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Novel Immuno-Oncology(IO) drugs evaluation by humanised immune cells and cancer co-inoculated models

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Abstract

Currently more and more immunotherapeutic drugs and engineered cells have been developed to boost the human immune system against cancer. While humanized peripheral blood mononuclear cell (huPBMC) reconstitution in immune deficient mice is a straight-forward model for evaluating therapeutic antibodies[1], this conventional model has several drawbacks to hinder its widespread use, including the onset of graft-versus-host-disease (GVHD) after one month of reconstitution, insufficient immune cell infiltration from reconstituted circulatory system and minimal retention of functional innate immune cells, such as macrophages and nature killer cells.

To overcome this, LIDE has developed a specific human immune cell and cancer cell co-inoculation model. Cancer-priming PBMC and/or its derivates were well mixed with the fresh cancer cells in MatriGel, co-transferring into NCG mice to form a relatively "hot tumor" tissue for immunotherapy, including drugs targeting T cells, dendritic cells and macrophages. This novel method has successfully helped evaluate biological function of immune checkpoint blockers (like PD1, Lag3, Tim3, Tigit antibodies) and immune agonists (GITR, CD40, 4-1BB antibodies) in multiple cancers, such as melanoma, breast cancer, lung cancer, hepatocellular carcinoma and ovarian 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.

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 its derived macrophages or dendritic cells) and cancer cells co-inoculated mice model for IO drug test: Activated PBMC cells (and differentiated macrophages or dendritic 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, given drug treatment based on the experiment design.

Flow cytometry detection: Tumor tissues after collagen digestion, and peripheral blood after RBC-lysis, were labelled with different fluorescent antibodies at 4℃ for 25min. After washing, all the samples were resuspended in PBS with 2%FBS and loaded into Attune NxT Flow Cytometer for analysis.

Results

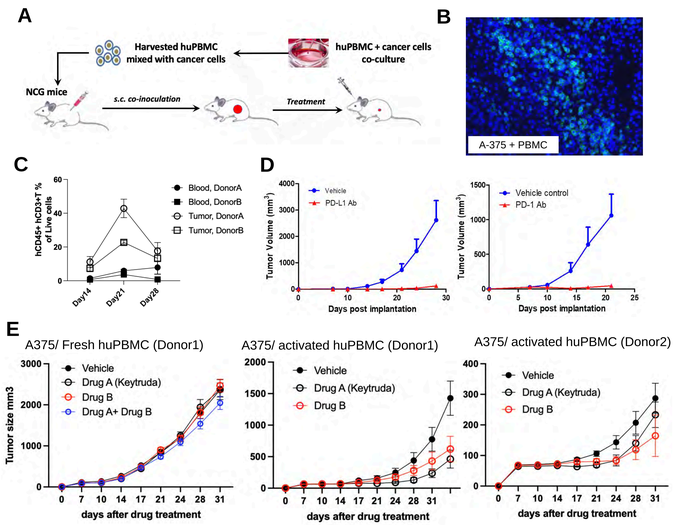


Figure 1. Establishment and validation of human PBMC and cancer cells co-inoculated model for immunotherapy

A. Schematic of human PBMC and cancer cell co-transfer in-vivo model. **B.** Example of immunofluorescent images of the mixed cells in human PBMC and A375 co-implantation model (DAPI: Blue, CD3: Green). **C.** Dynamic change of human T cells in huPBMC and cancer cell (A375) co-transfer by FACS. **D.** In-vivo efficacy of the therapeutic anti-PDL1, anti-PD1 antibodies in A375/huPBMC co-inoculated NCG mice model. **E.** The comparison of in-vivo efficacy for A375/ fresh PBMC co-transfer model and A375/ activated PBMC co-transfer model.

A

Selected PD-L1 NSCLC PDX in LIDE	
Model ID	**Expression**
LD1-0006-215648	High
LD1-0006-215709	High
LD1-0006-360741	Low
LD1-0006-360662	Low
LD1-0025-200694	Low
LD1-0025-200692	Low
LD1-0025-200691	Negative
LD1-0006-215621	Negative

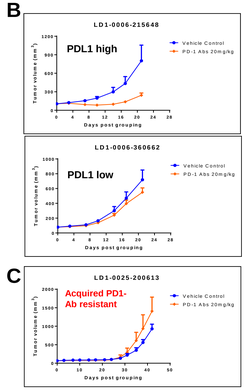
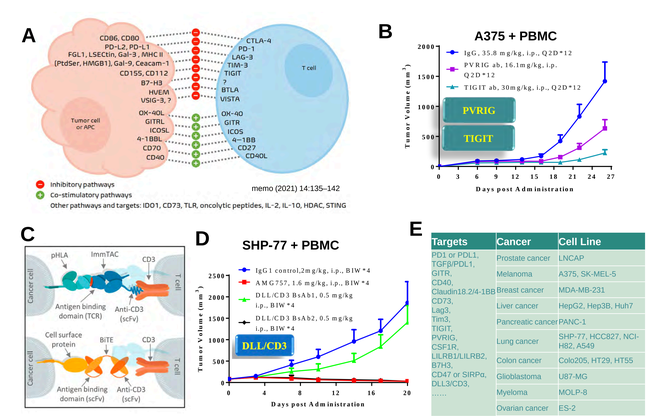


Figure 2. PDL1 expression as a biomarker for antiPD1 Ab therapy In huPBMC/PDX co-inoculated model

A. PDL1 expression level in NSCLC PDXs in LIDE. **B.** In vivo efficacy of anti-PD1 Ab in PDL1 high and low NSCLC PDX and huPBMC co-transfer model. **C**. In vivo PDX/huPBMC co-transfer model phenotypey copied clinical acquired anti-PD1 Ab resistant therapy.



E

Targets	Cancer	Cell Line
PD1 or PDL1, TGFβ/PDL1, GITR, CD40, Claudin18.2/4-1BB, CD73, Lag3, Tim3, TIGIT, PVRIG, CSF1R, LILRB1/LILRB2, B7H3, CD47 or SIRPα, DLL3/CD3, ...	Prostate cancer	LNCAP
	Melanoma	A375, SK-MEL-5
	Breast cancer	MDA-MB-231
	Liver cancer	HepG2, Hep3B, Huh7
	Pancreatic cancer	PANC-1
	Lung cancer	SHP-77, HCC827, NCI-H82, A549
	Colon cancer	Colo205, HT29, HT55
	Glioblastoma	U87-MG
	Myeloma	MOLP-8
	Ovarian cancer	ES-2

Figure 3. IO drug evaluation by human PBMC/CDX co-inoculated model.

A. Overview of popular cancer targets for immunotherapy[2]. **B.** In vivo efficacy of anti-TIGIT or anti-PVRIG in huPBMC/A375 co-transfer mice model. **C**. Design and work model of immTAC and BITEs[3]. **D.** In vivo function of DLL3/CD3-bispecific antibody or T cell engager in huPBMC/SHP77 co-transfer model. **H.** LIDE IO platform available for functional study.

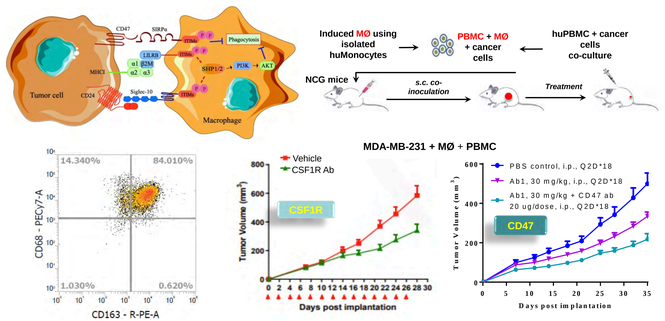
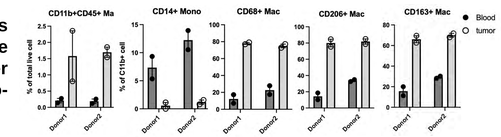


Figure4. IO drugs targeting macrophage evaluated in cancer and Mac/huPBMC co-transfer mice model.



A. Signal pathway for macrophage mediated tumor antigen recognition[4] **B.** Schematic of human PBMC, dendritic cell (or macrophage) and cancer cell co-transfer in vivo model. **C.** Flow cytometry analysis macrophage M2 polarization by CD68 and CD163 expression before in vivo injection. **D.** In vivo efficacy of AntiCSF1R Ab or Anti-CD47 Ab combo-therapy in triple negative breast cancer/M2/huPBMC co-inoculated mice model. E. FACS analysis of both blood and cancer tissues for human macrophage track on day21 after co-transfer in prostate cancer LNCAP/ M2/huPBMC model.

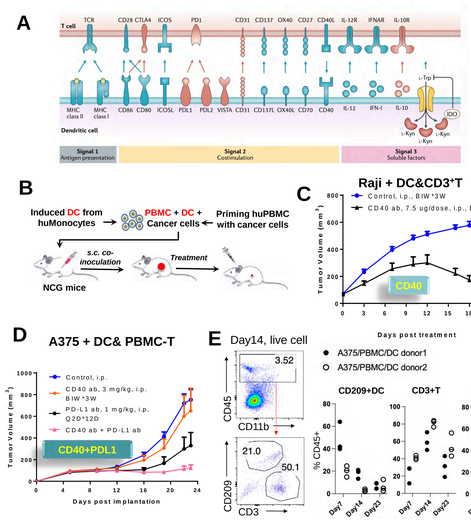


Figure 5. IO drugs targeting dendritic cell evaluated in huPBMC/cancer co-inoculated mice model.

A. Induction of T cell-mediated immunity or tolerance by DCs[5]. **B.** Schematic of human PBMC, dendritic cell (or macrophage) and cancer cell co-transfer in vivo model. **C.** In vivo efficacy of anti-CD40 antibody in huPBMC/DC/Raji co-inoculated NCG mice. **D.** Anti-CD40 and anti-PDL1 combo-therapy in huPBMC/DC/A375 cancer co-transfer model. **E.** FACS analysis and quantification of dendritic cell and T cell phenotype by CD209, CD3, CD25 and PD1 expression in A375 melanoma tumor microenvironment.

Summary and Conclusion

- A LIDE specific activated 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.
- Innate myeloid cells, like macrophage and dendritic cell could be well existed after 3 weeks in our Cancer cell and Immune cell co-inoculated models.

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.

4.Yingqi Qiu, et al. Next frontier in tumor immunotherapy: macrophage-mediated immune evasion. Biomark Res . 2021 Oct 9;9(1):72.

5. Stefanie K Wculek, et al. Dendritic cells in cancer immunology and immunotherapy. Nat Rev Immunol . 2020 Jan;20(1):7-24.

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