**Background**
The adenosine pathway is associated with immunosuppressive 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**
Gene expression of anti-CD3 stimulated human PBMCs in the presence of AMP with or without uliledlimab was measured using NanoString platform. Association of CD73 and immune signature pathways regulated by uliledlimab was analyzed in multiple cancer types in TCGA database. In vitro cytolysis by T cells or NK cells or phagocytosis by macrophages in the presence of uliledlimab alone and in combination with PD-1 antibody were evaluated by co-culture of CD73+ tumor cells and PBMCs or purified macrophages. Anti-tumor activity of uliledlimab combination was examined subsequently in xenograft tumor models.

**Results**
Expression of cell lineage markers in immunosuppressive cell subsets was up-regulated in human PBMCs cultured with AMP. Notably, the expression of various co-inhibitory checkpoint receptors was induced in T cells, including PD-L1 and CTLA-4, myeloid cells, including LILRB and SIGLEC1, and NK cells, including KIR and KLRD1. Signature genes involved in the recruitment of immunosuppressive cells and angiogenesis were also up-regulated. The induction of these genes was inhibited by uliledlimab. Similar to PD-L1, a panel of angiogenesis signature genes regulated by uliledlimab were found to positively correlate with CD73 expression in multiple solid tumors (p<0.05), and concurrent high expression of CD73 and VEGFA was associated with poor prognosis (p<0.05). Uliledlimab treatment led to increased cytotoxic activity of T cells and NK cells and phagocytic activity of macrophage in tumor killing. When combined with a PD-1 antagonist, uliledlimab elicited enhanced anti-tumor activity in vitro and in vivo through the regulation of infiltrating immune cells in tumor.

**Conclusions**
Our study has delineated the immune regulatory properties of uliledlimab as evident by its down-regulation of immunosuppressive pathways and restored activity of cytolysis and phagocytosis of various effector cells. In addition to the current combination therapy of uliledlimab with PD-L1 inhibitor, identification of selected pathways regulated by uliledlimab and their co-expression with CD73 that serves as a negative prognosis factor provides much needed scientific rationale to explore new combination therapies of uliledlimab in cancers.