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

#3222

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Abstract

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

Within the tumor microenvironment, CD39 is expressed on both tumor cells and immune infiltrating cells, including Treg and MDSC. Blockade of CD39 may promote anti-tumor immunity by directly accumulating immunostimulating ATP and indirectly by reducing adenosine accumulation (Figure 1).

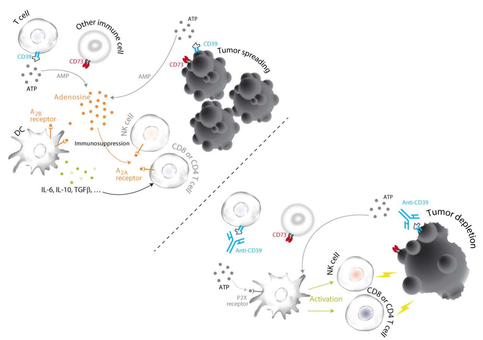
In this poster, we describe the preclinical development of an anti-huCD39 blocking antibody for cancer immunotherapy.

Parental anti-huCD39 mouse monoclonal antibody was humanized. The humanized mAb specifically binds to CD39, but not to related CD39-like proteins. The humanized mAb binds cell surface CD39 with nanomolar affinities, on both CD39-transfectants and tumor cell lines expressing endogenous CD39.

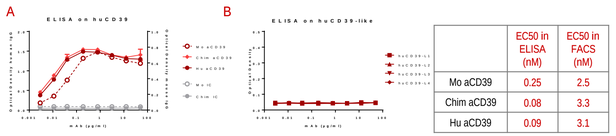
The humanized mAb blocked human CD39 ATPase activity *in vitro*, as demonstrated using transfected cells, CD39-expressing tumor cell lines, as well as human PBMC and *ex vivo* isolated fresh tumor samples. The humanized mAb also binds cynomolgus CD39, and blocks ATPase activity on cynomolgus PBMC. Finally, treatment with anti-CD39 blocking mAb inhibited tumor growth *in vivo* in a mouse xenogeneic tumor model.

Taken together, these data support the clinical development of anti-CD39 blocking mAb for cancer immunotherapy.

1. Mechanism of action



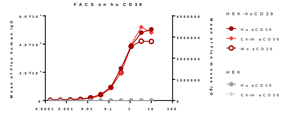
2. Humanized mAb binds CD39 with high affinity and specificity



	EC50 in ELISA (nM)	EC50 in FACS (nM)
Mo aCD39	0.25	2.5
Chim aCD39	0.08	3.3
Hu aCD39	0.09	3.1

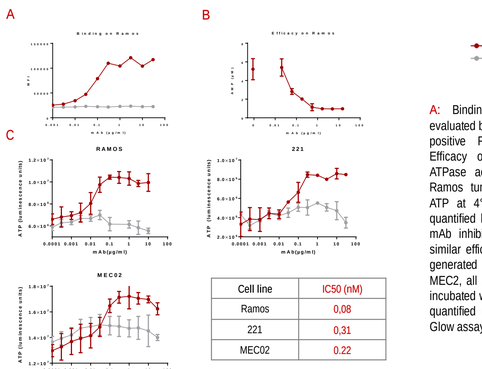
C

SPR on CD39	KD (nM)	ka (1/Ms)	kd (1/s)
Chim aCD39	17,6	0.97E+5	0,0017
Hu aCD39	12,1	1,14E+5	0,0014



A: Binding of the parental murine anti-huCD39 mAb (Mo aCD39), a chimeric human Fc-Mouse Fv (Chim aCD39) and humanized mAb (Hu aCD39) to human CD39 protein in ELISA. B: Lack of binding of Hu aCD39 to human CD39-like proteins L1 to L4 in ELISA. Similar results with the parental mAb (not shown). C: Monovalent affinities for CD39 protein measured by surface plasmon resonance. D: Specific binding to cell surface CD39 evaluated by flow cytometry on control HEK cells (CD39 negative) or HEK-human CD39 transfectants.

3. Humanized mAb inhibits human CD39 enzyme activity



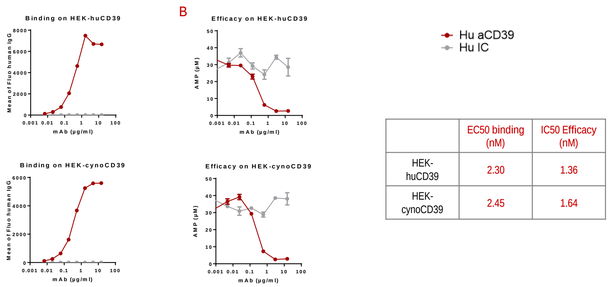
Cell line	IC50 (nM)
Ramos	0,08
221	0,31
MEC02	0.22

A: Binding of Hu aCD39 was evaluated by flow cytometry on CD39 positive Ramos tumor cells. B: Efficacy of Hu aCD39 to inhibit ATPase activity was evaluated on Ramos tumor cells, incubated with ATP at 4°C. AMP generated was quantified by Maldi Tof-MS. Parental mAb inhibited ATPase activity with similar efficacy (not shown). C: ATP generated from Ramos, 221 and MEC2, all CD39 positive tumor cells, incubated with ATP at 37°C, was also quantified using adapted Cell Titer Glow assay.

References

- Bastid et al. ENTPD1/CD39 is a promising therapeutic target in oncology. *Oncogene*. 2013
- Bonnefoy et al. CD39: A complementary target to immune checkpoints to counteract tumor-mediated immunosuppression. *Oncoimmunology*. 2015
- Bastid et al. Inhibition of CD39 enzymatic function at the surface of tumor cells alleviates their immunosuppressive activity. *Cancer Immunol Res*. 2015

4. Humanized mAb inhibits cynomolgus CD39 enzyme activity



	EC50 binding (nM)	IC50 Efficacy (nM)
HEK-huCD39	2.30	1.36
HEK-cynoCD39	2.45	1.64

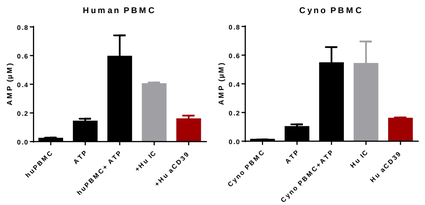
A: Binding of Hu aCD39 was evaluated in flow cytometry on HEK-human CD39 (HEK-huCD39) or -cynomolgus CD39 (HEK-cynoCD39). Similar results were obtained with parental mAb (not shown). B: Efficacy of Hu aCD39 to inhibit ATPase activity was evaluated on cells incubated with ATP at 4°C. AMP generated was quantified by Maldi Tof-MS.

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

A

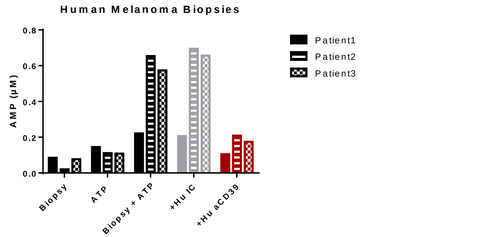
CD39 expression	Human Whole Blood	Cynomolgus Whole Blood
B cells	+++	++
CD4+ Tconv cells	low	low
CD4+ Treg cells	++	+
CD8+ T cells	low	+
NK cells	low	+
Monocytes	+++	+++
Granulocytes	+++	+++

B



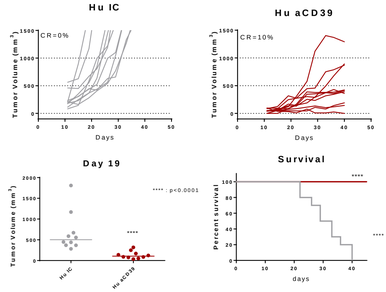
A: CD39 expression was evaluated on human and cynomolgus immune cell populations by flow cytometry using anti-huCD39 A1-PE. Low: <10% positive cells, +: <20%, ++: 20-90%, +++: >90%. CD39 was similarly expressed on human and cynomolgus immune cell populations, except that some CD39 negative B cells and CD39 positive NK cells were present in cynomolgus peripheral blood, but not in human. B: Efficacy of Hu aCD39 mAb to inhibit ATPase activity was evaluated on 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 3 patients with metastatic melanoma, digested at 37°C with Collagenase and DNAse. Cells were further 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 SCID mice, subcutaneously engrafted at d0 with CD39/CD73 positive Ramos cells. Mice were treated intraperitoneally with the indicated mAb twice a week, from d2.

Conclusion

- First-in-class humanized anti-CD39 mAb was generated with high affinity and specificity for CD39 and is blocking ATPase activity *in vitro* on both tumor cells and immune cells.
- *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 chemotherapy and/or immune checkpoint inhibitors should also demonstrate significant improvement in efficacy, as suggested by *in vivo* results obtained in our Poster #3218, « Disruption of the CD39 immune checkpoint pathway increases the efficacy of various anticancer therapies in syngeneic mouse tumor models. »
- In conclusion, our results warrant the development of a therapeutic blocking anti-CD39 mAb targeting the tumor microenvironment.