**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 (NSTE). 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 tumors1. 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) infection 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. In cell-based CD73 inhibition assays, CBO421 demonstrated potent and complete enzyme inhibition (IC50 of 0.94 nM), comparable to the small molecule inhibitor, AB680, (IC50 of 0.078 nM) and the anti-CD73 mAb oleclumab (IC50 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 determined by INFγ secretion (Fig. 1D) with an EC50 of 11 nM. CBO421 potently re-activates AMP-suppressed CD8+ T cells with a median EC50 (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*)).

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).

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). Differentially 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).

**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**

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