CD73 is an extracellular ectonucleotidase highly expressed by tumor or stromal cells in the tumor microenvironment. By inducing tumor cell death, conventional anti-cancer therapies induce extracellular release of adenosine triphosphate (ATP), which is degraded by CD39 into adenosine monophosphate (AMP) and then by CD73 into adenosine, an inhibitor of immune response. Blockade of CD73-mediated degradation of AMP may therefore stimulate anti-tumor immunity across a wide range of tumors through preventing the production of adenosine.

IPHS301 is a humanized effector-silent IgG1 monoclonal antibody that selectively binds to and inhibits the activity of both membrane-bound and soluble human CD73. IPHS301 is designed to enhance anti-tumor immune responses by inhibiting the enzymatic activity of CD73 in the tumor microenvironment, thus releasing tumor-infiltrating lymphocytes from adenosine-mediated suppression.

Here, we characterized IPHS301 properties and efficacy in vitro and described the expression of CD73 in human solid tumors.

**Background**

**Mechanism of Action**

**FIGURE 1: IPHS301 constrains CD73 in an inactive intermediate conformation**

(A) Electron microscopy negative staining of the CD73/IPHS301 complex. Right panel: CD73 dimer (N-term domain, yellow; C-term domain, green) and IPHS301 (Fab, cyan-blue; Fc, black) on a 2D class average calculated from the recorded pictures (representative of the main complex observed on the grid).


(C) Models extrapolated of the crystal structure of CD73/IPHS301 Fab complex. Left panel: complex in the open conformation of CD73. Right panel: complex in an intermediate conformation of CD73 (preferred model).

**FIGURE 2: IPHS301 blocks enzymatic activity of both soluble and membrane forms of CD73**

(A) D375 human melanoma cells were incubated with AMP (open square) and with increasing concentrations of indicated anti-CD73 mAbs or isotype control mAb (IC). AMP with no source of CD73 (closed square). After 1 h of incubation, AMP remaining in cell supernatant was quantified.

Blood from one representative healthy donor (D) and serum from healthy donors (C) were incubated with IC, IPHS301 (0.01 and 10 µg/mL) or ACP (100 µM) for 1 h and then incubated with AMP. Remaining AMP was measured with AMP-Glo kit. (B) Bars represent means +/- SD of triplicates. One representative experiment out of 3 is shown. (C) Each dot represents one donor. Lines represent means +/- SD. One Way ANOVA followed by Dunnet’s test, ***p<0.0001, **p<0.001.

**FIGURE 3: IPHS301 does not induce CD73 down-modulation and nor system B cell activation**

(A) A375 cells were incubated with IPHS301 (10 µg/mL) at 37°C up to 4h. CD73 expression was analyzed by flow cytometry and normalized to the expression on T0. The mAb CD73.4 was used as positive control.

(B) Peripheral blood mononuclear cells (PBMC) from 5 healthy donors were incubated for 16 hours with IPHS301 (5 µg/mL) or isotype control (IC). Expressions of CD83 and CD69 on B cells were analyzed by flow cytometry and normalized to untreated conditions. The mAb CP-006 was used as positive control. Each dot corresponds to one individual. Bars represent means +/- SD. One way ANOVA followed by Dunn’s test, *p=0.011, **p=0.0099.

These results indicate that IPHS301 blocks CD73 with a differentiated mechanism of action compared to benchmarked anti-CD73 clinical candidates and support the clinical development of IPHS301 for cancer immunotherapy, potentially in combination with chemotherapy or immune checkpoint inhibitors.