450 nt)
Lane 2: mRNA B (1445 nt)

≥ 90% Purity and integrity

tLNP production and QC



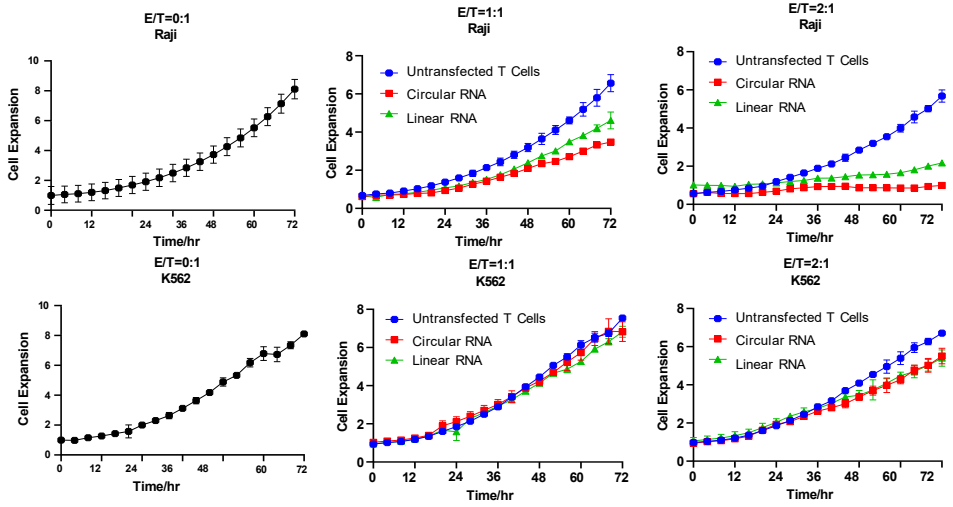
- >95% conjugation efficiency of CD3 antibody to LNPs
- consistent 80~120 nm particle size
- >85% encapsulation efficiency in LNPs post ultrafiltration

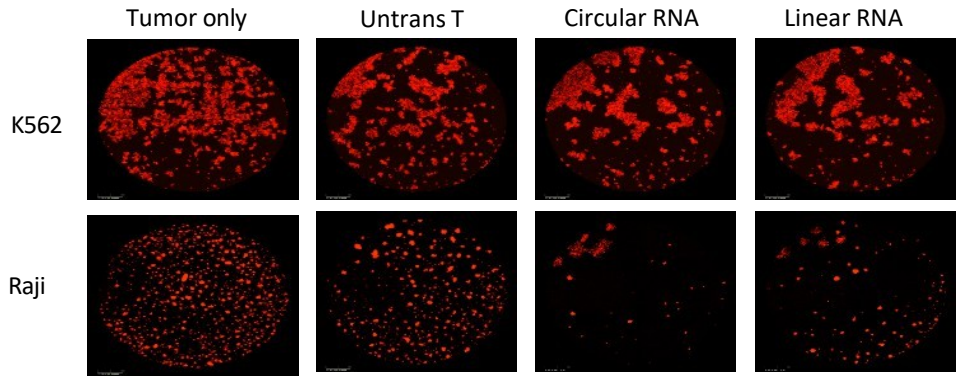
Source: PreciGenome, 2026

- The correlation between the design parameters of the delivery system and its functional performance can thus be preliminarily established, thereby providing a crucial empirical basis for subsequent in vitro and in vivo studies as well as the optimization of dosing strategies

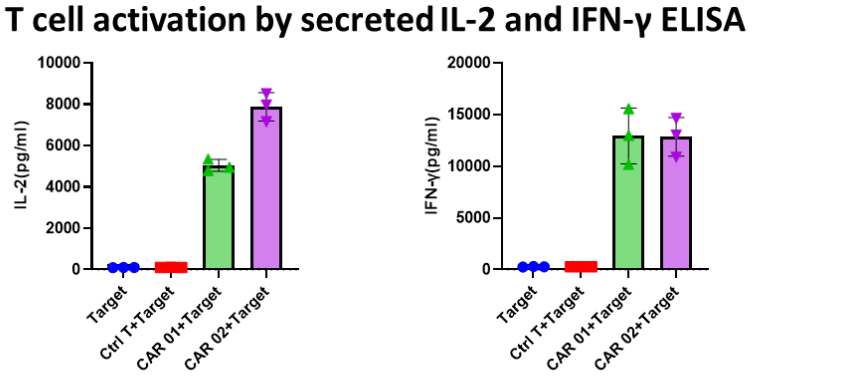
In Vitro Bioactivity Characterization

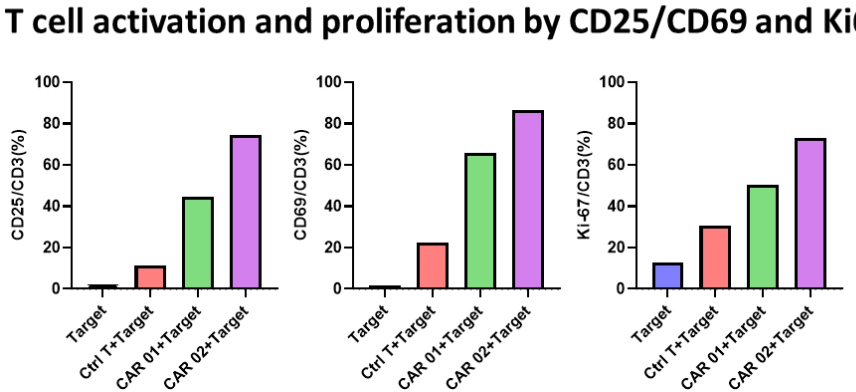
Co-culture Killing Assay





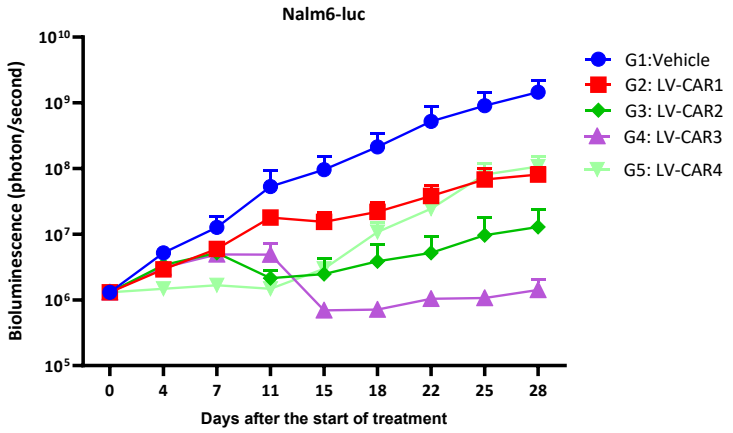
CAR-T Cell Activation and Proliferation

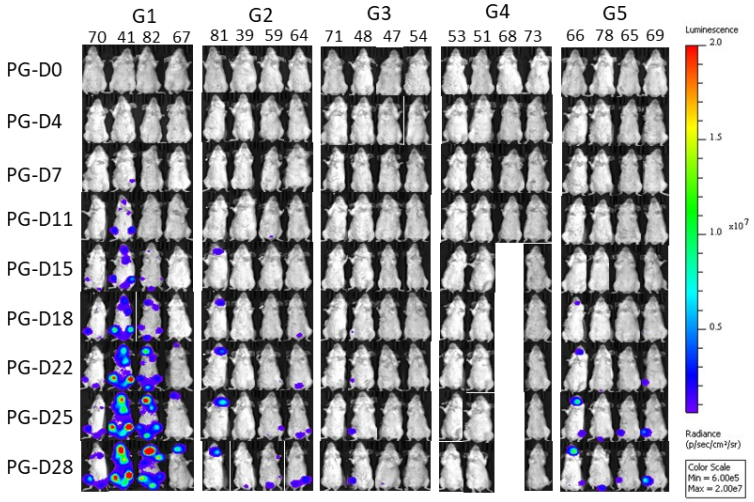




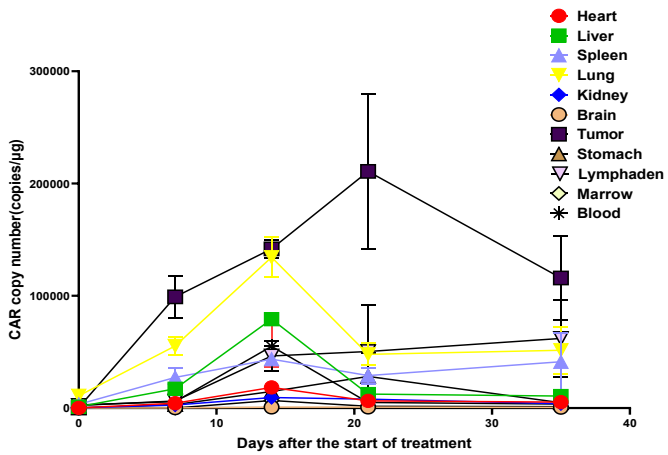
In vivo Anti-Tumor Efficacy And Biodistribution Evaluation

Anti-tumor efficacy





Biodistribution



Implant Nalm6-luc cells i.v. to PBMC humanized mice

- hPBMC and hHSC-humanized mice available for immune re-constitution.
- Established hPBMC-humanized CDX models in over 70 cell lines across 23 cancer types.
- Supports both LNP and LVV-based in vivo CAR-T pipelines.

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For questions and requests, please email to Pharmacology-BD-Translation@wuxiapptec.com

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