CBO421, a novel drug Fc-conjugate, inhibits the enzymatic activity of CD73 and triggers CD73 internalization

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BACKGROUND

CD73 (5'-nucleotidase or NT5E) is a cell-anchored enzyme responsible for the rate limiting step in adenosine production; the conversion of adenosine monophosphate (AMP) to adenosine[1] (**Fig. 1**). Accumulation of immunosuppressive adenosine in the tumor microenvironment contributes to immune evasion in solid tumors by inhibiting immune effector cells (e.g., $CD8^+$ T cells) via the A2AR-mediated signaling cascade. CD73 expression levels correlate with a worse prognosis in multiple cancers, including breast cancer. Adenosine production via CD73 can be therapeutically inhibited by two independent mechanisms: (1) enzyme inhibition, and (2) receptor internalization and subsequent degradation. CBO421, a drug Fc-conjugate (DFC), comprised of a novel small molecule CD73 inhibitor stably conjugated to an immune-silent human IgG1 Fc, employs both mechanisms, combining the strengths of small molecule inhibitors and CD73-targeting monoclonal antibodies (mAbs) with potential best-in-class activity.

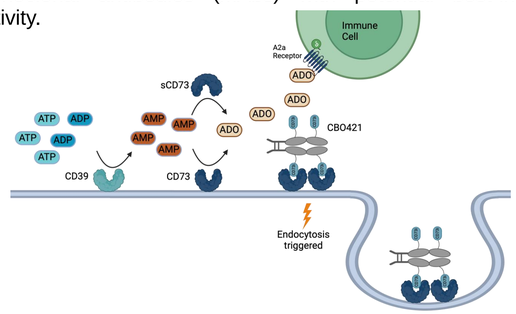


Figure 1. Adenosine pathway schematic. CBO421 is a catalytic inhibitor of CD73 that induces CD73 receptor downregulation via internalization.

METHODS

The activity of CBO421 was compared to clinical-stage CD73 inhibitors: commercially sourced small molecule inhibitor AB680 (quemliclustat)[2] and anti-CD73 mAb oleclumab biosimilar (MEDI9447)[3,4]. Enzymatic inhibition of CD73 was evaluated using recombinant, soluble CD73 and in a triple-negative human breast cancer cell line (MDA-MB-231) in the presence of AMP for 3 h. Receptor-mediated internalization of CD73 was measured using three methods: (1) MDA-MB-231 cells were treated with CBO421 or oleclumab and CD73 internalization was measured by flow cytometry as the decrease in CD73 surface receptor signal over time; (2) internalization of CD73 receptors expressed on MDA-MB-231 cells was assessed via a Fab-ZAP kit[4] (Advanced Targeted System) using a saporin-CBO421 conjugate; and (3) CD73 receptor internalization of MDA-MB-231 cells visualized by fluorescence microscopy.

RESULTS

CBO421 demonstrated dose-dependent and complete inhibition of soluble recombinant human CD73. CBO421 (IC_{50} = 13.8 nM) (**Fig. 2A**) demonstrated comparable activity to the small molecule CD73 inhibitor AB680 (IC_{50} = 4.0 nM) (**Fig. 2B**). Oleclumab demonstrated partial inhibition (with hook effect) against soluble recombinant CD73 (**Fig. 2C**). All three test articles demonstrated potent inhibition of cell-anchored human CD73 expressed on the surface of the human breast cancer cell line MDA-MB-231 (CBO421 IC_{50} = 0.77 nM, AB680 IC_{50} = 0.09 nM, oleclumab biosimilar IC_{50} = 0.17 nM). (**Fig. 2D**).

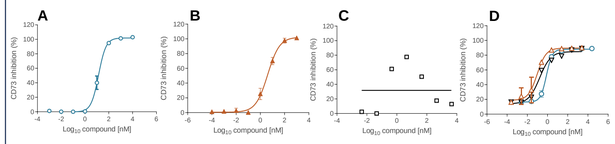


Figure 2. Inhibition of soluble recombinant human CD73 with the following compounds **(A)** CBO421 (blue), **(B)** AB680 (orange) and **(C)** Oleclumab (black) and **(D)** Inhibition of cell-anchored human CD73 in MDA-MB-231 cells.

CBO421 and oleclumab internalization percentages were measured by flow cytometry at 1 h (52.3% vs 26.7%), 4 h (80.8% vs 38.0%), and 6 h (84.8% vs 42.9%) respectively, with CBO421 demonstrating superior maximal receptor internalization. The magnitude of CD73 internalization by oleclumab was comparable to previously reported values[3]. The unconjugated Fc, negative control, did not exhibit any receptor-mediated internalization of CD73, as expected. IC_{50} values were calculated for CBO421 and oleclumab at 1 h (0.20 nM vs 0.88 nM; **Fig. 3A**), 4 h (0.07 nM vs 0.27 nM; **Fig. 3B**), and 6 h (0.08 nM vs 0.14 nM; **Fig. 3C**), respectively.

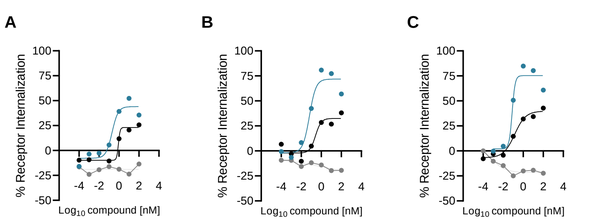


Figure 3. Time course of CD73 receptor internalization in MDA-MB-231 cells analyzed by flow cytometry at **(A)** 1 hour, **(B)** 4 hours, and **(C)** 6 hours. CBO421 (blue), oleclumab (black), unconjugated Fc (grey).

Internalization of CD73 receptors expressed on MDA-MB-231 cells was also assessed using a saporin-drug conjugate (**Fig. 4A**) indicated that CBO421 induced receptor internalization with an IC_{50} of 0.14 nM (**Fig. 4B**) at a 96 h time point.

RESULTS

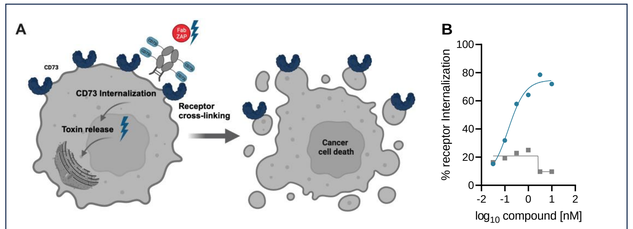


Figure 4. (**A**) Schematic of CD73 receptor-mediated internalization assay using the Fab-ZAP kit. (**B**) Receptor internalization in MDA-MB-231 cells at 96 h. CBO421 (blue) and unconjugated Fc (grey).

CD73 receptor mediated internalization was visualized using fluorescence microscopy. MDA-MB-231 cells were treated with a dilution series of CBO421 (**Fig. 5A**) or unconjugated Fc (**Fig. 5B**). After 4 h of treatment, surface CD73 was stained with a fluorescently-labeled anti-human CD73 detection antibody. CBO421 demonstrated potent reduction in surface CD73 signal. The calculated IC_{50} value of CBO421-induced receptor internalization was 0.26 nM (**Fig. 6**) with over 90% CD73 receptor internalization by microscopy.

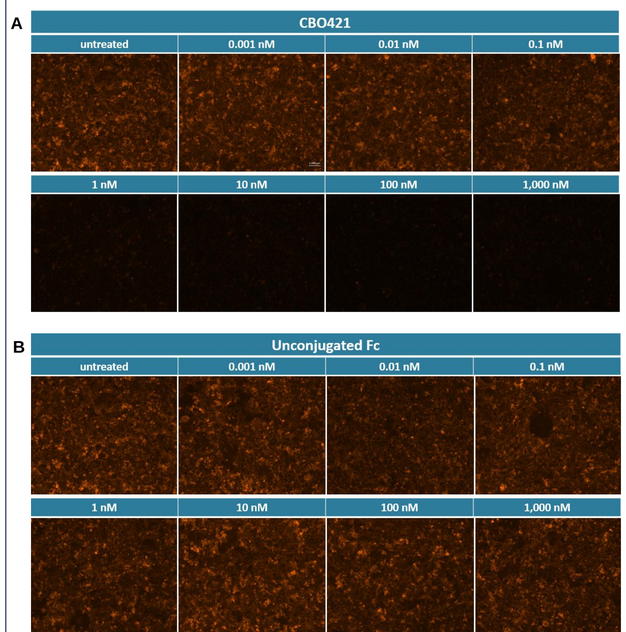
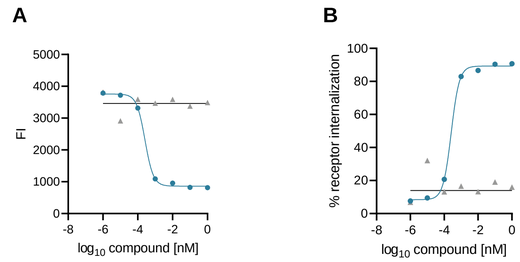


Figure 5. Surface CD73 was detected with an anti-human CD73 antibody and visualized after 4 h treatment with (**A**) CBO421 or (**B**) unconjugated Fc by fluorescent microscopy.

RESULTS



(**Figure 6**) Fluorescence intensity (FI) of surface CD73 after treatment with a dilution series of CBO421 (blue) or unconjugated Fc (grey) in MDA-MB-231 cancer cells. (**A**) A decrease in FI for CBO421 indicates internalization of surface CD73 receptors while no decrease in FI is observed with unconjugated Fc, as expected. (**B**) Calculated surface CD73 internalization based on normalization of fluorescence intensity in untreated control.

SUMMARY

CBO421 exhibited potent inhibition of both soluble and membrane-bound CD73 on MDA-MB-231 cancer cells, differentiating it from existing mAb CD73 inhibitors. In saporin-drug conjugate assays, flow cytometry, and fluorescence microscopy studies, CBO421 demonstrated potent CD73 receptor internalization and degradation as a secondary mechanism to prevent CD73-mediated adenosine production.

CBO421 has demonstrated high potency in functional cell-based assays (**Poster 2668**). This potency translated into robust antitumor efficacy in monotherapy that was further improved in combination with anti-PD-1 therapy in a syngeneic mouse tumor model[5].

Currently, CBO421 is completing IND-enabling studies to facilitate clinical evaluation of its efficacy in the treatment of solid tumors.

DISCLOSURE & REFERENCES

All authors are shareholders & employees of Cidara Therapeutics.

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[2]Lawson et al., 2020 PMID: 32614585
[3]Sachsenmeier et al.,US 0129108 A1, 2016.
[4]Hay et al., 2016 PMID: 27622077
[5]Cidara Therapeutics website