Preclinical development of a humanized blocking antibody targeting the CD39 immune checkpoint for cancer immunotherapy

Abstract

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CD39 (ENTPD1/CD39) is a cytosolic membrane ectonucleotidase that hydrolyzes extracellular ATP and ADP into AMP, which can be further hydrolyzed by CD73 into adenosine. While extracellular ATP released by dying cells promotes inflammation and immune response activation, adenosine secretion causes immune suppression and dysregulation of immune cell infiltrates resulting in tumor spreading.

Within the tumor microenvironment, CD39 is overexpressed on both tumor cells and immune infiltrating cells, including Treg and MDSC. Blockade of CD39 promotes anti-tumor immunity by directly accumulating immunostimulating ATP and indirectly by reducing adenosine accumulation (Figure 1). In this paper, we describe the preclinical development of an anti-huCD39 blocking antibody for cancer immunotherapy.

Parental anti-huCD39 mouse monoclonal antibody was humanized. The humanized mAb specifically targets in CD39, but not to related CD39-like proteins. The humanized mAb binds cell surface CD39 with nanomolar affinity, on both CD39-transfected and tumor cell lines expressing endogenous CD39. The humanized mAb blocked human CD39 ATPase activity in vitro, as demonstrated using transfected cells. CD39 expression was evaluated on both tumor cells and immune cells, as well on human PBMC and as in vitro isolated fresh tumor samples. The humanized mAb also binds cynomolgus CD39 and antihuCD39 activity in cynomolgus tumor model. Finally, treatment with anti-huCD39 blocking mAb inhibited tumor growth in vivo in a mouse xenografts tumor model.

Tumors, together with these data support the clinical development of anti-CD39 blocking mAb for tumor immunotherapy.

1. Mechanism of action

- Binding of the parental mAb anti-CD39 mAb with human CD39, a chimeric hu/vivo murine/Fc (Chim aCD39) and humanized anti-CD39 (huCD39) to human PBMC on CR (Figure 2A). Blocking of ATPase activity was evaluated on PBMC, incubated with ATP at 4°C and AMP generated was quantified by Maldi Tof-MS.

2. Humanized mAb binds CD39 with high affinity and specificity

- Blocking of huCD39 expression on human PBMC. Human PBMC were infected with huCD39, incubated with ATP at 37°C, and AMP generated was quantified by Maldi Tof-MS.

3. Humanized mAb inhibits human CD39 enzyme activity

- Blocking of huCD39 on human PBMC. Efficacy of Hu aCD39 to inhibit ATPase activity was evaluated on huPBMC, incubated with ATP at 4°C. AMP generated was quantified by Maldi Tof-MS.

4. Humanized mAb inhibits cynomolgus CD39 enzyme activity

- Blocking of huCD39 on cynomolgus PBMC. Efficacy of Hu aCD39 to inhibit ATPase activity was evaluated on cynomolgus PBMC, incubated with ATP at 4°C. AMP generated was quantified by Maldi Tof-MS.

5. Humanized mAb inhibits CD39 enzyme activity on human and cynomolgus PBMC

- Blocking of huCD39 on human and cynomolgus PBMC. Efficacy of Hu aCD39 to inhibit ATPase activity was evaluated on human and cynomolgus PBMC, incubated with ATP at 4°C. AMP generated was quantified by Maldi Tof-MS.

6. Humanized mAb inhibits CD39 enzyme activity in melanoma tumor biopsies

- Efficacy of Hu aCD39 to inhibit ATPase activity was evaluated on tumor biopsies, collected from patients with advanced melanoma, treated with CTLA4 (anti-Cytotoxic T lymphocyte-associated protein 4) and Ipilimumab. Cells were incubated with ATP at 4°C, and AMP generated was quantified by Maldi Tof-MS.

7. Humanized mAb inhibits tumor growth in xenogeneic tumor model

- In vivo efficacy of Hu aCD39 was evaluated in SCD mice, subcutaneously engrafted with CD39-expressing human melanoma cells. huCD39 was treated subcutaneously with the indicated mAb twice a week, from day 4.

Conclusion

- First-in-class humanized anti-CD39 mAbs was generated with high affinity and specificity for CD39 and is blocking ATPase activity.
- In vivo, humanized anti-CD39 mAb is able to significantly inhibit CD39/CD73 positive tumor growth, suggesting that blocking CD39 enzyme activity on tumors only is sufficient to ensure tumor inhibition.
- Combining anti-CD39 mAb with immunotherapy and/or immune checkpoint inhibitors should also demonstrate significant improvement in efficacy, as suggested by in vivo results obtained in our Preclinical Study.