# Revealing Novel Immune Modulatory Mechanism of Uliledlimab through the Blockade of CD73 Pathway

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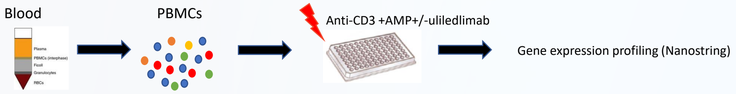
## INTRODUCTION

- Adenosine signaling has emerged as a key immunosuppressive mechanism in the tumor microenvironment, and CD73 is a rate-limiting enzyme for adenosine production.
- Uliledlimab is a differentiated CD73 antibody that binds to a unique epitope to achieve complete inhibition of CD73 and its anti-tumor activity is currently being evaluated in clinical studies in combination with checkpoint inhibitors.
- Here we investigated the immune modulatory mechanism of uliledlimab in different cell subsets and pathways to explore new therapeutic combinations of uliledlimab for cancer treatment.

## METHODS

- *In vitro* PBMC assay in the presence of AMP w/ or w/o uliledlimab




- Analysis of co-expression of CD73 and angiogenesis genes in multiple cancer types and their correlation with survival
  - Association of CD73 and uliledlimab-regulated angiogenesis signature genes identified via Nanostring platform was analyzed in multiple cancer types in TCGA database using Spearman's rank-order correlation test.
  - The Kaplan-Meier method log-rank test were used for the survival analysis of cancer patients with different expression level of CD73 and VEGFA.
- *In vitro* tumor killing assays
  - *In vitro* cytolysis by T cells and NK cells, or phagocytosis by macrophages in response to uliledlimab alone and in combination with PD-1 antibody were evaluated by co-culture of CD73$^+$ tumor cells and PBMCs or purified macrophages in the presence of AMP.
- *In vivo* tumor xenograft model
  - *In vivo* tumor growth inhibition of uliledlimab alone or in combination with PD-1 antibody were evaluated in a NCI-H358 lung cancer model injected with human PBMCs.

## RESULTS

### Immune inhibitory signature genes were up-regulated by AMP that were reversed by uliledlimab treatment

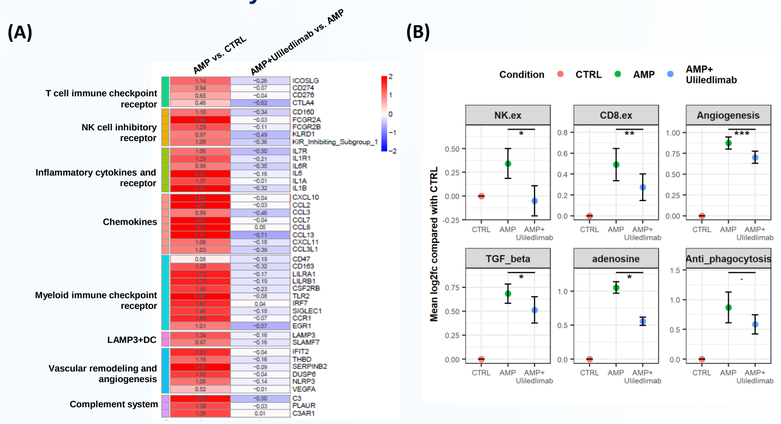



**Figure 1.** Differential gene expression in PBMC by AMP w/ or w/o TJD5 was evaluated using NanoString nCounter®. (**A**) Expressions of immune inhibitory genes classified by different pathways in T cells, myeloid cells and NK cells were up-regulated by AMP. (**B**) Signature genes involved in the recruitment of immuno-suppressive cells and angiogenesis as well were also increased by AMP. The induction of these signature genes by AMP was inhibited by uliledlimab.

### Co-expression of CD73 and angiogenesis genes was associated with poor prognosis in multiple solid tumors

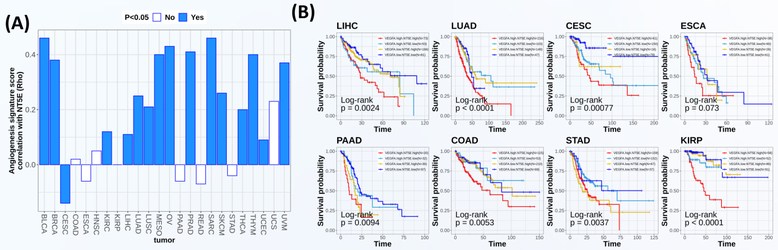



**Figure 2.** The signature genes involved in the angiogenesis pathway were positively correlated with CD73 expression in multiple tumors in TCGA database (p<0.05) (**A**), and co-expression of CD73 and VEGFA at a high level was associated with poor prognosis (p<0.05) (**B**).

### Uliledlimab increased the cytotoxic activity of T cells and NK cells as well as the phagocytic activity of macrophages

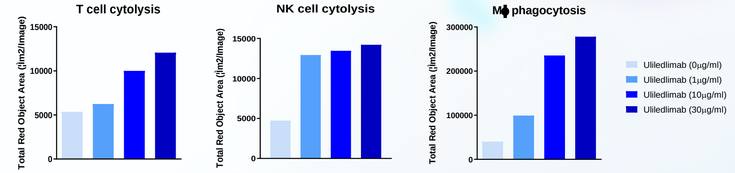



**Figure 3.** *In vitro* cytolysis or phagocytosis of immune cells in response to uliledlimab was investigated by co-culturing CD73$^+$ tumor cells with PBMCs or purified macrophages in the presence of AMP. The activity was measured by pHrodo Red staining.

### Uliledlimab enhanced anti-tumor activity in combination with PD-1 antibody

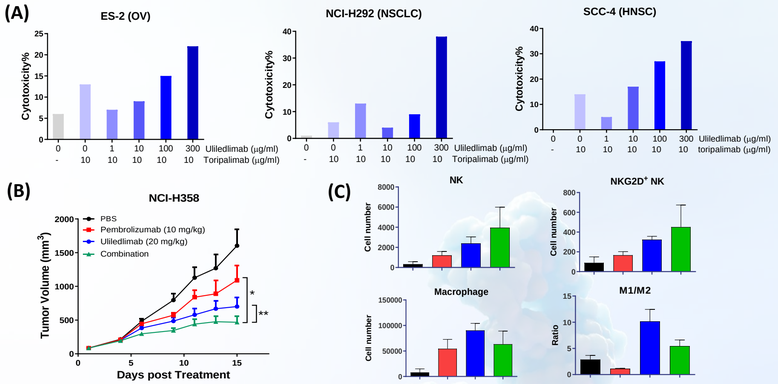



**Figure 4.** Enhanced anti-tumor activity of uliledlimab in combination with PD-1 antibody (A) *In vitro* tumor killing by PBMCs in the presence of uliledlimab and toripalimab measured by CellTiter-Glo (B) *In vivo* anti-tumor activity of uliledlimab and pembrozlimab alone or combination in NCI-H358 model and tumor infiltrating immune cell analysis by FACS (C).

## CONCLUSION

- Our study delineated the immune regulatory mechanism of uliledlimab, including down-regulation of immuno-suppressive pathways and increased cytolysis and phagocytosis by various effector cells.
- In addition to combination with PD-1 inhibitor, identification of new pathways regulated by uliledlimab provides the scientific rationale to explore new combination therapies of uliledlimab in cancer treatment.