Medicilon Cell & Gene Therapy R&D Service Platform

Cell & gene therapy (CGT) has developed by leaps and bounds in recent years, providing a possible treatment for many refractory diseases. With the rapid development of gene transduction and modification, delivery vector system, cell culture and other technologies, many breakthroughs have been made providing a new treatment concept and approach.

Medicilon's preclinical research services cover pharmacodynamic research, drug safety evaluation, pharmacokinetic research, and bioanalysis. The establishment of a complete gene therapy R&D platform can provide a one-stop solution for research on pharmacological efficacy, biodistribution and safety evaluation of cell and gene therapy products. Medicilon has established this service for the preclinical research and development of cellular immunotherapy drugs, covering a variety of methods including CAR-T, TCR-T, CAR-NK and TIL cells. Using a wealth of animal models and a variety of advanced analytical techniques, and comprehensively considering the characteristics of different research projects, we have completed a number of preclinical development projects for immunotherapy programs for clients.

Pharmacology & Pharmacodynamics of CGT

SAFETY PHARMACOLOGY

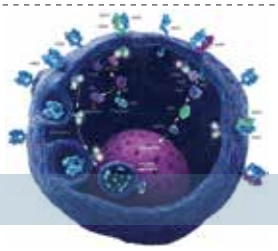
- Uncover potential undesired effects of the study drug on physiological function at doses within or above the therapeutic range
- Can include effects on the central nervous system, cardiovascular system, and respiratory system
- Research on other organ systems may need to be supplemented depending on product characteristics.

IN VITRO PHARMACODYNAMIC STUDIES

EFFICACY TESTING OF CELL THERAPY (CAR-T):

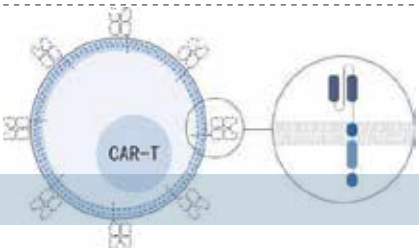
- Tumor killing rate or proliferation inhibition rate
- IFN-γ expression level
- Phenotype changes of CAR-T cells

PREPARATION AND EVALUATION OF CAR-T



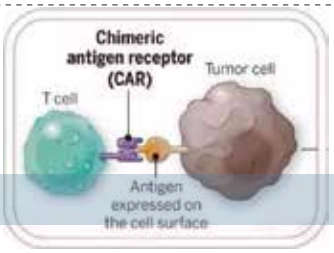
Cell Lines Establishment

Overexpression or Luc Cell Line
⊕
Single-clone and Mixed-clone



CAR-T Cell Preparation

Lentiviral Packaging & Infection
⊕
T cells Sorting & Activation

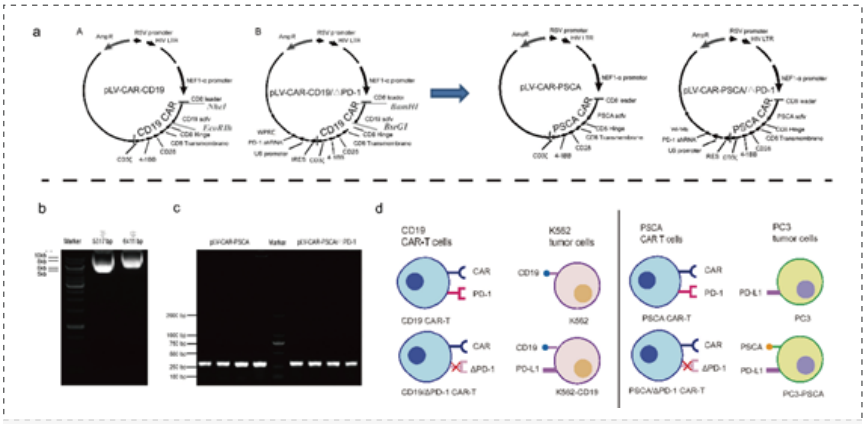


In Vitro Analysis

In Vitro killing test
⊕
Cytokine Release

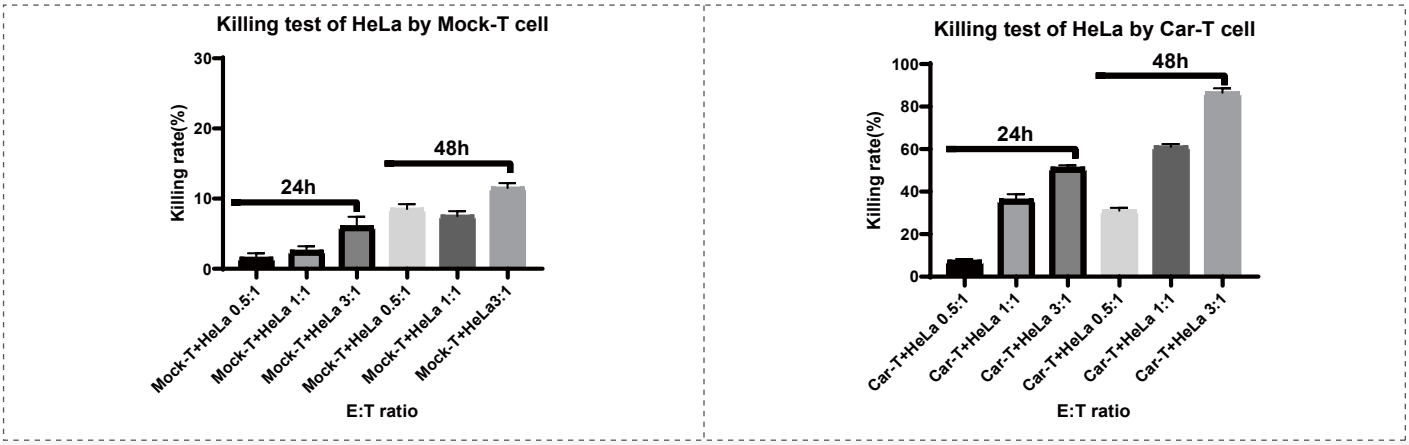
CASE STUDY: PLASMID VECTOR CONSTRUCTION

In this study, shRNA (short-hairpin RNA)-mediated gene silencing technology was used to block the influence of PD-L1/PD-1 immunosuppression axis on the proliferation and anti-tumor effect of CAR-T cells, thereby enhancing its therapeutic effect on subcutaneous prostate and leukemia xenograft. The PD-1 shRNA was integrated into the third-generation of CAR plasmid, which was then transduced into T cells by lentivirus to obtain CAR-T cells with PD-1 silencing function. The results showed that the efficient silencing of PD-1 significantly inhibited the immunosuppressive effect of the tumor microenvironment, and prolonged the activation duration of CAR-T cells, resulting in a long tumor-killing effect. The PD-1 silenced CAR-T cells significantly prolonged the survival period of subcutaneous prostate and leukemia xenograft bearing mice. This study proved that PD-1 silencing technology is a suitable solution for promoting the therapeutic effect of CAR-T cells on subcutaneous prostate and leukemia xenograft. The plasmids sequenced were fully identified by **Medicilon**.



Lentivirus vector plasmid construction

CAR-T CELL KILLING ASSAY



CAR-T cells showed greater cytotoxicity than Mock-T cells against Hela cells

IN VIVO PHARMACODYNAMIC RESEARCH

CELL THERAPY TEST SUBSTANCES

- Can be prepared from blood donated by healthy volunteers
- Proof-of-concept studies can be done with alternative products of animal origin
- Similarities and differences between non-clinical test substances and clinical samples should be explained in the new drug application

GENE THERAPY TEST SUBSTANCES

- Consider factors such as production process, key quality characteristics (such as titer), preparations for clinical use
- If there is species specificity, the activity of the test substance in non-clinical research should be evaluated
- If the vector uses an expression tag, the impact of the tag on the supportability of non-clinical trials should be analyzed

DETECTION METHODS AND EVALUATION INDICATORS

- Bioluminescent Imaging (BLI)
- Flow Cytometry: Detecting the number of tumor cells in animals
- Flow Cytometry, ELISA, MSD: Changes in tumor-related cytokines
- Related Parameters: Tumor volume, tumor weight, tumor cell localization, *in vivo* and median survival period of animals

BD LSRFortessa™- 20 Channels

IVIS Lumina III *In Vivo* Imaging System

ANIMAL MODELS

Example in the table below, more models could be consulted according to the last contact information

Cancer Type	Cell Lines	Cancer Type	Cell Lines
Brain Cancer	U-87 MG, LN-229, U-251 MG	Renal	786-O, OS-RC-2, A498, ACHN
Breast Cancer	BT474, HCC1569, HCC1954, HCC70, JIMT-1	Lung Cancer	A549, Calu-1, Calu-3, Calu-6, HCC827
Colon Cancer	COLO 205, DLD-1, HCT-116, HCT-15, HT-29	Lymphoma	SU-DHL-4, DB, Mino, Daudi, JeKo-1, Raji
Gastric	Hs 746T, NCI-N87, SNU-16, MKN-45	Myeloma	MM.1S, NCI-H929, RPMI-8226, OPM-2
Leukemia	CCRF-CEM, HEL, HL-60, K-562, MV-4-11	Ovary	A2780, OVCAR-3, SK-OV-3
Liver Cancer	Hep G2, HuH-7	Pancreatic	AsPC-1, Bx PC-3, Capan, CFPAC-1

CDX models

Cancer Type	Cell Lines
Breast Cancer	4T1, EMT6, JC, EO771
Colon Cancer	CT26.WT, MC-38, Colon26
Leukemia	C1498, L1210, WEHI-3
Lung Cancer	LLC1, KLN205
Lymphoma	A20, EL4, L5178-R, E.G7-OVA
Melanoma	B16-F10, Clone-M3

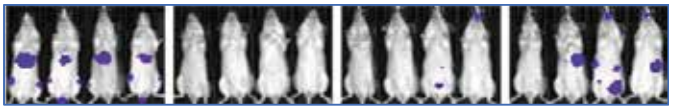
Syngeneic mouse models

Cancer Type	Cell Lines
Breast Cancer	HCC1954, MDA-MB-231, JIMT-1
Colon Cancer	HT29, LoVo, Ls174T, HT-15
Gastric Cancer	NCI-N87, NUGC-4
Lung Cancer	HCC827, NCI-H1975, NCI-H292, Raji,
Lymphoma	TMD8, MOLM-13
Myeloma	RPMI-8226, NCI-H929, MM.1S

HIS-reconstituted humanized mouse models (PBMC、HSC $CD34^+$)

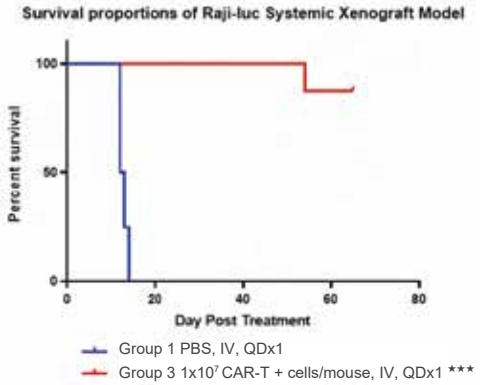
IMMUNE SYSTEM RECONSTITUTION HUMANIZED MOUSE MODEL

Control



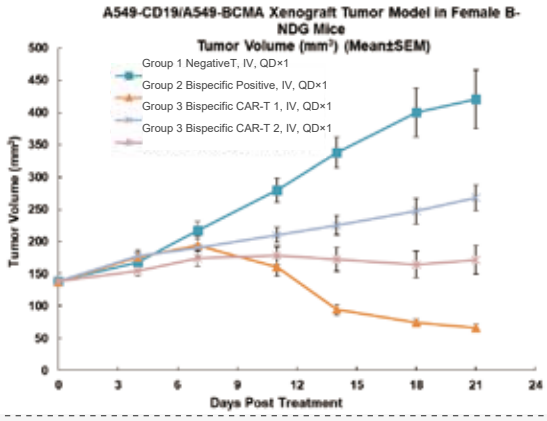
CAR-T + $1x10^7$ cells/mouse IV,QDx1

Groups	MST	%T/C_{MST}	P-Value
Vehicle	16.5	/	/
CAR T+	>65	>520	P<0.001



Pharmacodynamic study of Raji-luc fluorescein-labeled lymphoma cells-induced hPBMC immune system reconstruction mouse model

PHARMACODYNAMIC STUDY OF BISPECIFIC CAR-T



- **Animal:**
  Female B-NDG Mice，~20 g
- **Cell:**
  A549-CD19+/A549-BCMA (1:1 Mix)
  $5×10^6$ cells/mouse
- **Modeling:**
  SC Injection
- **Treatment:**
  CAR-T+ Cells, IV, QD×1

Pharmacodynamic Study of CD19/BCMA Bispecific CAR-T

Pharmacokinetic Study of CGT

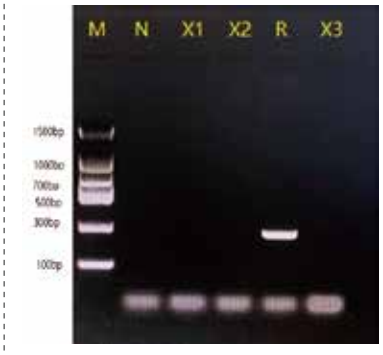
POINTS TO CONSIDER FOR PHARMACOKINETIC STUDY

- Exposure: Gene therapy products should be analyzed and evaluated according to the specific characteristics of the product considering the actual exposure in non-clinical research
- Biodistribution: Refers to the distribution, persistence and clearance of gene therapy products in target and non-target tissues *in vivo*
- Shedding: Assays should include testing for infectivity of excreted components

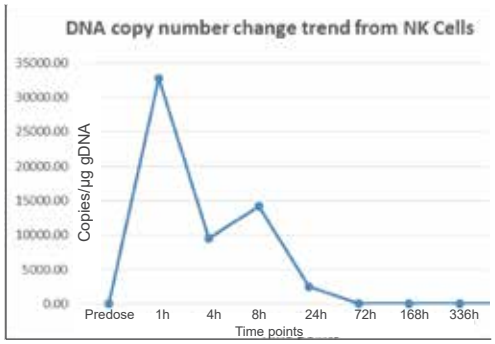
BIODISTRIBUTION DETECTION TECHNOLOGY

- Imaging Technology
- Flow Cytometry
- Immunohistochemistry
- Quantitative PCR Technology

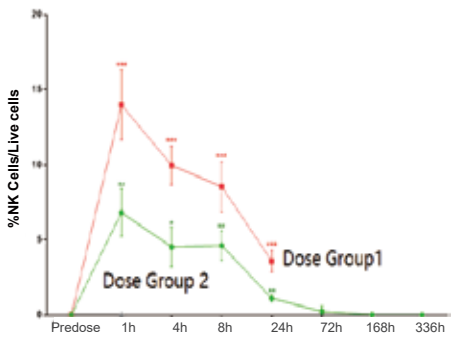
DETECTION OF DISTRIBUTION OF LUNG CELL THERAPY



PCR Validation of Cell Therapy Specific DNA



Biodistribution in The Lung (qPCR)



Biodistribution in The Lung (Flow Cytometry)

The Detection Results Obtained by qPCR & Flow Cytometry Are Consistent

Nonclinical Safety Evaluation of CGT

In toxicology research, a comprehensive safety analysis and evaluation of gene therapy products should be conducted, and the safety of the expression products of introduced genes should also be evaluated if necessary. Gene therapy products should be effectively introduced/exposed in relevant animal species. The non-clinical safety risks of cell therapy (such as CAR-T cells) mainly include cytokine release syndrome (CRS), reversible neurotoxicity, reduction of B cells, on-target/off-tumor, Graft-versus-host disease (GVHD), and tumorigenicity/tumorigenicity of CAR-T cells.

- General toxicology
- Immunogenicity
- Immunotoxicity
- Reproductive toxicity
- Genotoxicity
- Neurotoxicity
- Carcinogenicity
- Local tolerance

Project Supported by Medicilon

IBR854

IBR854 Cell Injection of iMBioRay obtained the permission of the NMPA for clinical trials. IBR854 Injection is a universal off-the-shelf CAR-raNK cell product derived from allogeneic peripheral blood. It is the first non-gene-modified CAR-raNK cell therapy for solid tumors in China, and the world's first FIC (First in class) products.

As a partner of IBR854 Cell Injection, Medicilon provided efficient, high-quality and comprehensive preclinical research services (including pharmacokinetic studies and safety evaluation) in compliance with GLP regulations.

References:

[1] Jing-E Zhou, et al. ShRNA-mediated silencing of PD-1 augments the efficacy of chimeric antigen receptor T cells on subcutaneous prostate and leukemia xenograft. Biomed Pharmacother. 2021 May;137:111339. doi: 10.1016/j.biopha.2021.111339.

MEDICILON

Email: marketing@medicilon.com **Website:** www.medicilon.com **Tel:** +1 (626) 986-9880

Global Headquarters: 585 Chuanda Road, Pudong, Shanghai, 201299, China

US Laboratory: 20 Maguire Road, Suite 103, Lexington, MA 02421, USA