#117

Improved PBMC humanised mouse models for evaluation of immuno-oncology drugs

[1]Bin Xie, [1]Xin Hou, [1]Pengfei Yang, [1]Yanbing Zhou and [1]*Danyi Wen

[1]LIDE Biotech, Shanghai, China; * Corresponding author: danyi.wen@lidebiotech.com

Abstract

Although anti-PD(L)1 therapy could significantly improve survival outcomes for patients with metastatic or unresectable solid cancers, such as melanoma, non-small cell lung cancer and MSI-H colorectal cancer, more than half of these patients do not respond to immune checkpoint blockade (ICB) therapy, and 90% of colorectal cancer patients have MSS/pMMR characteristics and almost have no response at all, which drives scientists to design novel drug candidates urgently and integrate tumor and tumor microenvironment (TME) signals systemically to rejuvenate exhausted immune system and overcome ICB resistance.

Along with establishing standard cancer biobanks (PDX, patient-derived xenografts; CDX, cell derived xenograft models), LIDE has developed IO-centric options, including traditional PBMC humanised mice models, and human PBMC/immune cells and cancer co-inoculated models. By leveraging LIDE's translational network for unique PBMC donor selection, this platform has helped decipher potential key factors in TME. For example, cancer biopsies with MDM2 amplification or DNMT3A alternation have been shown resistant to PD1-Ab therapy but sensitive to chemical combo-therapy. Also pMMR cancer PDX models seem responsive to EGFR targeted, CD39-CD73-adenosine targeted therapy. Human IL15 transgenic mice are also a useful tool to investigate in vivo function of Natural killer (NK) cells, NK engagers/modulators as well as drugs with ADCC effect.

The cancer-priming-immune cells and cancer co-transfer model can significantly delay graft-versus-host reaction, often for another 4 weeks, and achieves maximal therapeutic effect of the testing drugs by pre-activating T cells with specific tumor antigens. Dendritic cells or macrophages could be successfully introduced into this co-inoculated system to work together with T cells to mimic human tumor tissue complexity and help determine the overall effect induced by integrated signals. It seems CD40 agonist or CD47 antagonist could synergise well with anti-PD(L)1 to control melanoma and triple negative breast cancer development, respectively. This optimised PBMC humanised cancer-bearing mice models have been tested for various cancer targets, e.g. PD(L)1, Tim3, Lag3, Tigit, PVRIG, TGFβ1, CD47/Sirpa, DLL3/CD3, Claudin18.2/4-1BB, B7-H3, GITR, to better understand the interaction between cancer cells and immune cells.

Materials and Methods

Cancer cells: Cancer cell lines, e.g. A375, shp77, MDA-MB-231, were from either ATCC, China Cell Bank (Shanghai) or our collaborators. PDX models, fresh human tumors (surgery) were received from collaborated hospitals (HMEC approval). NCG mice are used to grow the primary tumor tissues.

Human peripheral blood mononuclear cell (PBMC) culture and activation: hPBMCs came from a commercial source. hPBMCs were used freshly or primed with target cancer cells with various E/T ratios plus IL2 for one week. For human macrophage M2 polarization and dendritic cell differentiation, human monocyte were isolated using CD14 microbeads and cultured with either M-CSF or GM-CSF+IL4, respectively. Human NK cells were directly expanded from PBMC via using IL21 expressing stroma system.

Human PBMC humanized traditional cancer-bearing mice model: PDX or CDX cancer cells were subcutaneously implanted into NCG mice and once the tumors reached proper size, selected human PBMC(and/or NK) donor cells were transferred by i.p. or i.v., mice could be dosed with drugs after the human immune system reconstructed successfully.

Human PBMC and cancer cells co-inoculated mice model: 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) and then subcutaneously co-transferred into immune deficient recipients (e.g. 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

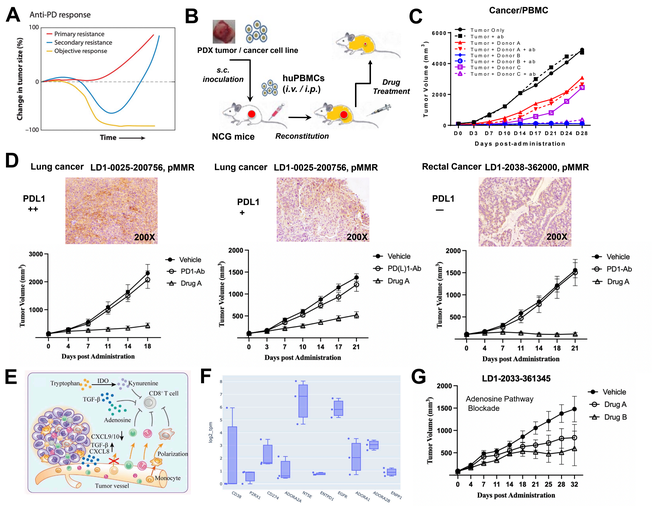

Figure 1. Novel cancer drugs evaluation in selected PBMC humanized cancer-bearing mice models.

A. Clinical responses to anti-PD therapy[1]. **B.** Schematic of PBMC humanized mice model. **C.** LIDE unique PBMC donors *in vivo* selection method. **D.** PDL1 IHC analysis and *in vivo* efficacy of drug A in three clinical PD(L)1-antibody therapy resistant PBMC/PDX models. **E.** TME contains diverse immunosuppressive cells that influence anti-PD(L)1 efficacy through inhibiting T cell reactivity[2]. **F.** PDL1, EGFR and adenosine pathway gene expression in three pMMR pancreatic models. **G.** Adenosine targeted therapy in PBMC/pMMR LD1-2033-361345 pancreatic cancer model.

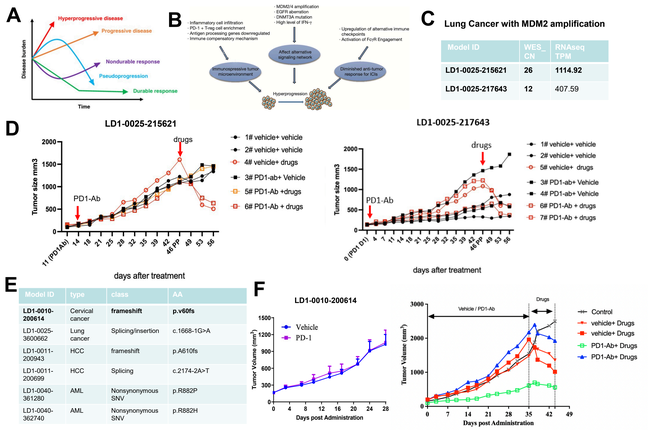

Figure 2. New combination therapy to overcome anti-PD(L)1 induced hyperprogressive disease (HPD).

A. Potential outcomes after ICB therapy over time py[3]. **B.** Potential mechanisms of HPD[4]. **C.** MDM2 gene amplification in two lung cancer PDX samples. **D.** Chemical combination therapy overcomes PD1 antibody resistance in MDM2amp models. **E.** DNMT3a mutation in LIDE PDX library. **F.** Targeted/chemical combo-therapy well suppresses PD1-Ab resistant cervical cancer LD1-0010-200614 development.

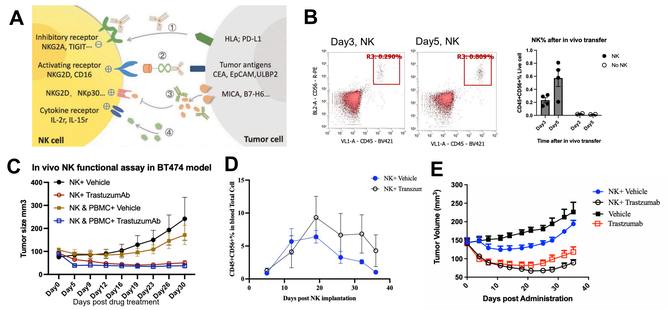

Figure 3. Evaluation of NK cell targeted therapy in hIL15-NXG mice.

A. Current strategies to harness NK cell in cancer immunotherapy[5]. **B.** Percentage of human NK cells in mice peripheral blood after in vivo transfer. **C.** In vivo efficacy of PBMC derived NK and trastuzumab combo-therapy. **D.** In vivo dynamics of human NK survival after trastuzumab treatment. **E.** Contribution of human NK and trastuzumab for in vivo breast cancer suppression.

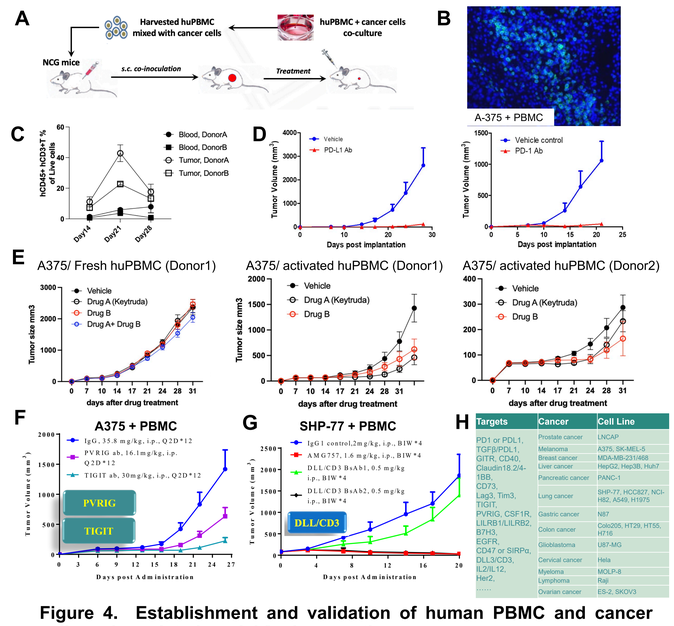

Figure 4. Establishment and validation of human PBMC and cancer cells co-inoculated model for immunotherapy

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-transfer model (DAPI: Blue, CD3: Green). **C.** Dynamic change of human T cells in huPBMC 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. **F.** In vivo efficacy of anti-TIGIT or anti-PVRIG in huPBMC/A375 co-transfer mice model. **G.** 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.

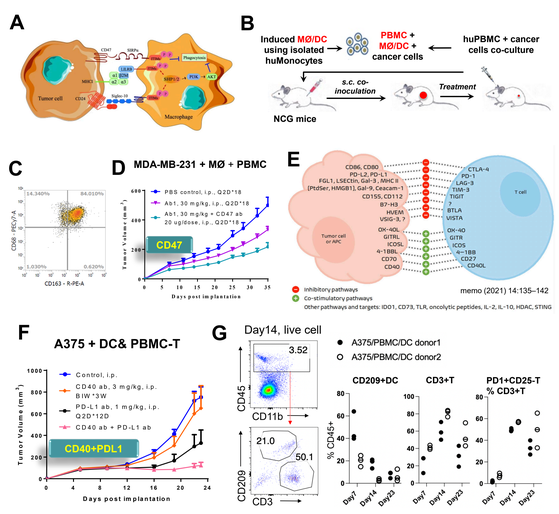

Figure 5. IO drugs targeting innate immune cells evaluated in huPBMC/cancer co-inoculated mice model.

A. Signal pathway for macrophage mediated tumor antigen recognition[6]. **B.** Schematic of human PBMC, macrophage (or dendritic cell) and cancer cell co-transfer model. **C.** FACS analysis macrophage M2 polarization by CD68 and CD163 expression before in vivo injection. **D.** In vivo efficacy of CD47 Ab combo-therapy in triple negative breast cancer/M2/huPBMC co-inoculated mice model. **E.** Overview of popular cancer targets for immunotherapy[7]. **F.** Anti-CD40 and anti-PDL1 combo-therapy in huPBMC/DC/A375 cancer co-transfer model. **G.** FACS analysis of dendritic cell and T cell phenotype by CD209, CD3, CD25 and PD1 expression in A375 melanoma tumor microenvironment.

Summary and Conclusion

- Novel IO and targeted therapies were successfully tested to overcome PD(L)1 antibody therapy resistance in optimized traditional PBMC humanized models.
- A LIDE specific activated human PBMC and cancer cell co-inoculated mice model succeeded in evaluating various IO drugs *in vivo,* including ICB and agonists.
- Innate myeloid cells, like NK, macrophage and dendritic cell could be well survived by using different strategies to evaluate new cancer therapies.

References

1. Vesely MD, et al. Resistance Mechanisms to Anti-PD Cancer Immunotherapy. Annu Rev Immunol. 2022 Apr 26;40:45-74.
2. Lei Q, et al. Resistance Mechanisms of Anti-PD1/PDL1 Therapy in Solid Tumors. Front Cell Dev Biol. 2020 Jul 21;8:672.
3. Adashek JJ, et al. Hyperprogression and Immune Checkpoint Inhibitors: Hype or Progress? Oncologist. 2020 Feb;25(2):94-98.
4. Lin M, et al. Hyperprogression under immunotherapy: a new form of immunotherapy response?-a narrative literature review. Transl Lung Cancer Res. 2021 Jul;10(7):3276-3291.
5. Wang F, et al. The role of natural killer cell in gastrointestinal cancer: killer or helper. Oncogene. 2021 Jan;40(4):717-730.
6. Yingqi Qiu, et al. Next frontier in tumor immunotherapy: macrophage-mediated immune evasion. Biomark Res . 2021 Oct 9;9(1):72.
7. Nina Zila, et al. Novel immune checkpoints beyond PD-1 in advanced melanoma. memo - Magazine of European Medical Oncology volume 14, pages135–142 (2021).