# CBO421: A novel Drug Fc-Conjugate to prevent tumor immune evasion via the CD73/adenosine pathway

Amanda Almaguer*, Doug Zuill*, Simon Döhrmann, James Levin, Nicholas Dedeic, Elizabeth Abelovski, Joanne Fortier, Qiping Zhao, Maria Hernandez, Karin Amundson, Madison Moniz, Hongyuan Chen, Dhanya Panickar, Thanh Lam, Tom Brady, Allen Borchardt, Grayson Hough, Jeffrey B. Locke, Jason N. Cole, and Leslie W. Tari^

Cidara Therapeutics, Inc., 6310 Nancy Ridge Drive, Suite 101, San Diego, CA, USA

2668

## BACKGROUND

Adenosine exhibits immunosuppressive properties and its accumulation in the tumor microenvironment contributes to immune evasion by solid tumors (**Fig. 1A**). CD73 (5'-nucleotidase or NT5E) is a cell surface enzyme responsible for the rate limiting step in adenosine production; the conversion of adenosine monophosphate (AMP) into adenosine through hydrolysis[1], making CD73 a promising target for immunotherapy. CBO421, a Drug Fc-Conjugate (DFC), comprises a novel small molecule CD73 inhibitor stably conjugated to an immune-silent human IgG1 Fc (**Fig. 1B**). CBO421 combines the strengths of small molecule inhibitors and CD73-targeting monoclonal antibodies (mAbs) for potential best-in-class activity to prevent adenosine-induced inhibition of CD8+ T cells and restore cancer cell killing (**Fig. 1C**).

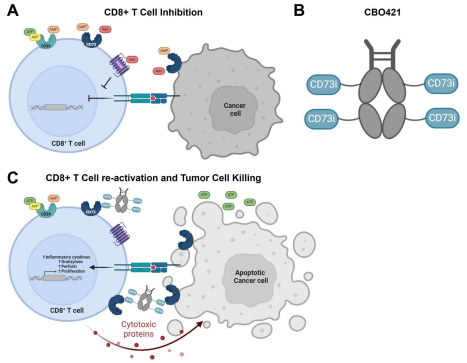


**Figure 1**. (**A**) CD8⁺ T cell inhibition by the adenosine pathway. (**B**) Schematic of CBO421 Drug Fc-Conjugate (**C**) CBO421-mediated re-activation of adenosine suppressed CD8⁺ T cells resulting in T cell-induced apoptosis.

## METHODS

CBO421's binding affinity to human CD73 and Fc receptors was assessed by surface plasmon resonance (SPR). Antibody-dependent cellular cytotoxicity (ADCC) was evaluated in the CD73-expressing MDA-MB-231 cells using the Promega ADCC Reporter Bioassay core kit. Inhibition of soluble and membrane-bound CD73 on human PBMCs and mouse mammary carcinoma EMT-6 cells was determined by measuring free phosphate levels using the Abcam Phosphate Detection kit. Immunophenotyping and binding to cancer cells was measured by flow cytometry and analyzed using t-SNE. Functional activity to reactivate either ATP- or AMP-suppressed T cells was measured in rescue assays. *In vivo* efficacy of CBO421 monotherapy was evaluated in a syngeneic mouse model using C57BL/6 mice. A dose response study was conducted using the colorectal cancer cell line, MC-38. CBO421 was dosed twice weekly at 2, 10, and 50 mg/kg.

## RESULTS

CBO421 exhibited a binding affinity to human CD73 ($K_D$ = 0.80 nM) that was comparable to, or greater than the affinity observed with small molecule inhibitors and CD73-targeting mAbs (**Table 1**).

**Table 1.** Binding of CBO421 and comparators to soluble human CD73 by SPR.

| Test Article | Description | $K_D$ (nM) |
|---|---|---|
| CBO421 | CD73-targeting DFC | 0.80 |
| Unconjugated Fc | Fc moiety of CBO421 | >125 |
| AB680 | CD73-targeting small molecule inhibitor | 2.64 |
| Oleclumab | Anti-CD73 mAb | 0.11 |

CBO421 utilizes a proprietary immune-silent Fc backbone. As expected, CBO421 binding to human Fc gamma receptors was abolished in the FcγRIIIA and FcγRIIA receptor variants and largely reduced in the FcγRI receptor variant compared to the control mAb trastuzumab biosimilar. No change in human FcRn affinity was observed for CBO421 compared to unconjugated Fc and a full-length wild type IgG1 control mAb, as anticipated (**Table 2**). ADCC activity was abolished in CBO421 ($EC_{50}$ = >6.7 nM) and unconjugated Fc ($EC_{50}$ = >6.7 nM) compared to the control mAb, cetuximab biosimilar ($EC_{50}$ = 0.037 nM) using a CD73-expressing cancer cell line (**Fig 2**). These binding results suggest CBO421 poses a minimal risk for undesired Fc-dependent responses.

**Table 2.** Binding of CBO421 and comparators to human Fc receptors by SPR.

| Test Article | $K_D$ (nM) Human Fc receptor | | | |
|---|---|---|---|---|
| | FcRn | FcγRIIIA / CD16a (V176) | FcγRIIA / CD32a (H167) | FcγRI / CD64 |
| CBO421 | 65.6 | None | None | 146 |
| Unconjugated Fc | 25.3 | None | None | 365 |
| Trastuzumab | 87.6 | 108 | 1010 | 5.36 |

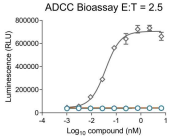


**Figure 2**. ADCC activity was determined at an Effector-to-Target ratio 2.5. CBO421 (**blue circle**), unconjugated Fc (**orange triangle**), and cetuximab (**grey diamond**).

In a free phosphate detection assay, CBO421 potently inhibited CD73 expressed on human PBMCs (**Fig. 3A, B**) and mouse EMT-6 mammary carcinoma cells (**Fig. 3C, D**) at 3 and 24 h. $IC_{50}$ values were comparable to small molecule CD73 inhibitors and CD73-targeting mAbs. CBO421 maintained sub-nanomolar $IC_{50}$ values in both cell types at 24 h and demonstrated the smallest shift in activity at 24 h compared to the other test articles (**Table 3**).

| Test Article | $IC_{50}$ (nM) PBMCs | | EMT-6 | |
|---|---|---|---|---|
| | 3 h | 24 h | 3 h | 24 h |
| CBO421 | 0.59 | 0.91 | 0.77 | 0.83 |
| Unconjugated Fc | >100 | >100 | >100 | >100 |
| AB680 | 0.03 | 0.07 | 1.06 | 2.29 |
| Oleclumab | 0.48 | 1.97 | 5.68 | >100 |

**Table 3.** Activity of CBO421 and comparators in a CD73 inhibition assay at 3 and 24 h using human PBMCs or mouse EMT-6 cancer cells.

## RESULTS

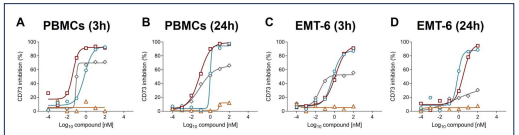


**Figure 3**. CD73 inhibition as measured by phosphate generation in human PBMCs at (**A**) 3 h and (**B**) 24 h and mouse EMT-6 cancer cells at (**C**) 3 h and (**D**) 24 h. CBO421 (**blue circle**), AB680 (**red square**), oleclumab biosimilar (**grey diamond**), and unconjugated Fc (**orange triangle**).

Immunophenotyping and t-SNE analysis of human PBMCs revealed that CD73 is predominantly expressed on B cells ($CD3^-CD19^+$) and $CD8^+$ T cells (**Fig. 4A, B**). CBO421 demonstrated potent reactivation of ATP-suppressed $CD8^+$ T cells ($EC_{50}$ = 51 nM) based on $CD25^+$; none of the comparator inhibitors of the adenosine pathway demonstrated PBMC re-activation ($EC_{50}$ = >1,000 nM): IPH5201 (anti-CD39 mAb), AB928 (A2AR/A2BR small molecule inhibitor), and CPI-444 (A2AR small molecule inhibitor) (**Fig. 4C**). CBO421 demonstrated potent reactivation of AMP-suppressed $CD8^+$ T cells ($EC_{50}$ = 20 nM) by $CD25^+$, comparable to AB680[2] ($EC_{50}$ = 10 nM), a potent small molecule CD73 inhibitor. Oleclumab biosimilar[3], a CD73-targeting mAb, showed minimal activity in this assay ($EC_{50}$ = >1,000 nM) (**Fig. 4D**).

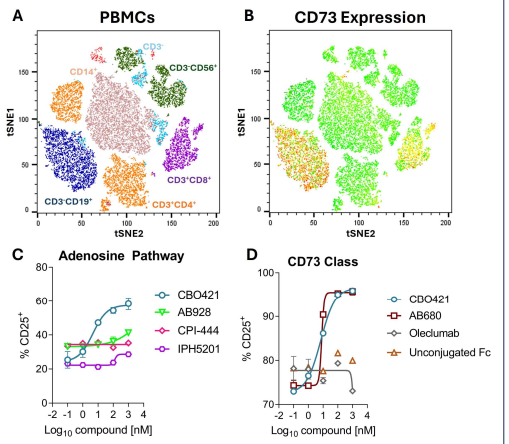


**Figure 4**. (**A**) Immune subsets of PBMCs and (**B**) relative CD73 expression by tSNE analysis. % $CD25^+$ of $CD8^+$ T cells following suppression with (**C**) ATP or (**D**) AMP

## RESULTS

To determine if the *in vitro* activity of CBO421 translates into *in vivo* efficacy, a dose response study was conducted in a syngeneic mouse model using the colorectal cancer cell line, MC-38. When CBO421 was dosed twice weekly at 2, 10, and 50 mg/kg there was a sequential decrease in tumor volume (**Fig. 5A**). This resulted in tumor growth inhibition (TGI) of 22.0, 36.8, and 59.0%, respectively; the TGI for the 50 mg/kg group was statistically significant (**Fig. 5B**). It was also noted that in the high dose group 25% of mice had tumors demonstrating cessation of growth. Collectively, these data demonstrate that CBO421 has significant *in vivo* efficacy at relatively modest dose levels. Ongoing studies are being conducted to determine its potency against additional tumor types.

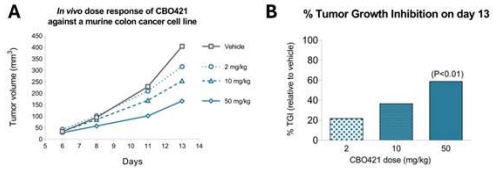


**Figure 5**. (**A**) *In vivo* efficacy of CBO421 at three doses in a syngeneic mouse tumor model through 13 days, (**B**) Percent tumor growth inhibition (TGI) for each CBO421 dose group on day 13.

## SUMMARY

CBO421 exhibited high affinity binding and potent inhibition of both soluble and membrane-bound CD73, differentiating it from mAb CD73 inhibitors. CBO421 demonstrated potent CD73 receptor internalization and degradation as a secondary mechanism to prevent CD73-mediated adenosine production (**Poster LB131**). The proprietary immune-silenced Fc of CBO421 did not affect FcRn binding and was confirmed to have reduced Fc gamma receptor binding, as expected. CBO421 demonstrated high potency in functional cell-based assays. This potency translated into robust antitumor efficacy as monotherapy that was further improved in combination with anti-PD-1 therapy (**Poster 2728**) in a syngeneic mouse tumor model. 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 shareholder & employees of Cidara Therapeutics.
* presenting authors
^ corresponding author
ltari@cidara.com

[1] Xia et al., 2023 PMID: 36859386
[2] Bowman et al 2019 PMID: 31334635
[3] Hay et al2016 PMID: 27622077