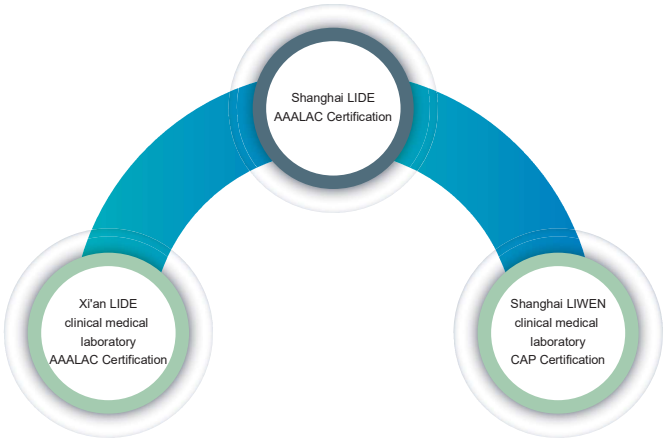
Company Introduction

Shanghai LIDE Biotech Co., Ltd. (hereinafter referred to as "LIDE Biotech") is committed to the research, service and product development of individualized precision medicine for cancer, providing pharmacological efficacy study of CRO services and assisting doctors in clinical personalized precision medicine. The company owns AAALAC accredited SPF level animal centers and world-class equipment. LIDE Biotech has two wholly-owned subsidiaries, “Shanghai LIWEN Biotech Co., Ltd."(hereinafter referred to as "Shanghai LIWEN") and "Xi'an LIDE Biotech Co., Ltd."(hereinafter referred to as "Xi'an LIDE"). Both Xi'an LIDE and Shanghai LIWEN have independently operated clinical medical laboratory.

Shanghai LIWEN is a third-party testing unit for individualized diagnosis and precision medicine of cancer. The service scope includes: molecular biomarker testing, pathology and companion diagnosis. The company has been certified by the American Association of Pathologists (CAP) with FDA-approved data reporting system and central lab qualifications. Shanghai LIWEN-Promega's Joint Laboratory was founded by Shanghai LIWEN and American Promega which is dedicated to the research and development of Immune Check Point biomarkers (MSI, dMMR, etc.).

As the public service platform of translational medicine at Xi'an Hi-tech Park, Xi'an LIDE also owns a SPF-level laboratorial animal center with AAALAC accreditation and the medical license of clinical testing lab.



LIDE

Immune-Oncology Platforms

SHANGHAI LIDE BIOTECH CO.,LTD.

Humanized tumor immune models can be used for pharmacodynamic studies using immunotherapies. Shanghai LIDE currently offers a one-stop solution of immune-oncology platform for antibodyies R&D.

Selected popular targets under development

Target	Approved	Phase III	Phase II	Phase I
PD-1/ PD-L1	PD-1 : BMS（Nivolumab） Merck（Pembrolizumab） Junshipharma（Toripalimab） Innoventbio（Sintilimab） Hengrui（Camrelizumab） BeiGene（Tislelizuma） PD-L1 : Roche（Atezolizumab） AZ（Duvalizumab）	PD-1: BMS, MRK, NVS, SNY, GSK, REGN PD-L1: AZN, ROG, PFE	PD-1: BMS, MRK, SNY PD-L1: AZN	PD-1: ROG, AMGN, AZN, ABBV, LLY PD-L1: LLY, GILD
TIM-3			NVS(MBG453)	ROG, BMS
CD40			ABBV(ABBV-428); Apexigen(APX005M）	ROG, AZN, Roche , Celldex
TGFβ/PD-L1				Hengrui(SHR-1701)
PD-L1/CD47				Innovent（IBI322）, Hanxbio （HX099）
CD47			GILD(magrolimab)	Innovent, I-Mab, Hengrui, BMS
LAG3		BMS（BMS-986016）	ROG, BMS, REGN, GSK	
Claudin 18.2		Astellas(Zolbetuximab)	Astellas, CARsgene, Aosaikang	Transcenta, LEGN, Amgen, I-Mab

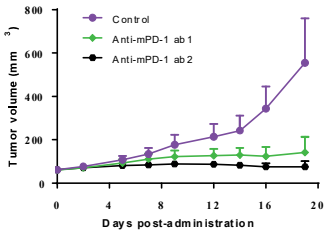
Immune/Oncology (I/O) Platforms in LIDE

- Syngeneic Murine Models
- Humanized GEMM
- huPBMC(+DC/MØ) Co-Inoculated CDX/PDX
- huPBMC(or NK cells) Reconstituted CDX/PDX
- CAR-T/Oncolytic Virus Efficacy Studies
- MØ/DCs in vitro induction or differentiation.
- Antibody-antigen binding and antibody-ligand competitive assay
- MØ phagocytosis assay
- Oncolytic virus in vitro assay
- T Cells activation and proliferation
- Helios: A CyToF system using heavy metal-conjugated Ab labeling, with around 40 channels per detection.
- Hyperion: An Imaging Mass Cytometry for extraction of over 30 measured markers in a single slice

Murine/humanized syngeneic models for ab prove of conception

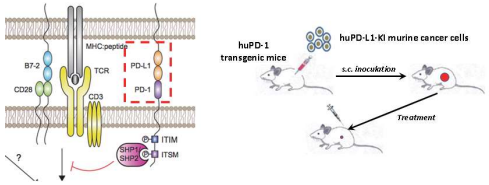
In vivo efficacy in WT mice that bearing commercialized murine cancer cells are widely used for early prove of conception for anti-mouse antibody on indicated targets.

Available murine cancer cells in LIDE			
Cancer Type	Name of Cell Line	Cancer Type	Name of Cell Line
Neuroblastoma	Neuro-2a	Renal	RAG
Melanoma	B16-F10	Lymphoma	E.G7-OVA, P388D1, EL4, YAC-1, L5178Y TK+/- clone (3.7.2C)
Breast	4T1	Myeloma	P3X63Ag8, SP2/0, FO
Lung	LLC	Leukemia	L1210
Liver	Hepa1-6	Mastocytoma	P815
Gastric	MFC	Prostate	RM-1
Colorectal	MC38, CT26.WT	Testis	MLTC-1
Sarcoma	K7M2 WT		

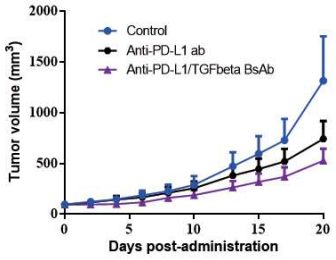


MC38 murine colon cancer cells was inoculated into the right flank in C57BL/6J mice. Two anti-mPD-1 antibody, ab1 and ab2, were given at 5 mg/kg bi-weekly for 6 injections via i.v. or i.p., respectively, and demonstrated good anti-tumor effect.

In order to evaluate anti-human antibody, syngeneic model with both mice and murine cancer cells humanized should be constructed and applied accordingly.



(Left) Extracellular domain of both murine cancer cells (e.g.> PD-L1) and mice (e.g.> PD-1 on murine T cells) were replaced with humanized structure, while the promoter region asd well as intracellular signaling were maintained as previous. (Right) Flowchart of in vivo efficacy in humanized syngeneic models.

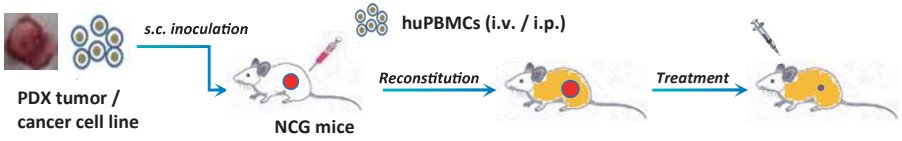


Commercial available GEMM mice	
huPD-1	huSIRPα
huPD-L1	huCD47
huCTLA-4	huTIGIT
huTNFRSF4(OX40)	huLAG-3
huCD137(4-1BB)	huTIM-3
huCD27	huBTLA

More TG mice models, feel free to contact LIDE

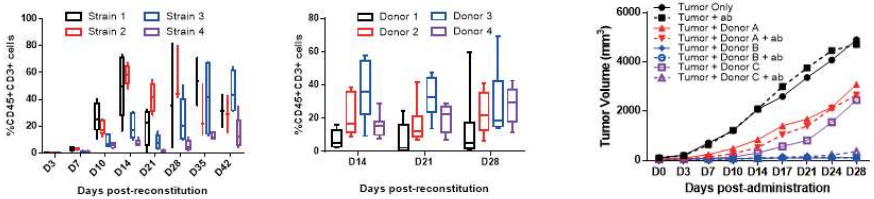
CDX/PDX with huPBMC reconstituted models for immunotherapy

CDX or PDX models can be utilized for immunotherapeutic evaluation when tumor-bearing mice were reconstituted with huPBMC via i.p./i.v. injection.



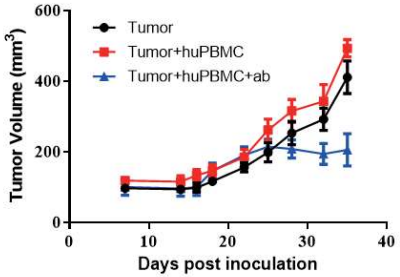
Flowchart of huPBMC reconstituted CDX/PDX model

Consistency of interaction between immune cells and cancer cells is of essential to success of immune-oncology (I/O) projects when using huPBMC reconstituted or co-inoculated (Page 5) platforms. LIDE performs initial investment for antibody validation in various batches of huPBMC and diverse mice strain, all of which enables LIDE to provide high-quality and stable platforms for client to complete their projects by utilizing the same batch of validated huPBMC.



LIDE has ordered over 10 batches of commercialized huPBMC, and performed validation in different mice strain (Left and middle). Validated strain has been reconstituted with varied donor's PBMC and treated with validation antibody to confirm the best donor for immunotherapy (right).

Using validated platforms, LIDE provide in vivo efficacy assay for client to evaluate immunotherapy about investigated antibodies.

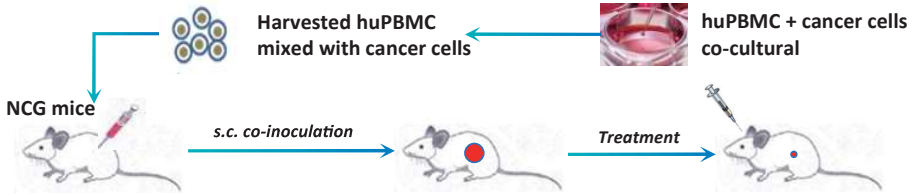


#LD1-0010-200614 human cervical PDX model was inoculated into the right flank in NOG mice. Mice were reconstituted with huPBMC via i.p. 7 days after tumor inoculation, while animals were randomized when average tumor volume grown up to ~150 mm3, with dosing initiated accordingly. An investigated BsAb was dosed at 0.125 mg/kg through i.p. once a day for a total of 18 injections. Tumor volume and body weight were measured twice a week.

CDX/PDX with huPBMC co-inoculated models for immunotherapy

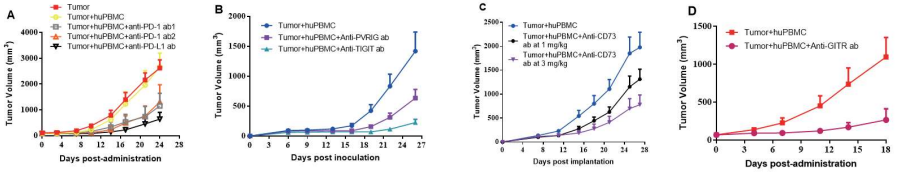
huPBMC reconstitution (Page 4) is a good model for evaluating therapeutic antibody, especially the BsAb with one arm of anti-CD3, that mediates immune cells. However, limited window of dosing regimen triggered by GvHD after 30-40 days of reconstitution, and insufficient tumor-infiltrated immune cells naturally from reconstituted immune cells in mice circulation hinders the widely application of the model in immunotherapy in development of multiple immune checkpoint inhibitors (e.g.> TIGIT, PVRIG, CD73, etc.).

To overcome this, LIDE has developed huPBMC-cancer cells co-inoculated models instead. In this platform, huPBMC will be firstly co-cultured with cancer cells. Then the mixture of the activated huPBMC and the cancer cells will be co-inoculated into mice to form a huPBMC well-infiltrated tumor tissue for immunotherapy.



Flowchart of huPBMC-cancer cells co-inoculated CDX/PDX model

A375 and huPBMC co-inoculating I/O model is an ideal platform for evaluation of many kinds of monoclonal antibodies.



huPBMC from indicated donors were co-cultured with A375 human melanoma cancer cells in vitro under specific condition. The activated huPBMC were then harvested and mixed with A375 cancer cells in certain E:T ratio, and co-inoculated into the right flank in NCG mice. Mice were randomized immediately after tumor co-inoculation or when average tumor volume grown up to 50-100 mm3, with dosing initiated accordingly, while tumor volume and body weight were measured twice a week. (A) Anti-PD-1 antibody from two vendors and anti-PD-L1 antibody were dosed at 10 mg/kg via i.p. bi-weekly for a total of 6 injections. (B) Anti-PVRIG antibody at 16.1 mg/kg or anti-TIGIT antibody at 30 mg/kg were dosed via i.p. every other day for a total of 12 injections. (C) Anti-CD73 antibody at 2 dose levels were dosed via i.p. every other day for a total of 11 injections. (D) Anti-GITR antibody at 1 mg/kg was dosed via i.p. weekly for a total of 3 injections.

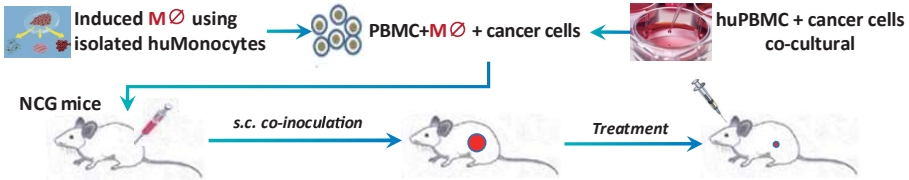
LIDE has developed several models based on the co-inoculated platform, and has supported a great number of client projects with multiple immune-oncology targets.

Humanized CDX/PDX model available in LIDE	
A375	MDA-MB-231
SHP77	Daudi
Molp8	SK-MEL-5
A549	Raji
ES-2	HepG2
MKN-45	U87
Lovo	HEK293-Claudin 18.2
Huh7	NCI-N87
PC-3	PDXs in LIDE
To be continued...	

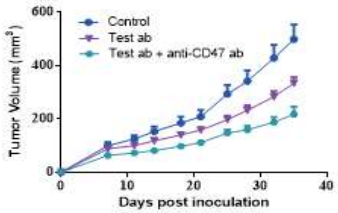
I/O target available in LIDE	
PD-1/PD-L1	TIGIT
GITR	PVRIG
TIM-3	CSF1R
CD29	CD47/SIRPa
CD38	CD39
CD40	B7H3
CD73	LAG3
IL2	IL-15
TGFβ/PD-L1	CD3/Claudin 18.2
CD3/GPC3	CD3/DLL3
PD-L1/CD47	CD3/EpCAM
To be continued...	

Application of macrophage-involved huPBMC+CDX co-inoculated in vivo model

Based on the co-inoculated model (Page 5), LIDE can provide in vitro induction using monocytes isolated from the same donor with huPBMC into indicated type of Mφ (e.g.> M2 type), and co-inoculation using activated huPBMC, induced Mφ, as well as cancer cells for immunotherapy that could mediate Mφ (de-)polarization and/or phagocytosis.



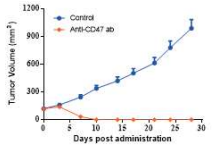
Flowchart of huPBMC+Mφ+cancer cells co-inoculating model



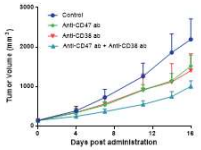
MDA-MB-231 human breast cancer cells were seeded in cultural dish, while human PBMC from a healthy volunteer was isolated and co-cultured with MDA-MB-231. Monocytes isolated from PBMC of the same donor were induced in vitro to form M2 type of macrophages. Then the activated huPBMC, induced M2 type of macrophages, and MDA-MB-231 cells were co-inoculated into the right flank in NCG mice. Mice were randomized right after tumor inoculation and dosing were initiated accordingly. Test ab was administrated at 30 mg/kg via i.p., whereas anti-CD47 ab was dosed at 20 μg/dose through i.v. All dosing were performed every other day for a total of 18 injections, while tumor volume and body weight were measured twice a week.

Application of "wildtype" mice for macrophage-involved in vivo efficacy evaluation

As cross reaction between humanized ab (e.g.> anti-CD47 ab versus murine macrophage, or Fc versus murine NK cells) and murine immune cells, specific strain of mice (e.g.> NCG mice with functional murine macrophage, despite the deficiency of T, B, and NK cells, or SCID mice with both NK cells and macrophage, etc.) can be utilized for in vivo evaluation of anti-tumor effect exerted by antibody.



NALM-6 human B lymphoblastic leukemia cells were inoculated into the right flank in NCG mice. Mice were randomized when average tumor volume grown up to ~120 mm3 and dosing were initiated accordingly. Anti-CD47 ab was dosed at 5 mg/kg through i.v. Dosing were performed bi-weekly for a total of 8 injections, while tumor volume and body weight were measured twice a week.



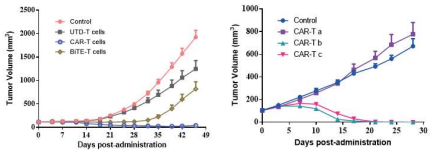
#LD1-0029-361915 human multiple myeloma PDX model was inoculated into the right flank in CB17.SCID mice. Mice were randomized when average tumor volume grown up to ~150 mm3 and dosing were initiated accordingly. Anti-CD47 ab and anti-CD38 ab, either in monotherapies or in combination, were dosed at 1 mg/kg through i.p. Dosing were performed bi-weekly for a total of 5 injections, while tumor volume and body weight were measured twice a week.

Macrophage-involved binding and ADCP in vitro using fresh tissues directly from patients

For macrophage-involved in vitro assay, LIDE provides unique platforms for binding and ADCP assays using fresh tissues generated directly from patients.See more about **Pre-Clinical Trial Using Fresh Tumor Directly from Patients**.

CAR-T in vivo efficacy evaluation

LIDE provides in vivo efficacy evaluation of various CAR-T therapies in either CDX or PDX models.

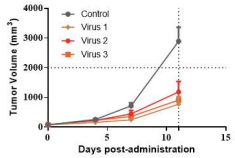


SK-OV-3 human ovarian cancer cells were inoculated into the right flank in NCG mice. Mice were randomized when average tumor volume grown up to ~120 mm3 and dosing were initiated accordingly. Varied T cells were dosed at *107 level through i.v. for 2 injections at Day0 and Day3, while tumor volume and body weight were measured twice a week.

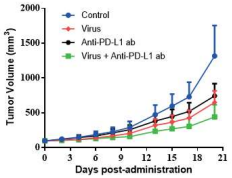
#LD1-0032-200670 human ovarian PDX model were inoculated into the right flank in NCG mice. Mice were randomized when average tumor volume grown up to ~110 mm3 and dosing were initiated accordingly. Varied CAR-T cells were dosed once at Day0, while tumor volume and body weight were measured twice a week.

Oncolytic virus in vivo efficacy evaluation

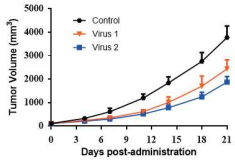
LIDE provides in vivo efficacy evaluation of oncolytic virus therapies in multiple models, including murine/humanized syngeneic models, CDX, PDX, or CDX/PDX with mice humanized.



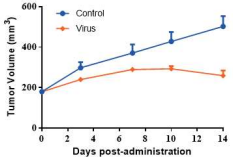
B16-F10 murine melanoma cells were inoculated into the right flank in C57BL/6n mice. Mice were randomized when average tumor volume grown up to ~90 mm3 and dosing were initiated accordingly. Varied oncolytic virus were dosed at *108 pfu/mouse through intratumorally every other day for 3 injections, while tumor volume and body weight were measured twice a week.



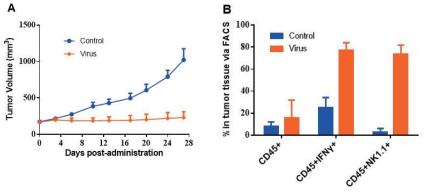
MC38 murine colon cancer cells were overexpressed with TAA1 (MC38-TAA1) and were inoculated into the right flank in huPD-L1 transgenic mice. Mice were randomized when average tumor volume grown up to ~90 mm3 and dosing were initiated accordingly. Oncolytic virus at *109 pfu/mouse through intratumorally for 3 injections at Day0, Day2, and Day4, and anti-PD-L1 ab at 3 mg/kg via i.p. bi-weekly for 9 injections were administrated in either monotherapy or combination way, while tumor volume and body weight were measured twice a week.



Huh7 human Hepatocarcinoma cells were inoculated into the right flank in Balb/c nude mice. Mice were randomized when average tumor volume grown up to ~100 mm3 and dosing were initiated accordingly. Varied oncolytic virus were dosed at *1010 pfu/mouse through intratumorally every other day for 10 injections, while tumor volume and body weight were measured twice a week.



PC-3 human prostate cancer cells were inoculated into the right flank in NSG mice, while the mice were reconstituted with huPBMC via i.v. injection when average tumor volume grown up to ~100 mm3. Mice were randomized 3 days after huPBMC reconstitution and dosing were initiated accordingly. Oncolytic virus were dosed at *105 pfu/mouse through intratumorally on Day0 and Day7 post-grouping, while tumor volume and body weight were measured twice a week.



#LD1-0025-200717 human NSCLC PDX model were inoculated into the right flank in NU/NU mice. Mice were randomized when average tumor volume grown up to ~200 mm3 and dosing were initiated accordingly. Oncolytic virus were dosed through intratumorally every other day for 5 injections, while tumor volume and body weight were measured twice a week (A). Indicated markers were analyzed via FACS using tumor tissues generated at the end of the study (B).