Discovery and characterization of new original blocking antibodies targeting the CD73 immune checkpoint for cancer immunotherapy

#2344

M. Giraudon-Paoli[1], S. Augier[1], M. Royannez-Blemont[1], C. Rodriguez[2,3,4,5], H. Rispaud-Blanc[1], S. Chanteux[1], N. Gourdin[2,3,4,5], L. Gauthier[1], C. Ménétrier-Caux[2,3,4,5], Y. Morel[1], C. Caux[2,3,4,5], C. Paturel[1] and I. Perrot[1]

[1] Innate Pharma, Marseille, France; [2] Team 11, INSERM U1052, CRCL; [3] Lyon University; [4] ISPB, Lyon 1 University; [5] Leon Berard Cancer Center, Lyon, France

Abstract

CD73 (NT5E) is a cell membrane ectoenzyme of the NTPDase family that plays a major role in the conversion of AMP into adenosine (Ado). Within the tumor microenvironment, accumulation of Ado causes immune suppression and dysregulation of immune cell infiltrates resulting in tumor spreading. CD73 expression in the tumor environment has been associated with poor disease outcome (1-3) and/or with a pro-metastatic phenotype (4, 5). Thus, targeting CD73 may promote anti-tumor immunity by reducing Ado accumulation and may block tumor cell metastasis by inhibiting CD73 on tumor cells.

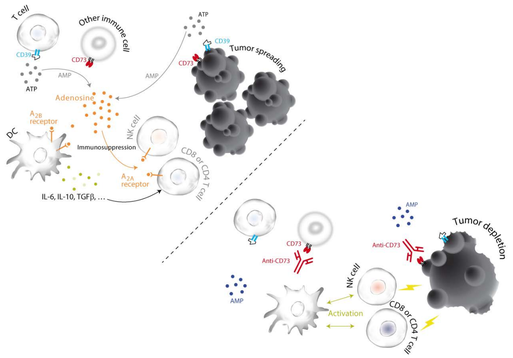
Here, we describe the generation and characterization of novel anti-human CD73 antibodies, intended for the treatment of a wide range of cancers.

Antibodies were discovered that inhibit CD73 function by different mechanisms, including direct blockade of CD73 enzymatic activity or the down-modulation of CD73 membrane expression. Epitope mapping revealed that antibodies acting by these different modes of action bind to distinct sites on CD73.

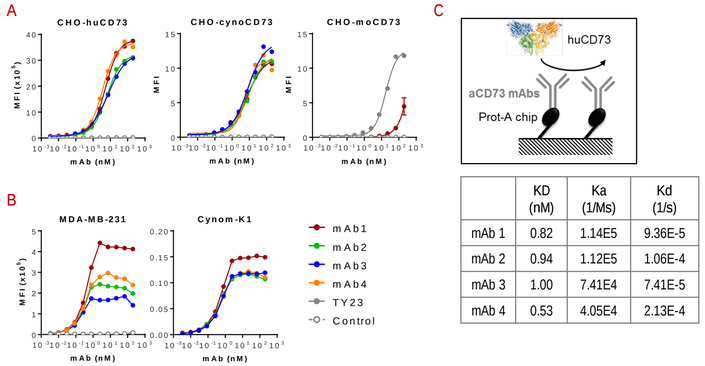
All selected antibodies cross-react with cynomolgus CD73 protein and have strong avidity and affinity for membrane or recombinant CD73, by flow cytometry and Surface Plasmon Resonance, respectively. Antibodies that inhibit CD73 enzymatic activity strongly reduce AMP catabolism by both recombinant and cellular CD73 with IC_{50} in the nanomolar range. They also efficiently reverse AMP-mediated T cell suppression in *in vitro* assays.

The antibodies displaying the most interesting features were humanized.

1. Mechanism of action



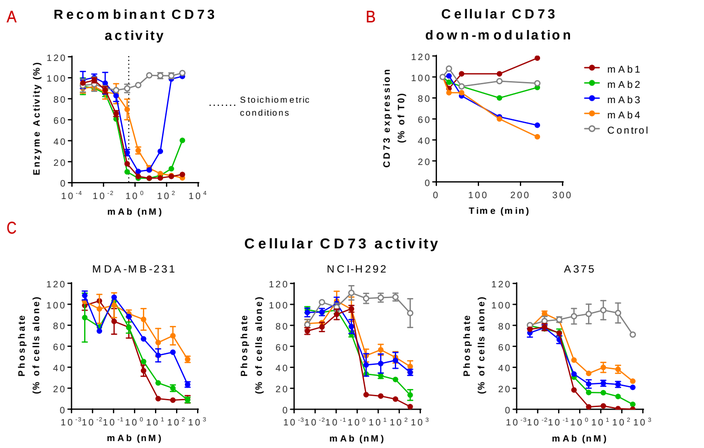
2. Anti-CD73 antibodies bind on human and cynomolgus CD73 with high affinity



	KD (nM)	Ka (1/Ms)	Kd (1/s)
mAb 1	0.82	1.14E5	9.36E-5
mAb 2	0.94	1.12E5	1.06E-4
mAb 3	1.00	7.41E4	7.41E-5
mAb 4	0.53	4.05E4	2.13E-4

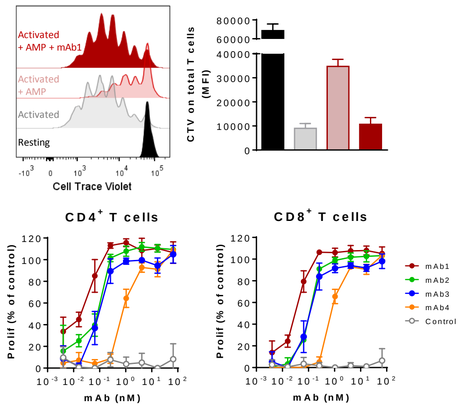
Binding of anti-CD73 mAbs was evaluated by flow cytometry A: on human, cynomolgus and mouse CD73-expressing CHO cell lines and B: on human (MDA-MB-231) and cynomolgus (Cynom-K1) cell lines that endogenously express CD73 . C: Affinity of anti-CD73 mAbs was measured by SPR.

3. mAb1, the most potent antibody to inhibit cellular CD73 blocks enzyme activity without "hook effect"



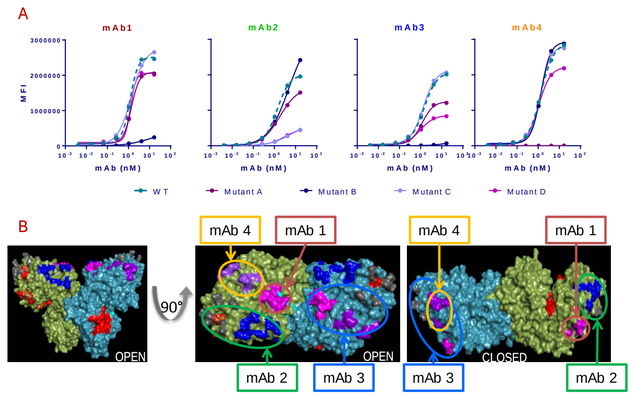
Recombinant CD73 was incubated with anti-CD73 mAbs then with AMP (125 µM) and ATP (12.5 µM). A: AMP degradation was indirectly measured with Cell Titer Glo reagent. B: CD73 expression after MDA-MB-231 incubation with anti-CD73 mAbs at 37°C was revealed by flow cytometry with non-competing anti-CD73 mAbs. C: Human tumor cell lines were incubated with anti-CD73 mAbs then with AMP (100 µM). Resulting phosphate was measured in the supernatant.

4. Anti-CD73 antibodies efficiently reverse AMP-mediated T cell suppression



Cell Trace Violet (CTV)-labelled lymphocytes were incubated with anti-CD73 mAbs, anti-CD3/CD28-coated beads and AMP (800µM). Cell division was followed by flow cytometry.

5. The 4 mAbs have distinct epitopes



A: Mutated human CD73 constructions were transfected in HEK293T cell line and binding of anti-CD73 mAbs was evaluated by flow cytometry. B: Position of anti-CD73 mAbs on both open (middle panel) and closed (right panel) conformation. Catalytic site is depicted in red.

6. Mode of action and characteristics of anti-CD73 antibodies

Antibodies	MOA	SPR (KD – nM)	Cellular binding* (EC_{50} – nM)	Recombinant CD73 activity (IC_{50} – nM)	Cellular CD73 Activity** (Inhibition level)	T cell proliferation (EC_{50} – nM)
mAb1	Activity blockade	0.82	0.57	0.100	93%	0.04
mAb2	Activity blockade	0.94	0.27	Hook effect	81%	0.11
mAb3	Activity blockade Down-modulation	1.00	0.29	Hook effect	64%	0.09
mAb4	Activity blockade Down-modulation	0.53	0.41	0.805	52%	0.81

* EC_{50} is a mean of 5 experiments on MDA-MB-231 cell line.
** Inhibition level of CD73 activity is the mean percentage reached at plateau phase on MDA-MB-231, NCI-H292 and A375 cell lines.

References

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Conclusion

- We have discovered new original antibodies targeting the CD73 immune checkpoint that block CD73 enzymatic activity and/or induce CD73 down-modulation
- The 4 mAbs efficiently reverse AMP-dependent T cell suppression *in vitro*
- The mAb that most potently inhibits CD73-mediated immune suppression (Ab1) is a strong blocker of CD73 enzyme activity
- The 4 mAbs have distinct epitopes all located on the apex of the N-terminal domain of CD73

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