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 NTSE) 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.

METHODS

The activity of CBO421 was compared to clinical-stage CD73 inhibitors: commercially sourced small molecule inhibitor AB680 (quemucilast)\(^2\) and anti-CD73 mAb oleclumab biosimilar (MED9447)\(^3,4\). Enzymatic inhibition of CD73 was evaluated using recombinant, soluble CD73 and 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\(^5\) (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).

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 4 h (84.8%) as the expected IC\(_{50}\) value. CBO421 demonstrated superior maximal receptor internalization. The magnitude of CD73 internalization by oleclumab was comparable to previously reported values\(^5\). 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.

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.

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.

DISCLOSURE & REFERENCES

All authors are shareholders & employees of Cidara Therapeutics.

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