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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. Herein, we describe a CD73 targeting DFC, CBO421. CBO421 is 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 (mAb) targeting CD73 with potential best-in-class activity.

METHODS

The activity of CBO421, a commercially sourced small molecule inhibitor, AB680, and a biosimilar anti-CD73 mAb, oleclumab, were investigated. Enzymatic inhibition of CD73 was evaluated in a human triple negative breast cancer cell line, MDA-MB-231, that expresses high levels of CD73 on its surface, in the presence of 100 µM AMP. Activity of CBO421 was measured in peripheral blood mononuclear cell (PBMC) rescue assay in the presence of 30 µM AMP using flow cytometry and ELISA. CD73 internalization was measured with MDA-MB-231 cells using a Fab-ZAP kit. Efficacy of CBO421 (10 mg/kg) and the murine anti-PD-1 mAb (20 mg/kg), RMP1-14, were evaluated as monotherapy and in combination dosed IP twice a week for two weeks in a syngeneic mouse model with a colorectal carcinoma (CRC) cell line, MC38.

RESULTS

DFCs are a novel class of therapeutics comprising small molecule inhibitors stably conjugated to an N-terminally extended Fc domain of human IgG1 (**Fig. 1A**). Exemplars from the DFC platform have reached clinical development. Our most advanced DFC is currently in a clinical phase 2a trial for the prevention of influenza (NCT05523089). CBO421 is a first-in-class DFC for oncology. CBO421 is a potent, AMP-competitive inhibitor of CD73[2]. In cell-based CD73 inhibition assays, CBO421 demonstrated potent and complete enzyme inhibition (IC_{50} of 0.94 nM), comparable to the small molecule inhibitor, AB680, (IC_{50} of 0.078 nM) and the anti-CD73 mAb oleclumab (IC_{50} of 0.17 nM) that are currently in clinical development (**Fig. 1B**). CD73 is predominantly expressed on B cells and $CD8^+$ T cells in human PBMCs (**Fig. 1C**). CBO421 demonstrated potent reactivation of AMP-suppressed human PBMCs as determined by INFγ secretion (**Fig. 1D**) with an EC_{50} of 11 nM. CBO421 potently re-activates AMP-suppressed $CD8^+$ T cells with a median EC_{50} (n = 3 unique donors) of 44 nM by $CD25^+$ (**Fig. 1E**) and 34 nM by granzyme B $(GZMB)^+$, a marker of cytotoxic $CD8^+$ T cells, (**Fig. 1F**) comparable to AB680 (11 nM ($CD25^+$) and 54 nM ($GZMB^+$)) and superior to oleclumab (>1,000 nM ($CD25^+$ or $GZMB^+$)).

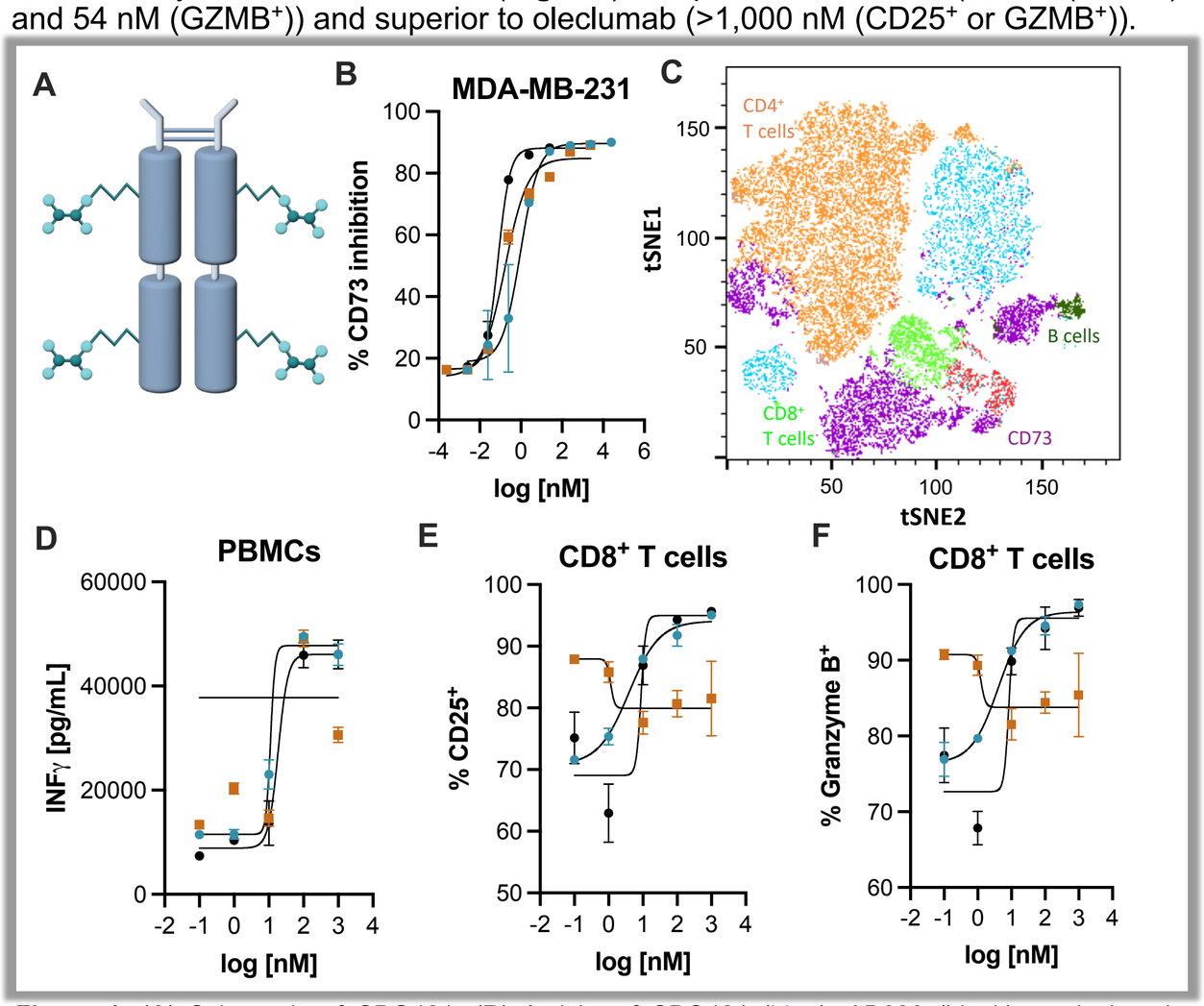

Figure 1. (**A**) Schematic of CBO421. (**B**) Activity of CBO421 (blue), AB680 (black) or oleclumab (orange) in CD73 inhibition with MDA-MB-231. (**C**) CD73 expression of immune subsets in human PBMCs by tSNE analysis. Rescue of AMP-suppressed PBMCs determined by (**D**) INFγ ELISA or of $CD8^+$ T cells as determined by (**E**) $CD25^+$ or (**F**) granzyme B^+ using flow cytometry.

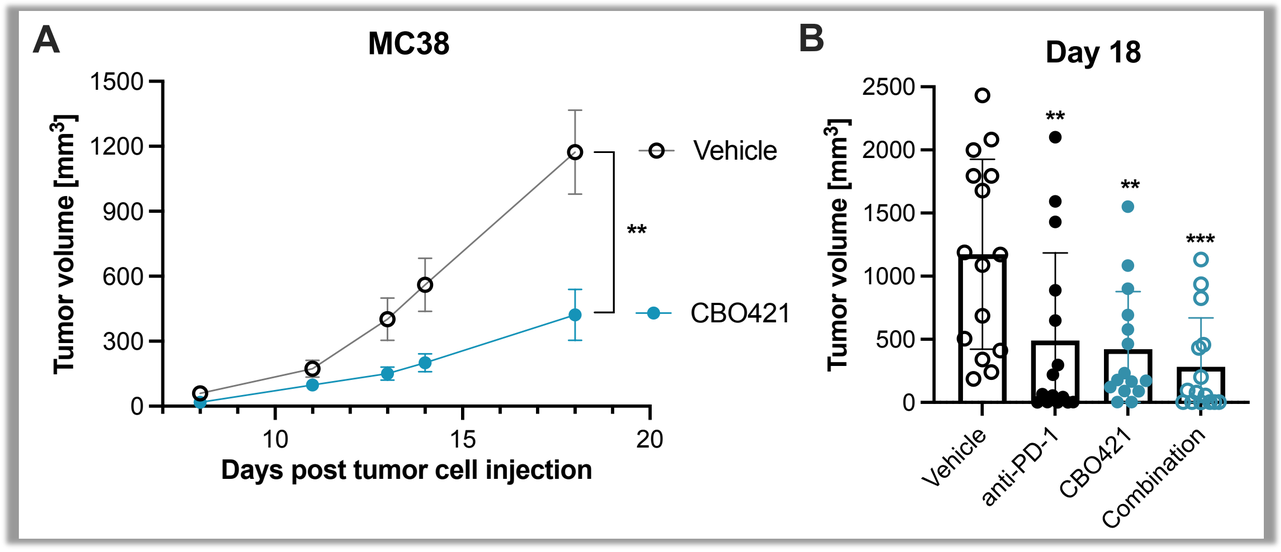

Figure 2. (**A**) Efficacy of CBO421 in monotherapy or (**B**) in combination with murine anti-PD-1 mAb in a syngeneic MC38 mouse efficacy model; statistical analysis by two- or one-way ANOVA in Prism.

CBO421 monotherapy demonstrated robust tumor growth inhibition (TGI) which improved when combined with the murine anti-PD-1 mAb RMP1-14 in a MC38 syngeneic mouse model (**Fig. 2**).

Table 1. TGI and %responders in CBO421 monotherapy and combination therapy with anti-PD-1 therapy on day 18 in the MC38 syngeneic mouse efficacy model.

Study Arm	Dose	%TGI	% Responders
Vehicle	n/a	0	0 (0/15)
CBO421	10 mg/kg	62.2	27 (4/15)
Anti-PD-1	20 mg/kg	56.0	47 (7/15)
Combination	as above	74.7	60 (9/15)

Moreover, combination therapy resulted in significant increases in response rate (60% of mice in the combination arm) when compared with the respective monotherapy cohorts (**Table 1**). Differentiated from small molecules (e.g. AB680), CBO421 triggered CD73 receptor internalization, a Fc-dependent mechanism requiring cross-linking of CD73 receptors, as a second mechanism to reduce CD73 activity and thereby adenosine production (**Table 2**).

Table 2. CD73 internalization in MDA-MB-231 cancer cells.

Test article	EC_{50} [nM]
unconjugated Fc	>10
CBO421	0.14
AB680	>10
Oleclumab biosimilar	<0.03

CONCLUSIONS

In preclinical models, CBO421 outperformed CD73 inhibitors currently in clinical development. Consistent with the DFC drug class, CBO421 combines the potent and complete enzymatic inhibition of small molecule inhibitors and the targeted receptor internalization of mAbs, resulting in enhanced TGI. The *in vitro* potency of CBO421 translated to robust antitumor activity in CRC mouse efficacy models, that was further improved in combination with an anti-PD-1 mAb. Based on these results and other emerging data, CBO421 is being advanced as a clinical development candidate for treatment of solid tumors.

DISCLOSURES

All authors are share-holder & employees of Cidara Therapeutics.

REFERENCES

[1]Allard et al., 2020 PMID: 32514148
[2]Cidara Therapeutics website
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