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

Abstract

CD73 (NT5E) is a cell membrane ectonucleotidase 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 cytomed NT5E 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 IC50 in the nanomolar range. They also efficiently reverse AMP-mediated T cell suppression.

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

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

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

5. The 4 mAbs have distinct epitopes

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

References

2. Li and X. Expression and clinical significance of CD73 and Tirofiban in gastric cancer. (2013)
5. Wang et al. 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.

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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