DISCOVERY OF CBO421, A FIRST-IN-CLASS DRUG-FC CONJUGATE (DFC), TARGETING CD73 IN CANCER

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BACKGROUND

Dying tumor cells release ATP, which is sequentially converted to adenosine monophosphate (AMP) by CD39 and to adenosine by CD73 (N5TE). By flooding the tumor microenvironment (TME) with immune-suppressive adenosine, where concentrations can reach µM levels, CD73, a rate-limiting enzyme in this process, contributes to immune evasion and drug resistance in solid tumors[1]. Adenosine has been shown to inactivate tumor infiltrating immune cells such as CD8⁺ T cells through its cognate receptor, A2AR, in the TME (**Fig. 1A**).

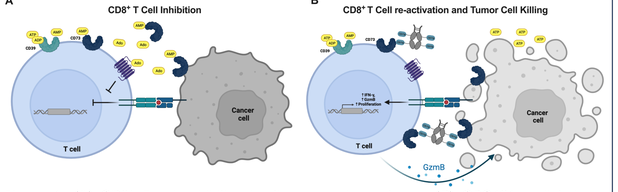

Figure 1. (**A**) CD8⁺ T cell inhibition by the adenosine pathway. (**B**) CBO421-mediated re-activation of CD8⁺ T cells by CD73 inhibition resulting in T cell-induced cancer cell apoptosis.

Furthermore, high CD73 expression levels are correlated with worse prognosis in multiple cancers (e.g. CRC, TNBC). Herein, we describe a CD73 targeting DFC, CBO421, a multivalent conjugate of a potent small molecule CD73 inhibitor stably linked to a proprietary immune-silent human IgG1 Fc. CBO421 combines the strengths of small molecule inhibitors and monoclonal antibodies (mAbs) targeting CD73 with potential best-in-class activity to prevent adenosine-induced inhibition of CD8⁺ T cells (**Fig. 1B**).

METHODS

The activity of CBO421, commercially sourced small molecule inhibitor, AB680 (Quemliclustat), and biosimilar anti-CD73 mAb, Oleclumab (MEDI9447), were investigated. Enzymatic inhibition of CD73 was evaluated using recombinant CD73, in a human breast cancer cell line (MDA-MB-231), a murine breast cancer cell line (EMT-6), or human PBMCs in the presence of AMP. CD73 expression on PBMCs was determined by flow cytometry. Activity of CBO421 was measured in a PBMC re-activation assay in the presence of AMP using flow cytometry (CD25⁺ or GzmB⁺), ELISA (INFγ), or CellTiter-Glo (CD73 inhibition). CD73 internalization was measured in MDA-MB-231 cells using a Fab-ZAP kit (Advanced Targeted System). Tumor spheroid penetration was conducted with MDA-MB-231 cells using confocal microscopy by PhenoVista Biosciences. Efficacy of CBO421 dosed at 10 mg/kg twice a week for two weeks was evaluated in a syngeneic mouse model with the CRC cell line, MC38. On day 18, tumor samples were collected and dissociated by mechanical and enzymatic dissociation (Miltenyi) to obtain single cell suspensions. Tumor infiltrating lymphocytes (TIL) were assessed for CD45⁺, CD3⁺, CD4⁺, CD25⁺, CD8⁺ and FOXP3⁺ staining by flow cytometry and analyzed in FlowJo.

RESULTS

CBO421 is a DFC (**Fig. 2A**) that targets CD73. CD73 is a GPI-anchored ectoenzyme, that forms homodimers and undergoes conformational changes from an open to a catalytically active closed conformation to convert substrate, AMP, to adenosine and phosphate (**Fig. 2B**).

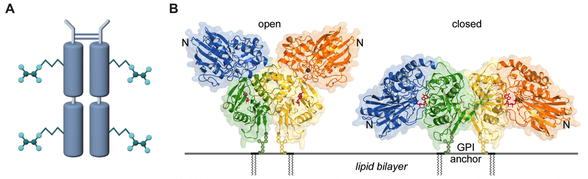

Figure 2. (**A**) Schematic of CBO421. (**B**) Crystal structure of CD73 with substrate (red) in the open and in the catalytically active closed conformation[2].

The first generation anti-CD73 mAb, oleclumab, currently in P2/3 clinical development, is a partial, non-competitive inhibitor that prevents CD73 from adopting the closed, active state[3,4]. CBO421 binds to CD73 expressed on MDA-MB-231 cancer cells with an IC_{50} of 0.17 nM (**Fig. 3A**), and showed a dose-dependent decrease in IC_{50} to 8.61 nM with 1,000 µM AMP (**Fig. 3B**) and to 62.6 nM with 100 nM AB680 (**Fig. 3D**) demonstrating CBO421 is a potent, complete, AMP-competitive and catalytic site inhibitor of CD73. The binding for oleclumab was unaffected by AMP or AB680 (**Fig. 3C, E**). CBO421 demonstrated complete enzyme inhibition with an IC_{50} of 13.8 nM (**Fig. 3F**), comparable to AB680 with an IC_{50} of 4.0 nM (**Fig. 3G**) against soluble CD73. Oleclumab demonstrated weak partial inhibition with hook effect (**Fig. 3H**). For cell-anchored CD73 using MDA-MB-231 cells, CBO421 demonstrated an IC_{50} of 0.77 nM, versus 0.09 nM for AB680 and 0.17 nM for oleclumab (**Fig. 3I**). Similarly, CBO421 demonstrated potent CD73 inhibition with an IC_{50} of 0.77 nM versus 1.06 nM for AB680 and 5.68 nM for oleclumab against CD73 expressing EMT-6 cells (**Fig. 3J**).

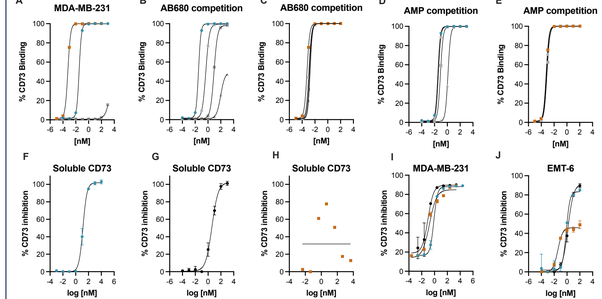

Figure 3. Binding to MDA-MB-231 cells (**A**) alone or with (**B**, **C**) 10–1,000 µM AMP (grey) or (**D**, **E**) 1–100 nM AB680 (grey). Soluble (**F-H**) and cell-anchored CD73 inhibition with (**I**) MDA-MB-231 or (**J**) EMT-6 cells. CBO421 (blue), AB680 (black), oleclumab (orange) or unconjugated Fc (grey) in **A**

RESULTS

CD73 is predominantly expressed on B cells ($CD3^-CD19^+$) and CD8⁺ T cells in human PBMCs (**Fig. 4A, B**) as shown by tSNE analysis. CBO421 showed complete inhibition of CD73 on human PBMCs with IC_{50}s of 0.62 nM to 1.75 nM, versus 0.02 nM to 0.05 nM with AB680 and 0.37 nM to 5.69 nM with oleclumab at 3h or 24h, respectively (**Fig. 4C-D**). Importantly, CBO421 demonstrated potent re-activation of human PBMCs in the presence of AMP with a median EC_{50} (n = 3) of 12 nM by INFγ (**Fig. 4E**) or 2.6 nM by CD73 inhibition (**Fig. 4F**) similar to AB680 (12 nM (INFγ) or 4.1 nM (CD73 inhibition)) and superior to oleclumab (>1,000 nM (INFγ or CD73 inhibition)) (**Fig. 4E-F**). Similarly, CBO421 demonstrated potent re-activation of CD8⁺ T cells with median EC_{50} of 44 nM by CD25⁺ (**Fig. 4G**) and 34 nM granzyme B⁺ (**Fig. 4H**) comparable to AB680 (11 nM (CD25⁺) and 54 nM (granzyme B⁺)) and superior to oleclumab (>1,000 nM (CD25⁺ or granzyme B⁺)).

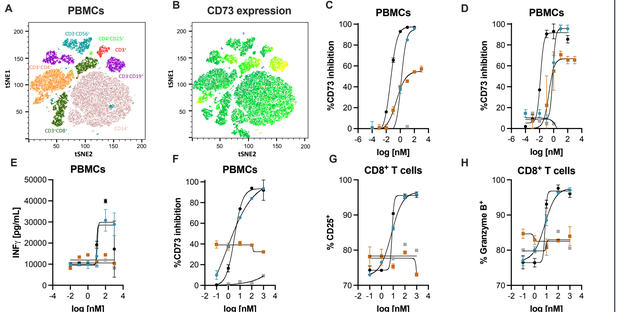

Figure 4. (**A**) Immune subsets of PBMCs and (**B**) relative CD73 expression by tSNE analysis. CD73 inhibition of PBMCs at (**C**) 3 h and (**D**) 24 h. Rescue of AMP-suppressed PBMCs determined by (**E**) INFγ ELISA, (**F**) CD73 inhibition or of CD8⁺ T cells as determined by (**G**) CD25⁺ or (**H**) granzyme B⁺. CBO421 (blue), AB680 (black), oleclumab (orange), unconjugated Fc (grey)

CD73 internalization via receptor cross-linking is a second mechanism that can be exploited to reduce adenosine production (**Fig. 5A**). CBO421 and oleclumab induced receptor internalization with EC_{50}s of 0.13 nM and <0.03 nM, respectively (**Fig. 5B**). AB680 did not trigger receptor internalization, as expected for a small molecule (**Fig. 5C**).

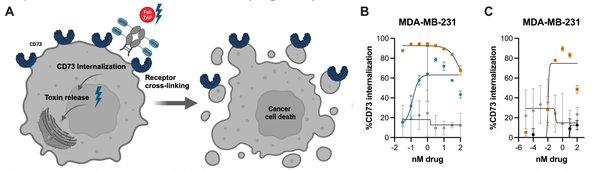

Figure 5. (**A**) Schematic of CD73 receptor-mediated internalization assay using the Fab-ZAP kit. (**B, C**) Receptor internalization in MDA-MB-231 cells at 96 h. CBO421 (blue), AB680 (black), oleclumab (orange), unconjugated Fc (grey)

RESULTS

CBO421 showed complete penetration into MDA-MB-231 spheroids compared to oleclumab, which was unable to reach the core (**Fig. 6A-C**).

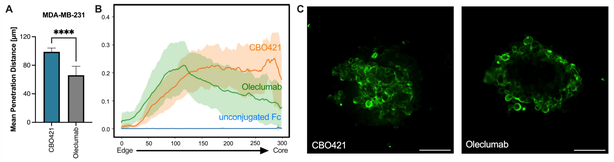

Figure 6. (**A**) MDA-MB-231 spheroid penetration of CBO421 (**A**) mean penetration distance, (**B**) radial plot analysis and (**C**) representative z-stack confocal microscopy images.

CBO421 monotherapy showed robust tumor growth inhibition (TGI) with 27% of mice achieving complete responses[5] in a MC38 mouse model (**Fig. 7A**). This TGI correlated with improved immune cell infiltration (**Fig. 7B-F**) and statistically significant increases in CD8⁺ T cell : T_{reg} ratio (**Fig. 7G**).

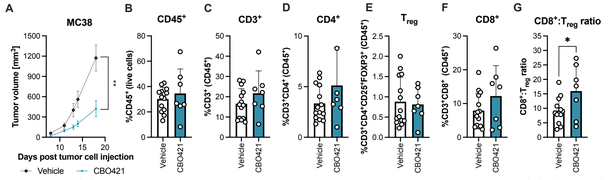

Figure 7. (**A**) Efficacy of CBO421 in monotherapy and TIL analysis on day 18 (**B**) CD45⁺ (all immune cells), CD3⁺ (T cells), $CD4^+CD25^+FOXP3^+$ regulatory T cells (T_{regs}), CD8⁺ and ratio of CD8⁺ to T_{regs} in a syngeneic MC38 mouse efficacy model; statistical analysis by unpaired t-test.

SUMMARY

In preclinical models, CBO421 outperformed anti-CD73 targeting molecules currently in clinical development. CBO421 combines the potent and complete enzymatic inhibition observed with small molecule inhibitors and targeted receptor internalization seen with the anti-CD73 mAb, oleclumab. The *in vitro* potency of CBO421 translated into robust anti-tumor efficacy in CRC mouse models, that was further improved in combination with an anti-PD-1 mAb[5]. Based on these results and other emerging data, CBO421 is being advanced as a clinical development candidate for treatment of solid tumors.

DISCLOSURE & REFERENCES

All authors are share-holder & employees of Cidara Therapeutics. *corresponding author: *sdoehrmann@cidara.com*

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