A novel CD73-blocking antibody reduces production of immunosuppressive adenosine and restores T cell function

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INTRODUCTION

ADENOSINE IS GENERATED BY CD73 AND CREATES AN IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT

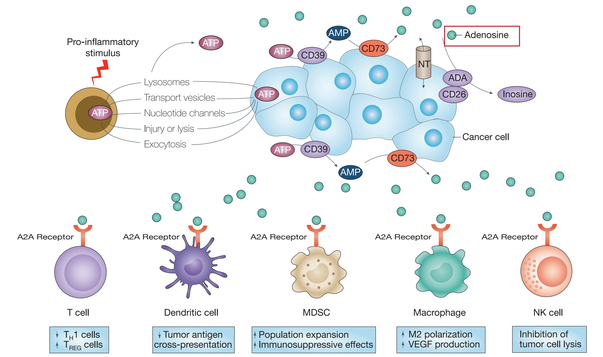


Figure adapted from Antonioli et al, Nat Rev Cancer. 2013[1]

CD73 IS AN ECTOENZYME THAT CONVERTS AMP TO ADENOSINE

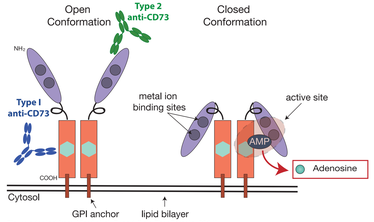


Figure adapted from Allard et al, Expert Opin Ther Targets. 2014[2]

- Switching from the open to closed conformation is required for catalytic activity[3]
- CD73 is overexpressed in multiple tumor types and high expression associates with poor prognosis[4,5,6]
- CD73 inhibition has demonstrated anti-tumor activity as a single agent and in combination with other IO agents in preclinical models[4,5,6]

CPX-006 AND CPX-016 BIND TO CD73 AND REDUCE ADENOSINE PRODUCTION

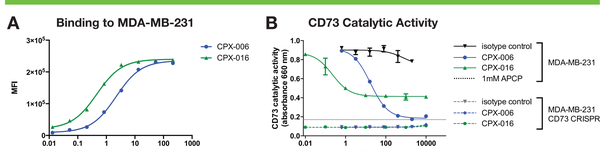


Figure 1 (A) MDA-MB-231 cells were incubated with CPX-006 or CPX-016 over a range of concentrations and antibody binding was detected with a PE-conjugated secondary antibody and flow cytometry analysis. Mean fluorescence intensity (MFI) of PE signal is reported. (B) MDA-MB-231 parental cells or CD73 CRISPR knockout cells were incubated with the indicated antibodies or APCP, a small molecule inhibitor of CD73 enzymatic activity, prior to addition of 250 µM AMP. Phosphate levels were measured in the cell culture supernatant using the Sensolyte Malachite Green phosphate assay kit.

CPX-006 AND CPX-016 BIND TO DISTINCT EPITOPES ON CD73

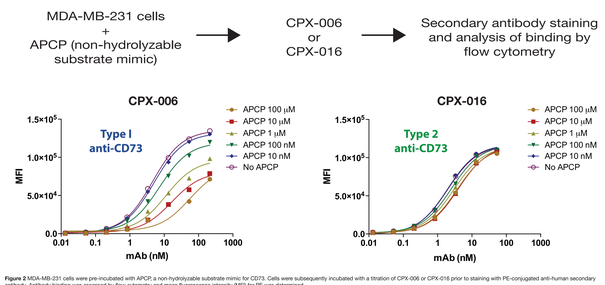


Figure 2 MDA-MB-231 cells were pre-incubated with APCP, a non-hydrolyzable substrate mimic for CD73. Cells were subsequently incubated with a titration of CPX-006 or CPX-016 prior to staining with PE-conjugated anti-human secondary antibody. Antibody binding was assessed by flow cytometry and mean fluorescence intensity (MFI) for PE was determined.

CPX-006 REVERSES SUPPRESSIVE EFFECTS OF AMP ON T CELL ACTIVITY

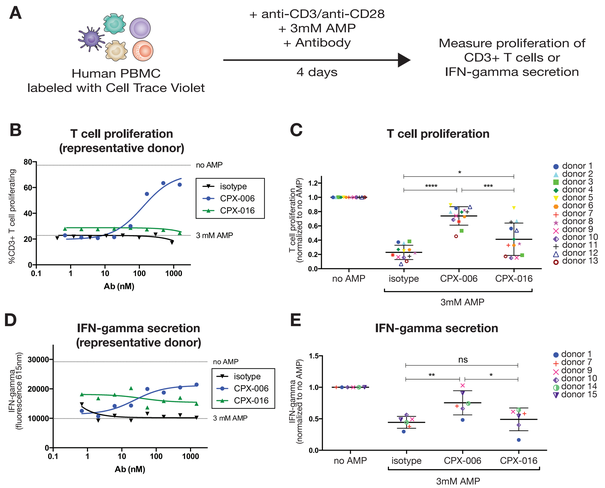


Figure 3 (A) Schematic of experimental design. PBMC were isolated from healthy donors and labeled with Cell Trace Violet prior to culture with 1 µg/mL anti-CD3 and anti-CD28, 200 units/mL IL-2, 3mM AMP, and antibody treatment as indicated. After 4 days in culture, IFN-gamma levels in the supernatant were measured by AlphaLISA. Proliferation of CD3+ T cells was followed by flow cytometry analysis of Cell Trace Violet dilution and was defined by gating relative to unstimulated PBMC. (B) T cell proliferation for a representative donor. (C) T cell proliferation for 13 donors treated with 500nM CPX-006 or CPX-016 or 890nM isotype control. (D) IFN-gamma production (reported as raw AlphaLISA values) for a representative donor. (E) IFN-gamma production for 6 donors treated as described in panel (C).

CPX-006 REVERSES SUPPRESSIVE EFFECTS OF AMP ACROSS A BROAD RANGE OF CD73 EXPRESSION

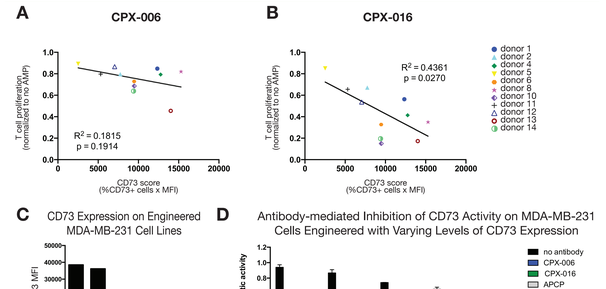


Figure 4 (A, B) CD73 expression levels in each donor were determined by flow cytometry and were plotted as a function of the effect of CPX-006 or CPX-016 (500nM) on restoring T cell proliferation in the presence of AMP as reported in Figure 3C. (C) MDA-MB-231 cells were engineered to reduce or eliminate CD73 expression via shRNA or CRISPR, respectively. CD73 protein levels were measured by flow cytometry analysis. (D) CD73 catalytic activity was measured for each cell line in the presence of 1µM CPX-006 or CPX-016 or 1mM APCP as described Figure 1B.

CD73 IS HIGHLY EXPRESSED IN MULTIPLE TUMOR TYPES

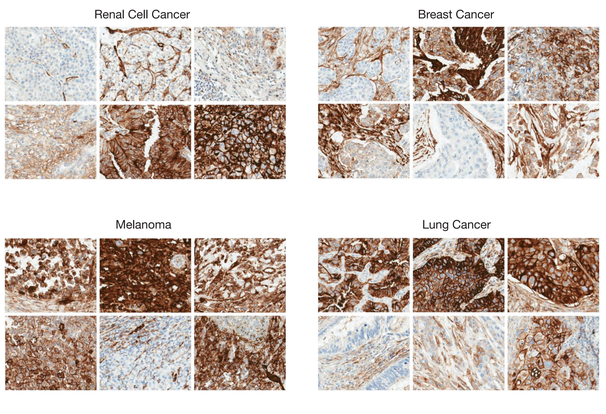
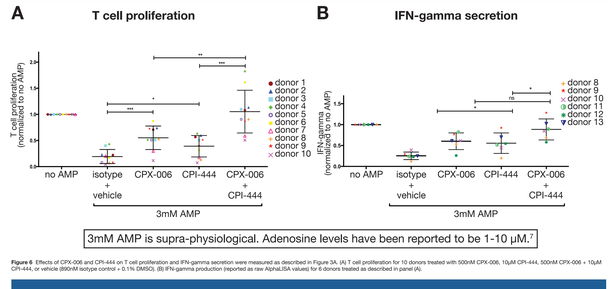


Figure 5 CD73 expression was analyzed by immunohistochemical staining of tissues from renal cell cancer (n=62), NSCLC (n=68), melanoma (n=68), and breast cancer (n=94). Six representative tissues are shown from each histology.

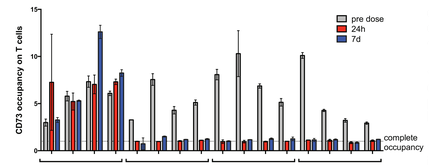
CPX-006 AUGMENTS A2AR ANTAGONIST, CPI-444



3mM AMP is supra-physiological. Adenosine levels have been reported to be 1-10 µM.[7]

Figure 6 Effects of CPX-006 and CPI-444 on T cell proliferation and IFN-gamma secretion were measured as described in Figure 3A. (A) T cell proliferation for 10 donors treated with 500nM CPX-006, 10µM CPI-444, 500nM CPX-006 + 10µM CPI-444, or vehicle (890nM isotype control + 0.1% DMSO). (B) IFN-gamma production (reported as raw AlphaLISA values) for 6 donors treated as described in panel (A).

NO TOXICITY OBSERVED WITH CPX-006 IN NON-HUMAN PRIMATES



- Cynomolgus monkeys dosed weekly for 5 consecutive weeks at 10, 40, or 120 mg/kg
- No changes observed in clinical chemistry, gross pathology, organ weights, histopathology
- Complete CD73 coverage is achieved with CPX-006 at all doses tested

Figure 7 CD73 occupancy was determined by measuring ex vivo staining of CD8+ T cells with Alexa-Fluor 647 labeled CPX-006. Ratio of AF647 staining on CD73+/CD73- cells is reported.

CONCLUSIONS

- We describe a unique Type 1 anti-CD73 antibody that binds to the active site of CD73 and completely inhibits CD73 activity in contrast to all other described antibodies which bind to the N-terminal lobe (Type 2)
- CPX-006 inhibits AMP-mediated suppression of T cell proliferation and IFN-gamma secretion and effects are augmented with CPI-444
- CPX-006 inhibits the immune-suppressive activity of CD73 completely even at high levels of CD73 found in tumors, in contrast to CPX-016
- CPX-006 is well-tolerated with no observed toxicities in multi-dose non-human primate studies where complete CD73 occupancy is achieved
- IND for CPX-006 is planned for Q1 2018

REFERENCES
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