Preclinical development of humanized CD39 (IPH52) and CD73 (IPH53) blocking antibodies targeting the ATP/Adenosine immune checkpoint pathway for cancer immunotherapy

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Abstract

The immunosuppressive role of CD39, expressed on both Tregs and tumor cells, has been largely demonstrated. CD39 expression in the tumor environment has been associated with poor disease outcome and/or with a pro-metastatic phenotype. Blockade of CD39 and CD73 may promote anti-tumor immunity by reducing adenosine (Ado) accumulation and increasing levels of ATP, which possesses immunomodulatory properties.

Blockade of CD73 enzymatic activity has recently been reported to improve immune checkpoint inhibitor anti-tumor activity. In addition, we show that in vivo blockade of ATP/Ado pathway in CD39 KO mice resulted in improved tumor-specific immune checkpoint therapies (i.e. PD-1, CTLA-4) and chemotherapy such as Osimaplin.

Immunohistochemistry (IHC) and flow cytometry showed that CD73 is rather expressed by tumor cells and that CD39 is frequently upregulated on tumor infiltrating lymphocytes (TILs) compared to PBMC or adjacent non-tumor tissue.

We have generated anti-human CD39 (IPH52) and anti-human CD73 (IPH53) blocking antibodies (Abs) expressing unique properties for cancer immunotherapy. These Abs potentially inhibit the enzymatic activity of both the soluble and membrane-associated forms of their respective target. Both Abs efficiently reverse adenosine-mediated T cell suppression in vitro, by markedly enhancing high concentrations of ATP in the extracellular compartment. The anti-CD73 IPH53 Ab enhances dendritic cells (DC) activation and subsequent T cell proliferation in vitro, probably by maintaining high concentrations of ATP in the extracellular compartment. The anti-CD73 IPH53 Ab is more potent than benchmark Abs currently in phase I clinical development for the blockade of soluble and membrane-associated CD73 enzymatic activity and for AMP-mediated T cell suppression reversal. Finally, we showed that combining IPH52 and IPH53 Abs at sub-optimal doses leads to a strong reversal of immune cell inhibition in the presence of ATP.

Taken together, these data support the rationale for clinical development of anti-CD39 and anti-CD73 neutralizing Abs for cancer immunotherapy, potentially in combination with chemotherapy or immune checkpoint blockade.

1. Deletion of CD39 improves anti-tumor efficacy of immune checkpoint blockade and chemotherapies

A. 1×10^6 MCAs25 (upper panel) or 1×10^6 B6-F10 (lower panel) mouse cells were subcutaneously engrafted in C57BL/6 mice type or CD39 KO mice. At day 6 after tumor cell graft mice were treated with anti-PD-1, anti-CTLA-4 (MCAs25 only) or control Ab (10 mg/kg). MCA205 mouse cells were hematopoietically reconstituted in (A). At day 6 after tumor cell graft, mice were treated or not with Osimaplin (10 mg/kg, ip) and the day after with anti-PD-1 or control Ab (10 mg/kg). Post of 2 experiments. A/J, Back bombing, Cell and Tumor (CTV). B. Complete Tumor Regression, Tumor Free.

2. In human tumors CD39 is mostly upregulated on tumor infiltrated lymphocytes

A. Soluble CD39 was quantified in serum from healthy donors (HD) and Head & Neck patients (HN) by ELISA. Anti-CD39.

3. In human tumors CD73 is rather expressed on tumor cells

A. CD73 expression was assessed by flow cytometry on blood and infiltrated tumor adjacent tissue from Head & Neck patients. Data are from two independent patients, one non-irradiated (a/s) + treated with (b/t) + anti-CD39 mAb 100 ng/kg (B). CD73 expression was evaluated by IHC on FFPE Head and Neck tumor samples. D. Soluble CD39 was quantified in serum from healthy donors (HD) and Head & Neck patients (HN) by ELISA. Anti-CD39.

4. IPH52 (CD39) and IPH53 (CD73) Abs block enzymatic activity of both membrane-bound and soluble CD39 and CD73

A. B6-F10 (Control Ab) and Anti-PD-1 treated tumor-bearing WT mice (CD39 KO mice).

5. IPH52 and IPH53 blocking Abs reverse Ado-mediated T cell suppression

A and B: Cells (A) or supernatant (S/N) (B) from the Ramos human B cell line were incubated with 20 µM ATP in presence of IPH52 or control Abs. Rested FAP was quantified using Cell Titer Glo® reagent. Data are representative of at least 5 experiments. C and D: Cells from the A275 human melanoma cell line (D) or from serum from healthy donors (B) were incubated with 10 (A) or 100 (B) µM ATP in presence of PD1 Ab or control Ab. Maximum FAP was quantified using the AMP GM reagent. Data are representative of at least 5 experiments. CR = 6/10 (A) and 20/20 healthy donors (B). CR: C/TOD: 1:1000 (S) (µg/ml/mg/protein).

6. IPH52 enhances ATP-medicated DC activation and resulting T cell proliferation

A. Anti-PD-1 (control) and Anti-PD-1 + Osimaplin treated DC from patients' blood were assessed for DC activation and T cell proliferation.

7. Combined CD39 and CD73 blockade strongly reverses Ado-mediated T cell suppression

A. CTV-labeled lymphocytes were cultured with anti-CD39/CD73 double- and 100 µM ATP in presence of IPH52 or control Abs (A). CD4+ T cell apoptosis A/B (n=4). CD73/CD39 double- and 100 µM ATP in presence of anti-CD73 (B) and (C). In (B) Ab presence on independent donors was evaluated at 15 days. Data in B and C are representative of at least 5 experiments.

Conclusion

**IPH52 (anti-CD39 Ab)**
- Humanized Fc-silent IgG1 antibody, blocking membrane and soluble CD39
- Anti-CD39 blocking mAb allows (i) to sustain extracellular ATP that promotes immune responses and (ii) to block the generation of adenosine that is immunosuppressive

**IPH53 (anti-CD73 Ab)**
- Humanized Fc-silent IgG1 antibody, blocking membrane and soluble CD73
- Differentiated and superior in vitro activity compared to MEDI and BMS CD73 blocking Abs

**IPH52/IPH53 combination**
- In vitro synergy in T cell suppression assay