Dying tumor cells release ATP, which is sequentially converted to adenosine monophosphate (AMP) by CD39 and to adenosine by CD73 (NITE). By flooding the tumor microenvironment (TME) with immunosuppressive adenosine, where concentrations can reach levels >10 μM, T cells become unresponsive. This effect is mediated by CD73, a rate-limiting enzyme in this process, contributes to immune evasion and drug resistance in solid tumors. 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).

The activity of CBO421, commercially sourced small molecule inhibitor, AB680 (Quentelimmun GmbH, mAb, Oxeloidum (MED186477), 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 (CD3, CD25, CD127, and IL-2/ELISA (INF), or CellTiter-Glo (CD73 inhibition). CD73 internalization was measured in MDA-MB-231 cells using a Fab-ZAP Kit (Advanced Targeting System). Tumor spheroid penetration was conducted with MDA-MB-231 cells using confocal microscopy by PhenoVista Biosciences. Efficacy of CBO421 against tumor cell killing was assessed using an ELISA-based, chemiluminescent substrate assay as a readout.

**RESULTS**

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

**METHODS**

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

**DISCUSSION & REFERENCES**

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**DISCOVERIES & REFERENCES**

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