Poster 4645

Identification of conditionally active antibodies that selectively block CD39 activity in the acidic tumor microenvironment

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AACR Annual Meeting
April 14-19, 2023

Background

CD39 expression is upregulated in the tumor microenvironment (TME), which is characterized by high levels of extracellular ATP (eATP) and low pH. CD39 catalyzes the rate-limiting degradation step of this immunostimulatory ATP, leading to a rise in immunosuppressive adenosine (ADO). CD39 displays broad expression on endothelial cells and macrophages, which represents a significant peripheral sink for CD39 targeting antibodies. Our strategy aims to circumvent this problem through delivery of pH-selective anti-CD39 blocking antibodies that will achieve a high target occupancy in the tumor, maintaining eATP and inhibiting ADO generation to enhance anti-tumor immunity.

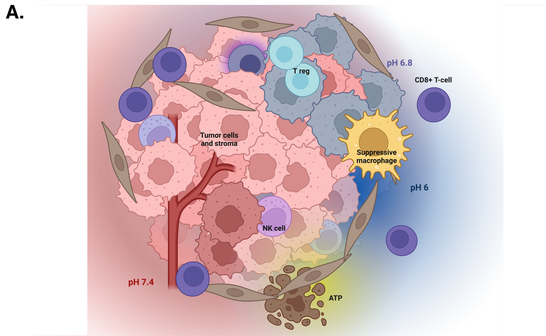

Figure 1. CD39 function in the tumor microenvironment. A) Tumors present a complex microenvironment containing numerous cell types including tumor cells, stromal cells and immune cells. Local hypoxia and altered metabolism generate localized acidic (~ pH 6) regions. B) Cell death in the TME results in release of large amounts of immunostimulatory ATP. CD39 metabolizes ATP to ADP and AMP, which is then degraded to the immunosuppressive molecule adenosine by CD73. Inhibition of CD39 in the TME results in accumulation of ATP and a decrease in adenosine, favoring immune responses and tumor cell killing. High eATP may also have direct effects on tumor cell survival.

Methods

Anti-CD39 antibodies were generated through a yeast-based screening platform and characterized for efficacy of blocking CD39 activity. Cell lines (HEK293 stably overexpressing CD39, parental HEK293, CD39⁺ SK-MEL-28, and CD39⁺ ARH77) or soluble CD39 protein were incubated with candidate antibodies. CD39 enzymatic activity was determined with complementary assays (see **Figure 5**).

Figures created in part with BioRender.com

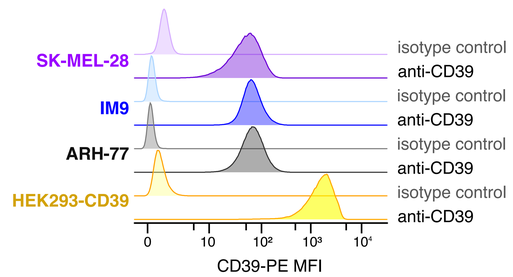

Figure 2. Expression of Endogenous and Stably Overexpressed CD39 in Cell Lines. HEK293 cells stably overexpressing CD39 were compared to natively expressing SK-MEL-28 (melanoma) and IM9 and ARH-77 (B lymphoblastoid) cells by flow cytometry. HEK293-CD39 (stable pool) expresses ~20-fold higher surface CD39 (based on ΔMFI).

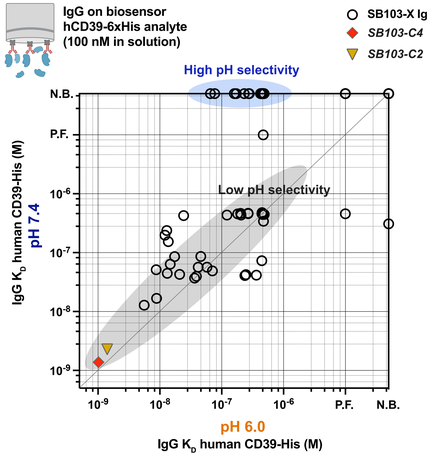

Figure 3. Primary characterization of initial candidate antibodies. In collaboration with Adimab, anti-CD39 antibodies were identified by yeast-based screening. 83 candidates were further tested for CD39 binding at both pH 7.4 and pH 6.0 using Octet® Bio-Layer Interferometry (BLI). Colored symbols represent positive control high affinity anti-CD39 antibodies provided by Adimab. N.B., no observable binding; P.F., poor fit for binding kinetics.

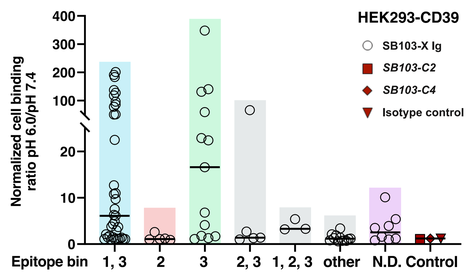

Figure 4. Cell binding of candidate antibodies to HEK293-CD39 cells. Candidate antibodies were tested for cell surface binding by flow cytometry under neutral (pH 7.4) and acidic (pH 6.0) conditions. Data are expressed as the ratio of binding at pH 6.0 to binding at pH 7.4 and grouped according to epitope bin.

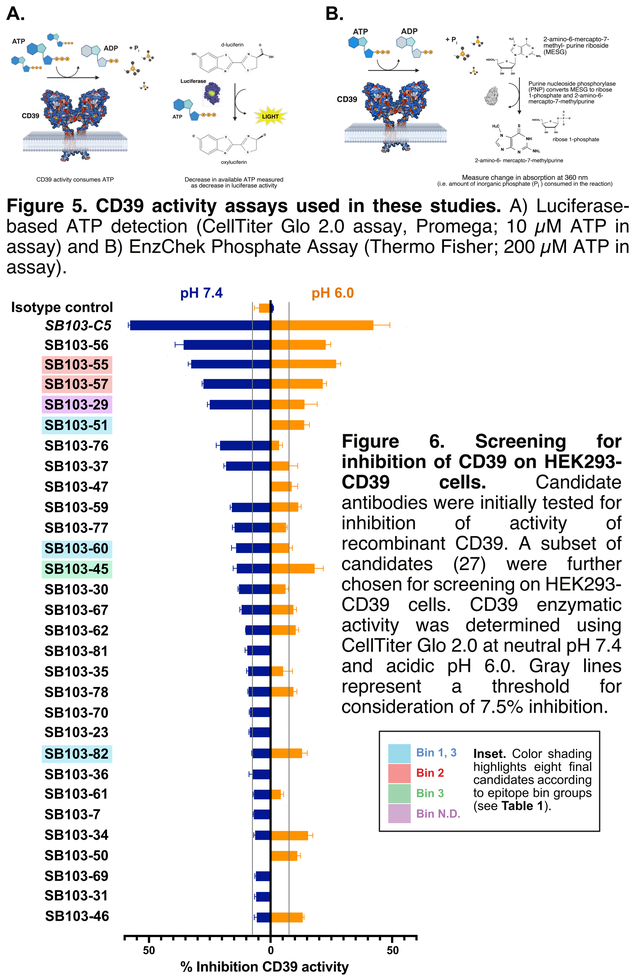

Figure 5. CD39 activity assays used in these studies. A) Luciferase-based ATP detection (CellTiter Glo 2.0 assay, Promega; 10 μM ATP in assay) and B) EnzChek Phosphate Assay (Thermo Fisher; 200 μM ATP in assay).

Figure 6. Screening for inhibition of CD39 on HEK293-CD39 cells. Candidate antibodies were initially tested for inhibition of activity of recombinant CD39. A subset of candidates (27) were further chosen for screening on HEK293-CD39 cells. CD39 enzymatic activity was determined using CellTiter Glo 2.0 at neutral pH 7.4 and acidic pH 6.0. Gray lines represent a threshold for consideration of 7.5% inhibition.

Bin 1, 3
Bin 2
Bin 3
Bin N.D.

Inset. Color shading highlights eight final candidates according to epitope bin groups (see **Table 1**).

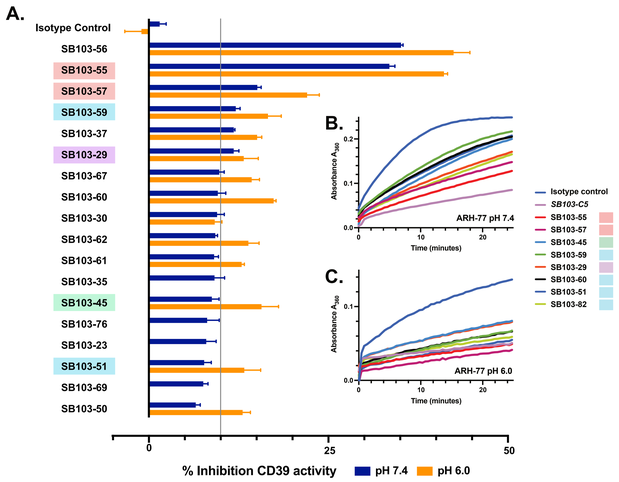

Figure 7. Screening for inhibition of endogenous CD39 in ARH-77 cells. A) A narrowed set (15-18) of candidate anti-CD39 antibodies were tested for inhibition on CD39⁺ ARH-77 B lymphoblastoid cells. CD39 enzymatic activity was determined using CellTiter Glo 2.0 at neutral pH 7.4 and acidic pH 6.0. Gray line denotes a threshold of 10% inhibition. B-C) The final 8 candidates were tested for inhibition of endogenous CD39 activity on ARH-77 cells by EnzChek phosphate assay at B) pH 7.4 and C) pH 6.0.

Antibody ID	CD39 inhibition [cell based @ pH 6.0; %]			Inhibition Index	Binding Affinity (Octet BLI)			Cell Binding (Flow Cyt)	Epitope Bin
	HEK293-CD39	SK-MEL-28	ARH-77	Avg fold Δ pH 6.0 vs pH 7.4	K_D (nM) pH 7.4	K_D (nM) pH 6.0	#Fold K_D	#Fold	
SB103-55	27	21	41	1	37	37	1	1	2
SB103-57	21	6	22	1	449	472	1	1	2
SB103-45	18	11	16	2	86	17	5	133	3
SB103-59	11	17	17	2	51	9	6	6	1, 3
SB103-29	14	1	9	0.5	57	58	1	1	N.D.
SB103-60	8	12	17	2	64	15	4	4	1, 3
SB103-51	14	8	13	8	426	24	18	11	1, 3
SB103-82	13	–	10	3	456	445	1	8	1, 3
SB103-C5	42	77*	N.D.	1	1	1	1	N.D.	1

*performed at 100 nM vs 200 nM for others
Ratio between measurements at pH 6.0 vs pH 7.4

Table 1. Summary results for top candidate antibodies. Eight candidates were selected for further optimization.

Summary and Conclusions

- A panel of 83 antibodies were identified and characterized for inhibition of CD39 enzymatic activity at neutral and acidic pH.
- 8 candidate antibodies were selected based on inhibitory activity, affinity, pH-dependence, and epitope and CDR sequence diversity. These are currently undergoing lead optimization.
- We believe that our pH-dependent TME targeting strategy can alleviate undesirable properties of on-target/off-tumor binding, particularly target-mediated drug disposition that results in suboptimal pharmacokinetic properties.

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