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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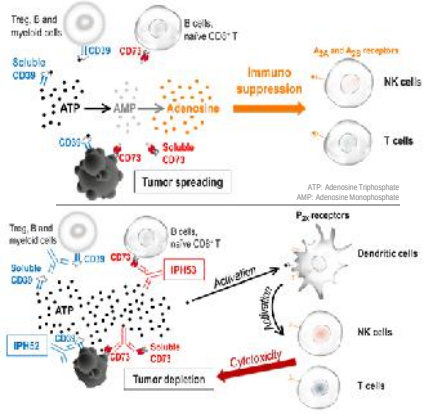
Abstract



The immunosuppressive role of CD39, expressed on both Tregs and tumor cells, has been largely demonstrated. CD73 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 immunostimulatory properties.

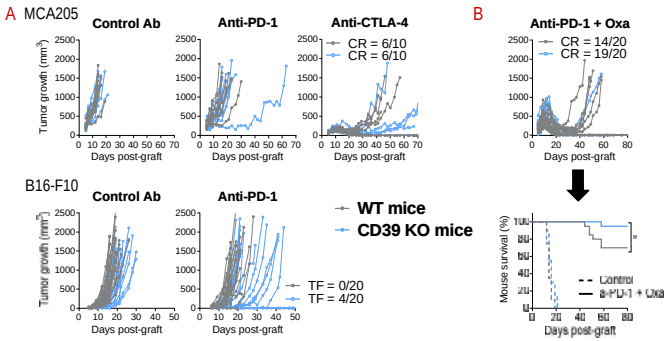
Blockade of CD73 enzymatic activity has recently been reported to improve immune checkpoint inhibitor anti-tumor activity. In addition, we show that in vivo blockade of ATP/Ado pathway in CD39 KO mice resulted in improved anti-tumor efficacy of immune checkpoint therapies (i.e. PD-1, CTLA-4) and chemotherapy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

Immunohistochemistry (IHC) and flow cytometry staining showed that CD73 is rather expressed by tumor cells and that CD39 is frequently up-regulated 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) with unique properties for cancer immunotherapy. These Abs potently 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 in presence of ATP and CD39- and CD73-expressing immune cells. The anti-CD39 IPH52 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 reversion. Finally, we showed that combining IPH52 and IPH53 Abs at sub-optimal doses leads to a strong reversion of immune cell inhibition in the presence of ATP.

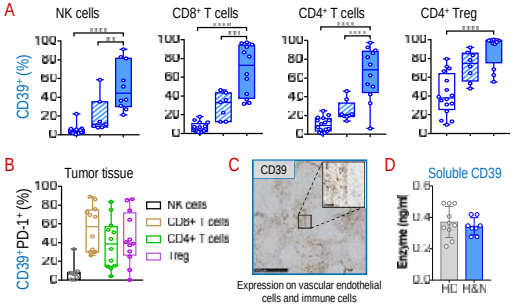
Taken together, these data support the rationale for clinical development of anti-CD39 and anti-CD73 neutralizing Abs for cancer immunotherapy, potentially in combination with chemotherapy or immune checkpoint blockade.

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



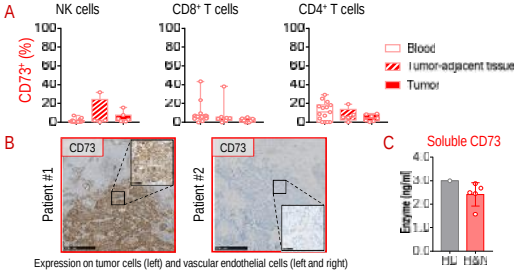
A/ $1x10^6$ MCA205 (upper panel) or $5x10^4$ B16-F10 (lower panel) mouse cells were subcutaneously engrafted in C57BL/6 wild type or CD39 KO mice. At day 6 after tumor cell graft mice were treated with anti-PD-1, anti-CTLA-4 (MCA205 only) or control Abs (10 mg/kg; ip). MCA205 model, representative of 2 experiments. B16-F10 model, pool of 2 experiments. B/ $1x10^6$ MCA205 mouse tumor cells were engrafted as described in (A). At day 5 after tumor cell graft, mice were treated or not with Oxaliplatin (Oxa, 10 mg/kg; ip) and the day after with anti-PD-1 or control Abs (10 mg/kg; ip). Pool of 2 experiments. Log-Rank Mantel-Cox test (*=0.035). CR: Complete tumor Regression; TF: Tumor Free.

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



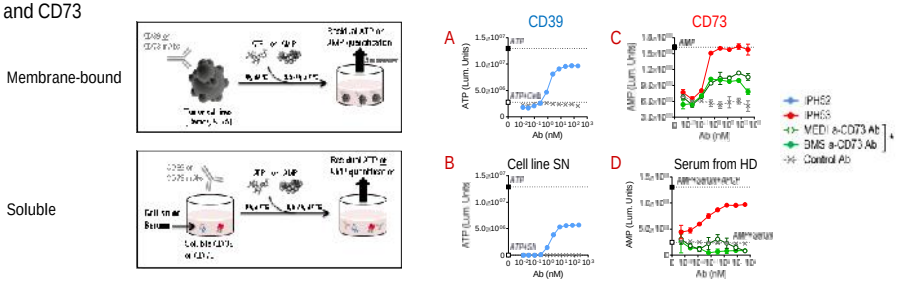
A and B/ CD39 (A) and CD39-PD-1 (B) expression was assessed by flow cytometry on blood and digested tumor or adjacent tissue from Head & Neck patients. Box and whiskers (min. to max.). One-way ANOVA + Tukey multiple comparisons test (**<0.01, ***<0.001, ****<0.0001). C/ CD39 expression was evaluated by IHC on FFPE Head and Neck tumor samples. D/ Soluble CD39 was quantified in serum from healthy donors (HD) and Head & Neck patients (H&N) by ELISA. Mean with SD.

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



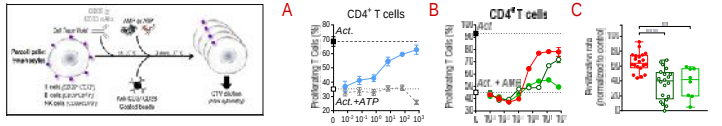
A/ CD73 expression was assessed by flow cytometry on blood and digested tumor or adjacent tissue from Head & Neck patients. Box and whiskers (min. to max.). B/ CD73 expression was evaluated by IHC on FFPE Head and Neck tumor samples. 2 examples shown, one with (letf) and one without (right) expression of CD73 on tumor cells. C/ Soluble CD73 was quantified in serum from healthy donors (HD) and Head & Neck patients (H&N) by ELISA. Mean with SD.

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



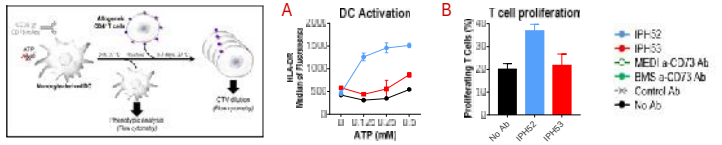
A and B/ Cells (A) or supernatant (SN) (B) from the Ramos human B cell line were incubated with 20 µM ATP in presence of IPH52 or control Abs. Residual ATP was quantified using Cell Titer Glo® reagent. Data are representatives of at least 5 experiments. C and D/ Cells from the A375 human melanoma cell line (C) or serum from healthy donors (D) were incubated with 12,5 µM (C) or 10 µM (D) AMP in presence of IPH53, control Abs or APCP. Residual AMP was quantified using the AMP Glo® reagent. Data are representatives of at least 5 experiments (C) and of 2 healthy donors (D). APCP: CD73 inhibitor (Adenosine 5'-(α,β-methylene)diphosphate).

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



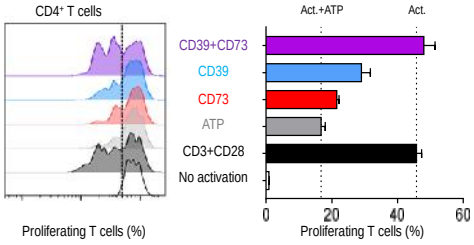
CTV®-labelled lymphocytes were cultured with anti-CD3/CD28-coated beads and 100 µM ATP in presence of IPH52 or control Abs (A) or 200 µM AMP in presence of anti-CD73 Abs (B and C). In (C) Ab potency on independent donors was evaluated at 10 µg/ml. Data (A and B) are representatives of at least 5 experiments.

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



Monocyte-derived DC were stimulated with 100 µM ATP in presence of IPH52, IPH53 or control Abs. Phenotypic changes were evaluated by flow cytometry (A) and conditioned-DC were used to stimulate CTV®-labelled allogeneic CD4+ T cells (B). Data are representative of 3 experiments

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



CTV®-labelled lymphocytes were cultured with anti-CD3/CD28-coated beads and ATP in presence or not of a suboptimal dose of both anti-CD39 and anti-CD73 Abs. T cell proliferation was assessed as described in figure 4. Mean with SD (right panel). Data are representatives 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

The anti-CD73 program is developed within the TumAdoR project (www.tumador.eu) and funded under the European Community's seventh framework Program (European Community's Seventh Framework Program (FP7/2007-2013) under grant agreement n°602200).

*Competition Ab sequences were obtained from public patents:
- #US2016/0129108A1 (MedImmune)
- #US2015/061639 (BMS)

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