IPH5301, a CD73 blocking antibody targeting the adenosine immunosuppressive pathway for cancer immunotherapy

Poster #P323

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Background

CD73 is an extracellular ectonucleotidase highly expressed by tumoral 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.

IPH5301 is a humanized effector-silent IgG_1 monoclonal antibody that selectively binds to and inhibits the activity of both membrane-bound and soluble human CD73. IPH5301 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 IPH5301 properties and efficacy *in vitro* and described the expression of CD73 in human solid tumors.

Mechanism of Action

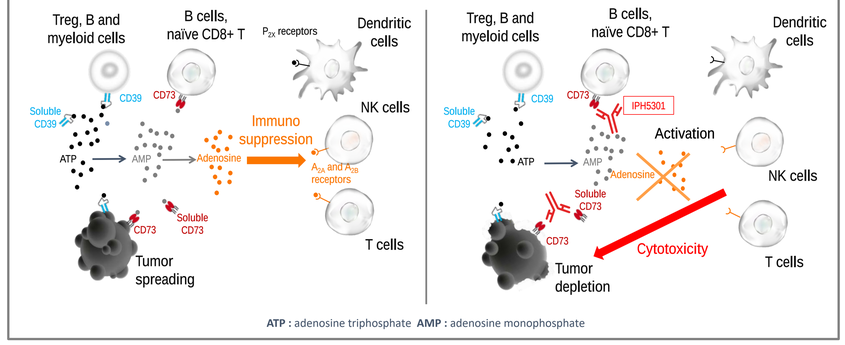
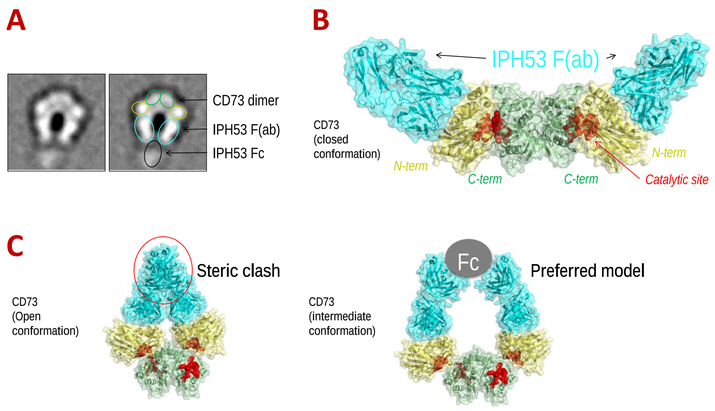
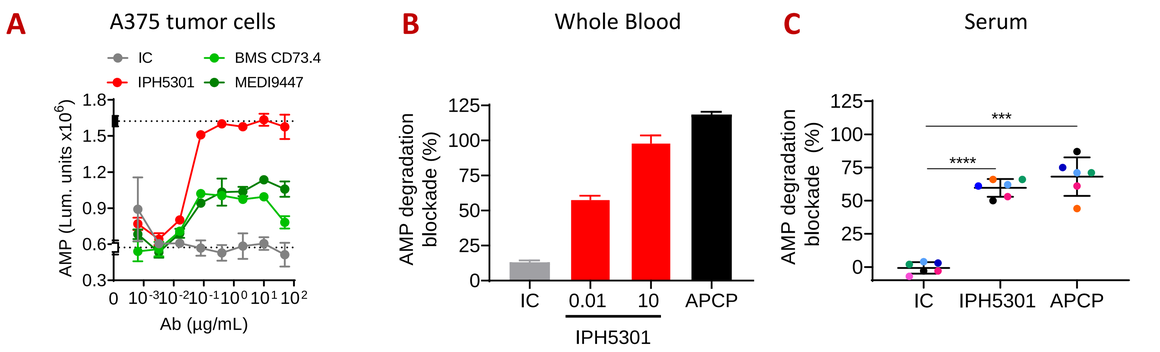


FIGURE 1: IPH5301 constrains CD73 in an inactive intermediate conformation



(A) Electron microscopy negative staining of the CD73-IPH5301 complex. Right panel: CD73 dimer (N-term domain, yellow; C-term domain, green) and IPH5301 (Fab, cyan-blue; Fc, black) on a 2D class average calculated from the recorded pictures (representative of the main complex observed on the grid).
(B) Crystal structure of CD73 and IPH5301 Fab complex. Yellow: N-term domain of CD73 dimer, Green: C-term domain of CD73-dimer, Cyan-blue: IPH5301 Fab, Red: Catalytic site of CD73.
(C) Models extrapolated of the crystal structure of CD73-IPH5301 Fab complex. Left panel: complex in the open conformation of CD73. Right panel: complex in an intermediate conformation of CD73 (preferred model).

FIGURE 2: IPH5301 blocks enzymatic activity of both soluble and membrane forms of CD73



(A) A375 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 (B) and serum from 6 healthy donors (C) were incubated with IC, IPH5301 (0.01 and 10 µg/mL) or APCP (100 µM) for 1h 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 Dunett's test, **** p<0.0001, *** p=0.0001.

FIGURE 3: IPH5301 does not induce CD73 down-modulation and nor systemic B cell activation

(A) A375 cells were incubated with IPH5301 (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 IPH5301 (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 CPI-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.

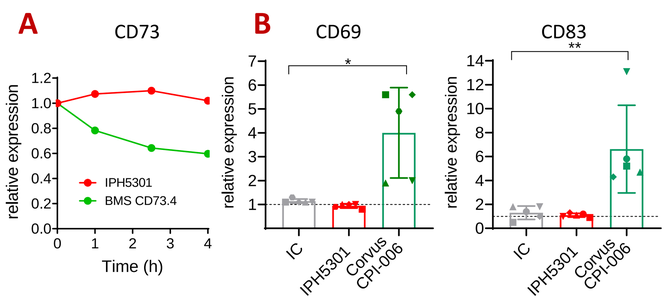
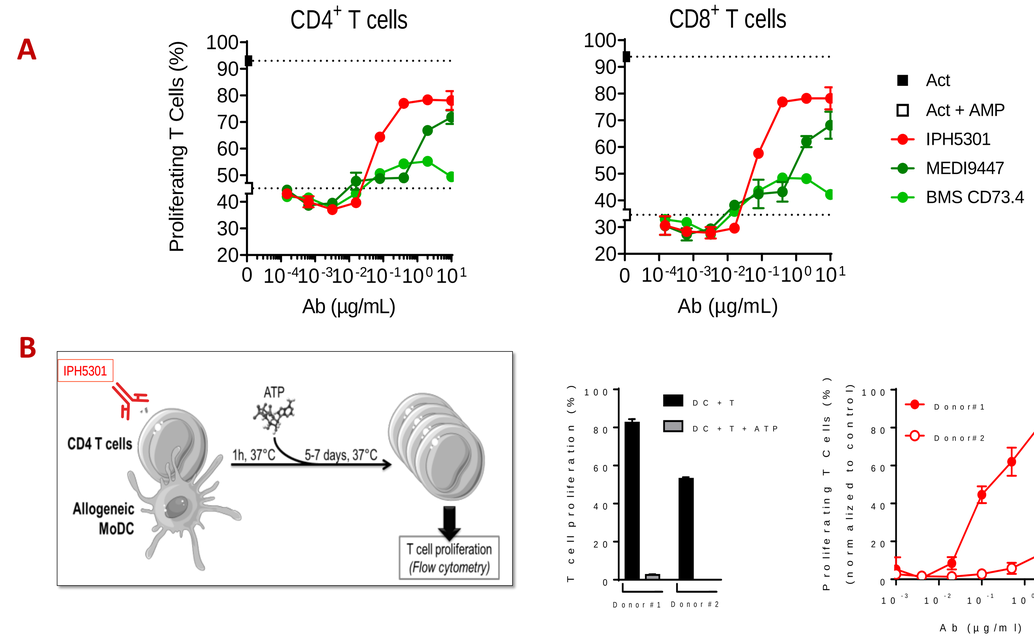
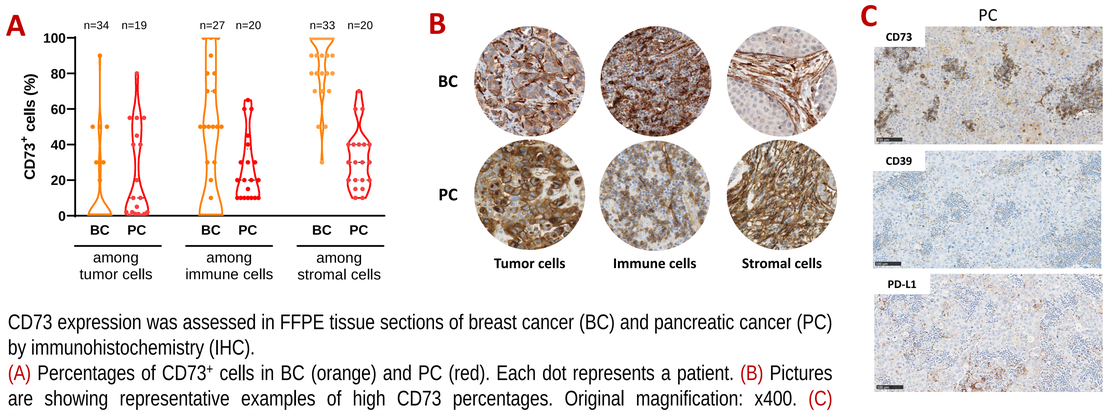


FIGURE 4: IPH5301 reverses adenosine-mediated suppression of T cell proliferation, in the presence of AMP



(A) Human lymphocyte-enriched peripheral blood cells were activated with anti-CD3/anti-CD28 antibody-coated beads in the presence of AMP and anti-CD73 mAbs. T cell proliferation was assessed by flow cytometry. Data representative of at least 3 experiments.
(B) MoDCs were cultured with allogeneic $CD4^+$ T cells with or without IPH5301 and an immunosuppressive dose of ATP (100 mM). T cell proliferation was evaluated by flow cytometry. Percentages of T cells proliferating in the presence or absence of ATP (middle panel, two independent experiments) and in the presence of increasing concentrations of IPH5301 mAb (right panel) are shown.

FIGURE 6: CD73 is widely expressed in human TME, with a different expression pattern from CD39 and PDL1



CD73 expression was assessed in FFPE tissue sections of breast cancer (BC) and pancreatic cancer (PC) by immunohistochemistry (IHC).
(A) Percentages of $CD73^+$ cells in BC (orange) and PC (red). Each dot represents a patient. (B) Pictures are showing representative examples of high CD73 percentages. Original magnification: x400. (C) Representative pictures of CD73, CD39 and PD-L1 IHC staining in PC showing the different expression patterns of the three markers. Black scale bars represent 100 µm.

These results indicate that IPH5301 blocks CD73 with a differentiated mechanism of action compared to benchmarked anti-CD73 clinical candidates and support the clinical development of IPH5301 for cancer immunotherapy, potentially in combination with chemotherapy or immune checkpoint inhibitors.