#1391

PBMC Humanized Mice Model for Immuno-Oncology Drug Evaluation

[1]Bin Xie, [1]Xin Hou, [1]Hanglu Chen, [1]Pengfei Yang, [1]Yanbing Zhou and [1]*Danyi Wen
[1]LIDE Biotech, Shanghai, China; * Corresponding author: danyi.wen@lidebiotech.com

Abstract

Since the first immune checkpoint blocker ipilimumab was approved by the US FDA in 2011, more drug companies have sought to develop their own immune-therapy drugs. Humanized peripheral blood mononuclear cell (PBMC) reconstitution in immune deficient mice is becoming a valuable model for evaluating therapeutic antibodies[1], especially bispecific antibodies (BsAbs), which can mediate immune cells as well as target tumor antigens.

However, this model has several drawbacks, including a limited dosing regimen window due to graft-versus-host-disease (GvHD) after 30-40 days of reconstitution, and insufficient natural immune cell infiltration from reconstituted circulatory system. This has hindered wide application of the model in the development of multiple immune checkpoint inhibitors or immune agonists.

To overcome this, LIDE has developed a unique human PBMC and cancer cell co-inoculation model. Cancer-priming PBMCs were mixed with the fresh cancer cells in MatriGel, co-inoculated into NCG mice to form a huPBMC well-infiltrated tumor tissue for immunotherapy. Additionally, we also developed a unique PBMC donor selection method for investigation of immuno-oncology (IO) drugs in classic PBMC humanized models, which could maximize in-vivo efficacy within the right dosing window. This novel method has successfully helped evaluate biological function of PD1, Tigit, PVRIG, CD73, CD47, CD38, CD40, GITR antibodies, Tgfb1/PDL1 BsAb, DLL3/CD3 BsAb in multiple cancers, such as melanoma, breast cancer and lung cancer.

Materials and Methods

Cancer cell lines: Cancer cell lines, such as A375, shp77, MDA-MB-231, Raji were from either ATCC, China Cell Bank (Shanghai) or our collaborators. Hek293-CLDN18.2 and N87-CLDN18.2 were provided by our collaborators.

PDX model from surgery tumor tissues: Fresh human tumors were received from collaborated hospitals (HMEC approval). NCG mice were used to grow the primary tumor tissues.

Human peripheral blood mononuclear cell (PBMC) culture and activation: All human PBMCs came from a commercial source. HuPBMC were primed with target cancer cells (either CDX or PDX) with various E/T ratios dependent on different models with IL2 for one week. For human macrophage M2 polarization and dendritic cell differentiation, human monocyte were isolated using CD14 microbeads (Miltenyi) and cultured with either M-CSF or GM-CSF plus IL4, irrespectively.

Human PBMC and cancer cells co-inoculated mice model for IO drug test: Activated PBMC cells mixed with the target cancer cells based on a particular E/T ratio in MatriGel (BD *or* Gibco) and then co-transferred (subcutaneous injection) into immune deficient recipients (such as NCG). When tumors reached 100mm^3, mice were randomly divided into several groups and were given drug treatment according to the experiment design.

Donor selected Classic PBMC humanized mice model for IO drug test: Target cancer cells (CDX or PDX) were subcutaneously engrafted into NCG mice. Once tumor growth reached to 100mm^3, selected huPBMC donor cells were intravenously injected into tumor-bearing mice and drugs were investigated during or after human immune system reconstitution.

Results

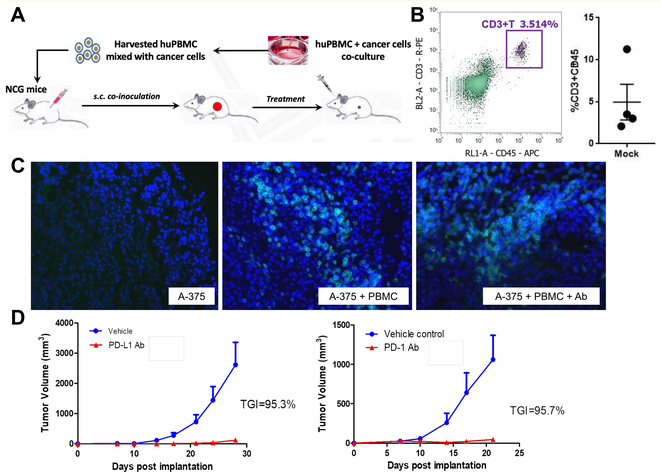

Figure 1. Establishment of human PBMC and cancer cells co-inoculated model for immuno-oncology drug investigation.
A. Schematic of human PBMC and cancer cell co-transfer in-vivo model. **B.** Flow cytometry analysis of tumor infiltrating CD3+T cell percentage in total live cells at day 25 after human PBMC and cancer cell (A375) co-transfer. **C.** Example of immunofluorescent images of the mixed cells in human PBMC and A375 co-implantation model (DAPI: Blue, CD3: Green). **D.** In-vivo efficacy of the therapeutic anti-PDL1, anti-PD1 antibodies in A375/huPBMC co-inoculated NCG mice model.

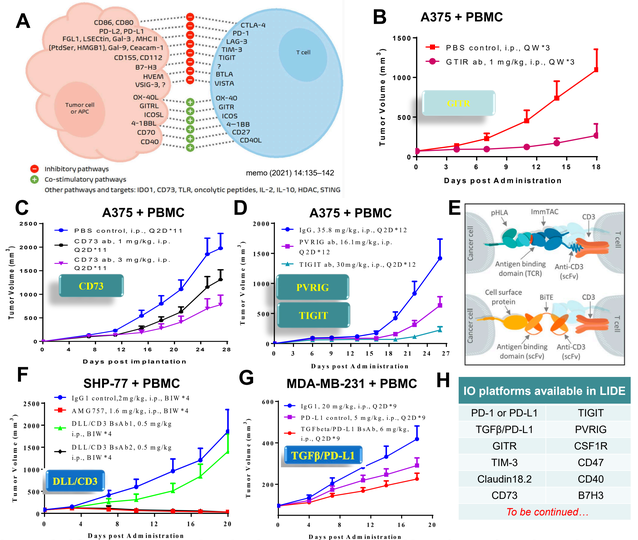

Figure 2. IO drug evaluation by human PBMC/CDX co-inoculated model.
A. Overview of popular cancer targets for immunotherapy[2]. **B-D.** In vivo efficacy of Anti-GITR, anti-CD73, anti-PCRIG and anti-Tigit antibodies in human PBMC/A375 co-transfer mice model. **E.** Design and work model of immTAC and BITEs[3]. **F-G.** In vivo function of DLL/CD3-bispecific antibody or T cell engager in huPBMC/SHP77 co-transfer model, and anti-Tgfb1/PDL1 bsAb in huPBMC/MDA-MB-231 mice model. **H.** IO targets available for functional study on LIDE platform

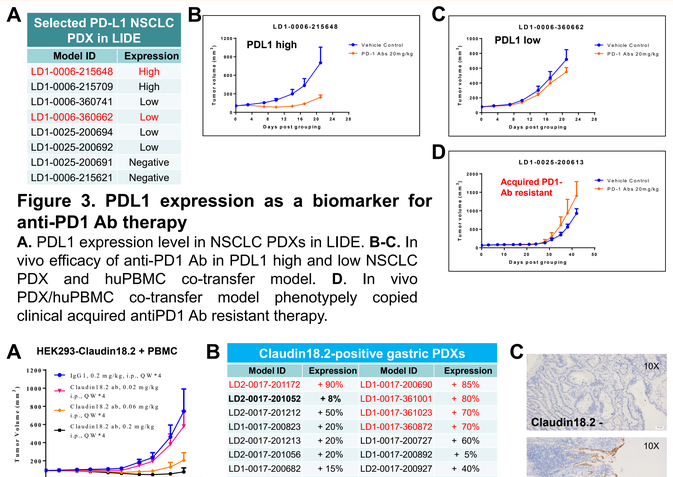

Figure 3. PDL1 expression as a biomarker for anti-PD1 Ab therapy
A. PDL1 expression level in NSCLC PDXs in LIDE. **B-C.** In vivo efficacy of anti-PD1 Ab in PDL1 high and low NSCLC PDX and huPBMC co-transfer model. **D.** In vivo PDX/huPBMC co-transfer model phenotypely copied clinical acquired antiPD1 Ab resistant therapy.

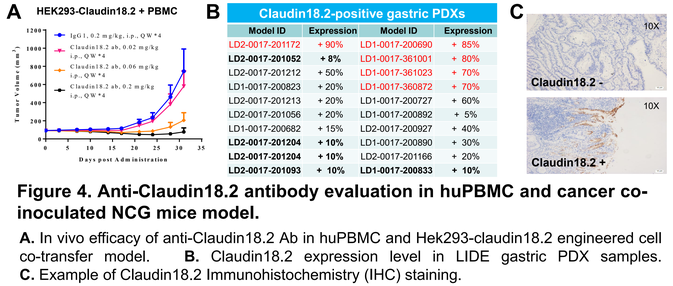

Figure 4. Anti-Claudin18.2 antibody evaluation in huPBMC and cancer co-inoculated NCG mice model.
A. In vivo efficacy of anti-Claudin18.2 Ab in huPBMC and Hek293-claudin18.2 engineered cell co-transfer model. **B.** Claudin18.2 expression level in LIDE gastric PDX samples. **C.** Example of Claudin18.2 Immunohistochemistry (IHC) staining.

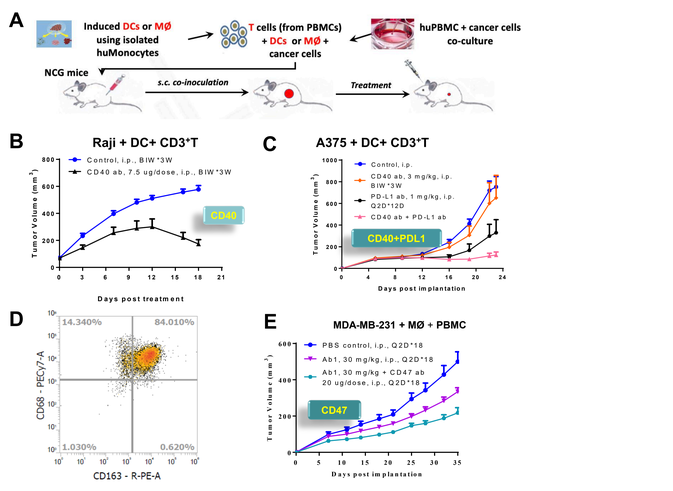

Figure 5. Immuno-oncology drugs targeting dendritic cell and macrophage evaluated in huPBMC/cancer co-inoculated mice model.
A. Schematic of human PBMC, dendritic cell (or macrophage) and cancer cell co-transfer in vivo model. **B.** In vivo efficacy of anti-CD40 antibody in huPBMC/DC/Raji co-inoculated NCG mice. **C.** Anti-CD40 plus anti-PDL1 antibody combination therapy in human PBMC/DC/A375 cancer co-transfer NCG model. **D.** Flow cytometry analysis macrophage M2 polarization by CD163 and CD68 expression. **E.** Anti-CD47 Ab synergized with other antibody in triple negative breast cancer/M2/huPBMC co-inoculated mice model.

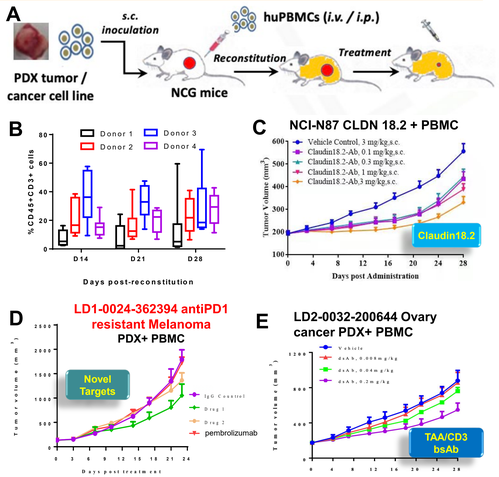

Figure 6. Donor selected Classic PBMC humanized mice model for immuno-oncology drug in vivo evaluation
A. Schematic of donor selected classic PBMC humanized NCG model: Selected fresh PBMC cells were transferred into tumor-bearing NCG mice, then mice were treated with different groups of medicine. **B.** Flow cytometry analysis of human immune system (CD3+T cell) reconstitution efficiency at different days after PBMC implantation. **C.** In vivo efficacy of α-Claudin18.2 Ab in PBMC humanized NCI-N87-CLDN18.2 model. **D.** In vivo function of novel drugs in pembrolizumab resistant humanized melanoma PDX model. **E.** In vivo function of a TAA/CD3 bsAb in humanized ovary cancer PDX model.

Summary and Conclusion

- A LIDE specific human PBMC and cancer cell co-inoculated mice model were well established, succeeded in evaluating various immuno-oncology drugs in vivo, including immune checkpoint blockers and immune agonists.
- Classic PBMC humanized PDX mice model were optimized after donor selection, ready for *in vivo* testing of different kinds of IO drugs.

References

1. Silvia Guil-Luna, et al. Humanized Mouse Models to Evaluate Cancer Immunotherapeutics. Annual Review of Cancer Biology. 2021, Vol. 5:119-136.
2. Nina Zila, et al. Novel immune checkpoints beyond PD-1 in advanced melanoma. memo - Magazine of European Medical Oncology volume 14, pages135–142 (2021).
3. Kate L Lowe, et al. Novel TCR-based biologics: mobilising T cells to warm 'cold' tumours. Cancer Treat Rev. 2019 Jul;77:35-43.

Shanghai LIDE Biotech, Co. Ltd.
887 ZuChongZhi Rd, Building 77-78, 3rd F, Pudong, Shanghai 201203. P.R. China
Call 1-276-296-LIDE
Email business@lidebiotech.com

Poster Download

www.lidebiotech.com