A novel anti-tumor anti-PDL1-IL7 immunocytokine targeting lymphocytes

Feifei Cui, Lei Fang, Haijuan Gu, Yuanuyan Yang, Zhengyi Wang, Taylor B. Guo
Suite 500, 2275 Research BLVD, Rockville Maryland 20850, I-Mab Biopharma US
I-Mab Biopharma, Inc., Shanghai, China

ABSTRACT
Lymphocyte counts in the peripheral immune system and tumor are correlated with positive clinical outcomes in PD-(L)1 immunotherapy. Interleukin 7 (IL7) is an immune homeostasis cytokine for T cells. Treatment with recombinant human IL7 preferentially expands recent thymic emigrants, naive and central memory T cells, but spares regulatory T cells. Here we constructed a series of immunocytokine L1I7 comprised of a PDL1 blocking mAb fused with an IL7 molecule with varying degree of attenuated potency. The aim of the study was to identify the right molecule that achieves enhanced biodistribution at the tumor site and an optimal balance between synergistic anti-tumor activity and an acceptable safety profile.

MATERIALS AND METHODS
Affinity measurement by Biacore. PDL1 binding to human PDL1-his protein were evaluated by BioCore T200.
PDL1/PDL1 cell-based functional assay. Jurkat-PDL1-NFAT-reporter cells were incubated with Raji-PDL1 cells for 4 hours in the presence of TCR stimulation and a-PDL1 or L1I7 fusion proteins. NFAT-reporter induced florescence signal were detected by One-Glu (Promega).

IL7 receptor (IL7R) binding and internalization assay. Human primary CD4+ T cells were isolated from PBMC. The cells were incubated at 4 °C in the presence of L1I7 or α-PDL1. PE-conjugated anti-human Fc was used to detect the binding of L1I7 to cells. For internalization assay, the cells were incubated at 37 °C in the presence of L1I7 or α-PDL1 for 15 minutes followed by staining with anti-human IL7R/ FACS antibody.

IL7 induced pSTAT5 signaling assay. Human primary CD4+ T cells were incubated in the presence of L1I7 or free IL7 at 37 °C for 15 minutes. P-STAT5 was detected by FACS staining.

IL7-induced T cell proliferation assay. Human primary CD4+ T cells were incubated with L1I7 or free IL7 at 37 °C for 7 days. Ki67 was detected by FACS staining.

T cells and dendritic cells (DCs) coculture assay. Human CD14+ monocytes were isolated from PBMCs and then differentiated into immature DCs in vitro. Human CD4+ T cells were isolated from a different donor and cocultured with DCs in the presence of α-PDL1, IL7, combination of α-PDL1 and IL7, or L1I7. The concentration of IFNγ in the supernatant was detected by LANCE (PerkinElmer).

In vivo tracking assay. Fluorescence-labeled L1I7 was injected into HCC827-transplanted CD3+ hematopoietic stem cells humanized mice when the tumor grew up to about 500 mm³. The distribution of the labeled proteins was monitored by in vivo imaging.

In vivo B16F10 melanoma syngeneic mice model. C57/B16 mice were subcutaneously implanted with 1×10⁶ B16F10 cells on day 0. Mice were randomized into indicated treatment groups (N=8/group) and intraperitoneally administered indicated antibodies every 4 days for 4 doses. Tumor volumes were monitored by caliper measurement twice per week for the duration of the experiment. Absolute numbers of spleenic and tumor-infiltrating CD4+ T and CD8+ T cells were analyzed by FACS at the end of the study.

RESULTS
Characterization of α-PDL1 activity in L1I7

Figure 1. (A) Affinity measurement of α-PDL1 and L1I7 to human PDL1-his by BioCore T200. (B) Binding of α-PDL1 and L1I7 to human PDL1 protein by ELISA. (C) Binding affinity of α-PDL1 and L1I7 to PDL1 overexpressed on Raji. (D) Cell-based functional assay to evaluate the anti-PDL1 activity of α-PDL1 and L1I7.

In vitro characterization of IL7 activity in L1I7

Figure 2. (A) Binding of various receptor forms of L1I7 and α-PDL1 (negative control) to IL7R expressed on human primary CD4+ T cells. (B) Internalization of IL7R expressed on human primary CD4+ T cells induced by IgG1 of L1I7 or α-PDL1 (negative control). (C) P-STAT5 signaling on human primary CD4+ T cells induced by L1I7 or Fc-IL7 (positive control). (D) Human IL7 or L1I7 induced CD4 proliferation as measured by Ki67 expression.

Figure 3. (A) In vivo tracking assay to evaluate the distribution of L1I7 compared with α-PDL1 or IL7 (B) Organ distribution of L1I7.

In vitro and in vivo efficacy of L1I7

Figure 4. (A) The activation of T cells was measured by IFNγ secretion to evaluate the synergistic effect induced by L1I7 by using T cells and DC cell co-culture assay. (B) In vivo efficacy of surrogate IL7 or monotherapy as measured by tumor growth. (C) The absolute numbers of CD4+ and CD8+ T cells in spleen and tumor were measured by FACS analysis. Shown are mean±SEM.

CONCLUSIONS
- L1I7 series of immunocytokine maintained PDL1 binding and antagonist function when compared with PDL1 mAb.
- As compared with wildtype L1I7, L1I7 variants showed attenuated IL7 activity as evidenced by reduced IL7R binding/internalization, p-STAT5 activation and CD4+ T cell proliferation.
- Both wildtype and the attenuated variants were more effective in promoting CD4 T cell activation than anti-PDL1 or IL7 monotherapy in T cells and DC coculture assay.
- Similar with PDL1 mAb, L1I7 was enriched in the tumor site whereas IL7 was widely distributed in in vivo tracking experiment.
- In an anti-PDL1-resistant B16F10 tumor model, L1I7 showed superior efficacy as compared with either anti-PDL1, IL7 alone or in combination, which was correlated with an increased number of tumor-infiltrating CD4+ T and CD8+ T cells.