Activating CD73 on B cells as a target for immunotherapy of COVID-19 and viral associated cancers: Clinical activity in human papilloma virus positive (HPV) head and neck squamous cell cancers (HNSCC)

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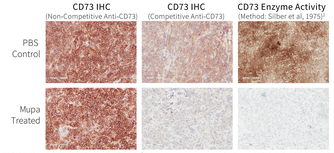
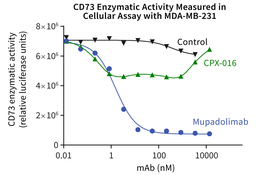
MUPADOLIMAB, ANTI-CD73 ANTIBODY, BLOCKS CD73 ENZYMATIC ACTIVITY AND ACTIVATES B AND T CELLS

MUPADOLIMAB (MUPA) BINDS TO TUMOR CELLS AND BLOCKS THE PRODUCTION OF ADENOSINE WITHOUT CAUSING CD73 INTERNALIZATION[2]

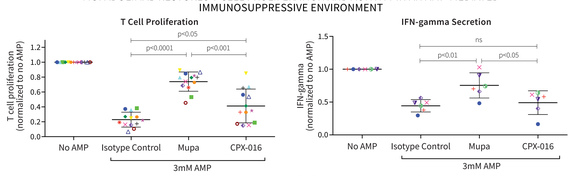


Mupadolimab is a humanized FcγR binding-deficient IgG1 anti-CD73 antibody. Mupadolimab administered intraperitoneally to nude mice bearing human breast cancer cell line MDA-MB-231. Left and middle panels demonstrate binding to CD73 and presence of antigen detected by non-competitive antibody indicating lack of antigen internalization. Right panel demonstrates complete blockade of adenosine production. CD73 enzymatic activity in tissue sections was determined using the method described by Silber, et al. (1975). CD73 activity is visualized by the black lead sulfide precipitate.



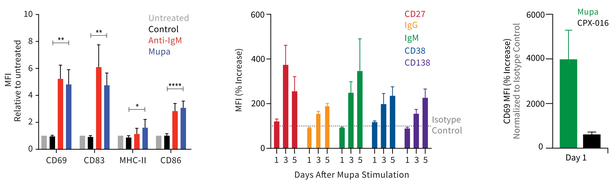
Mupadolimab completely inhibits CD73 enzymatic activity, with no hook effect, measured using a luciferase assay that is dependent on levels of AMP. CPX-016 is an antibody in advanced clinical testing that shows a hook effect.

MUPADOLIMAB RESTORES T CELL PROLIFERATION AND ACTIVITY IN AN AMP-MEDIATED IMMUNOSUPPRESSIVE ENVIRONMENT



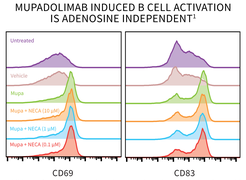
Peripheral blood mononuclear cells (PBMC) were obtained from healthy donors and cultured under T cell activating conditions. Addition of AMP, which is converted to adenosine by CD73, resulted in decreased T cell proliferation and cytokine secretion. Addition of mupadolimab restored T cell proliferation in all donors tested. The anti-CD73 antibody in advanced clinical testing (CPX-016) restored T cell proliferation in a subset of donors but did not reach the magnitude of mupadolimab response.

MUPADOLIMAB ACTIVATES B CELLS AND PROMOTES DIFFERENTIATION[1,3]



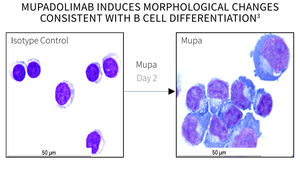
Left panel: In vitro studies show mupadolimab upregulates MHC-II and CD86 on B lymphocytes, suggesting potential for increased antigen presentation. Middle panel: Increased cell surface expression of markers consistent with B cell differentiation into plasma cells. Right panel: B cell activation is unique to mupadolimab as other anti-CD73 antibodies do not induce CD69 expression.

MUPADOLIMAB INDUCED B CELL ACTIVATION IS ADENOSINE INDEPENDENT[1]



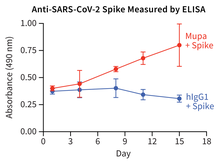
Addition of NECA, an adenosine agonist, does not interfere with mupadolimab-induced B cell activation and differentiation.

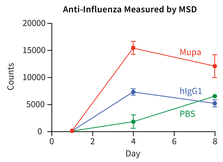
MUPADOLIMAB INDUCES MORPHOLOGICAL CHANGES CONSISTENT WITH B CELL DIFFERENTIATION[3]



Incubation of human peripheral blood B cells with mupadolimab results in transformation into plasmablasts.

MUPADOLIMAB ENHANCES ANTIBODY RESPONSE TO SARS-CoV-2 AND INFLUENZA ANTIGENS





- Humanized mice (NSG-SGM) were immunized with the SARS-CoV-2 spike protein or influenza H1N1 hemagglutinin on Day 1, and treated with mupadolimab or human IgG1 control.
- Mupadolimab treatment enhanced a specific antibody response to SARS-CoV-2 (spike protein) or Influenza (H1N1 hemagglutinin).
- Universal treatment: mupadolimab may be useful in augmenting immune responses to viral antigens.

Results from a Randomized Study in Hospitalized COVID-19 Patients

	2 mg/kg + SOC (N=15)	1 mg/kg + SOC (N=14)	Placebo + SOC (N=11)
Primary Endpoint			
Free from Respiratory Failure or Death (%)	93.3	85.7	81.1
Secondary Endpoints			
Median Days to Improvement (95% CI)	7.0 (4-9)	5.5 (3-14)	11.0 (2-14)
Median Days Sustained Improve (95% CI)	8.0 (4-12)	6.0 (3-14)	11.0 (2-21)
Median Days to Discharge (95% CI)	6.0 (4-12)	4.0 (2-5)	7.0 (2-12)

- A randomized, placebo controlled, double-blind, multicenter, stratified study of a single dose of mupadolimab (1 mg/kg and 2 mg/kg) plus standard of care (SOC) versus placebo plus SOC was conducted in mild to moderately symptomatic hospitalized COVID-19 patients (NCT04734873).[4]
- Consistent trend in improved clinical outcome measures seen in treatment arms.
- Anti-viral antibody response studies consistent with proposed mechanism.

CD73 EXPRESSION IN VARIOUS CANCERS

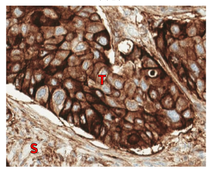
CD73 Immunohistochemistry Staining

	Membrane	Cytoplasm	Stroma
HNSCC (N=31)			
HPV+	1/25	2/25	25/25
HPV-	0/6	0/6	6/6
NSCLC (N=75)	42/75	19/75	74/75

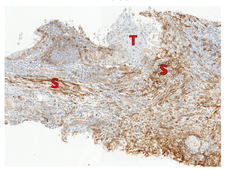
Patient tumor biospsies were examined by IHC (number patient samples positive/number patient samples tested). Calculated by H score, a weighted average of the 4 categories = 3x% 3+ staining + 2x% 2+ staining + 1x% of 1+ staining + 0x% 0 staining, giving a range of 0 to 300. Positivity defined by H score above 100. All NSCLC are adenocarcinoma. In normal lung (N=3, data not included), rare to infrequent staining mostly associated with vessels was observed.

- Nearly all NSCLC patient samples demonstrate stromal staining; subsets also show staining of tumor membrane and cytoplasm.
- All HNSCC patient samples examined show stromal staining and rare cases have membrane and cytoplasmic staining.
- HPV status does not appear to affect stromal expression of CD73.

NSCLC



HNSCC



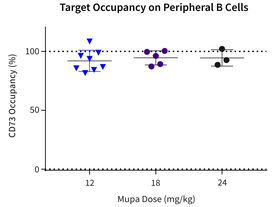
IHC of NSCLC demonstrating stromal and tumor cell staining; IHC of HNSCC demonstrates stromal staining and minimal tumor staining.
S = Stroma, **T** = Tumor

PHASE 1/1B PROTOCOL DESIGN SUMMARY

In an ongoing Phase 1/1b clinical trial (NCT03454451), mupadolimab alone and in combination with ciforadenant (A2A receptor antagonist) and/or pembrolizumab is being evaluated in patients with advanced refractory cancers. These studies have shown that a dose of ≥ 12 mg/kg intravenous every 3 weeks is well-tolerated and leads to complete CD73 target occupancy. Data from the Phase 1 dose escalation portion of the study are shown below. Fifteen NSCLC patients and ten HNSCC patients received doses of ≥ 12 mg/kg. Additional NSCLC and HNSCC patients are being evaluated in ongoing expansion cohorts.

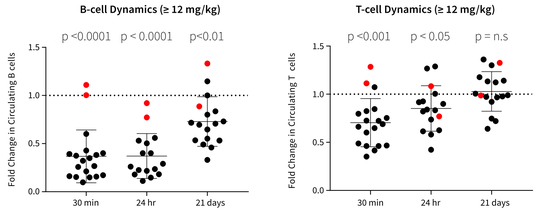
PHARMACOKINETICS AND PHARMACODYNAMICS[3]

SUSTAINED CD73 OCCUPANCY OF PERIPHERAL BLOOD AT ≥ 12 MG/KG



- Mupadolimab occupancy of peripheral blood B cells was evaluated using flow cytometry and showed complete saturation at trough levels (Day 21).
- Shown below, administration of mupadolimab caused changes in circulating B cells and T cells consistent with redistribution. Only CD73 positive B cells were affected. This supports presence of CD73 engaged by mupadolimab drives immune cell redistribution.
- Each data point represents a patient. Red data points (below) are patients who exhibited low levels of CD73 pretreatment (<20%) expression.

TREATMENT INDUCED RAPID CHANGES IN BLOOD B AND T CELLS



HNSCC AND NSCLC PATIENT CHARACTERISTICS AND ADVERSE EVENT SUMMARY

Patient Characteristics

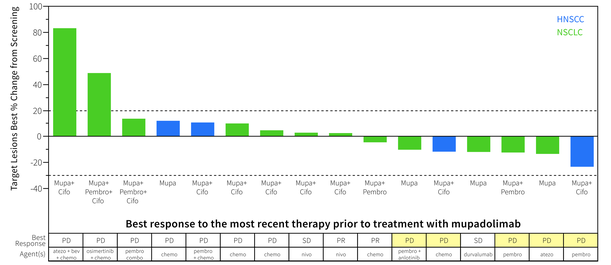
	Subject Count	Age (yrs), Median (range)	Gender, male N (%)	No. of prior therapies Median (range)	Prior PD-(L)1 Therapy N (%)
HNSCC	10	65 (43, 87)	9 (90)	3 (1, 5)	10 (100)
NSCLC	15	64 (53, 80)	6 (40)	3 (2, 4)	14 (93)

Adverse Event Summary

- Treatment related adverse events (AEs) were reported in 17 (68%) of the NSCLC and HNSCC patients.
- Severe AEs (Grade 3 or above) were reported in 4 (16%) patients.
- Treatment related serious AEs were reported in 2 (8%) patients.
- No changes in serum quantitative immunoglobulins were observed.

ANTI-TUMOR ACTIVITY IN HNSCC AND NSCLC

HNSCC AND NSCLC AT MUPADOLIMAB DOSES ≥ 12 MG/KG



Best response to the most recent therapy prior to treatment with mupadolimab

Cifo = ciforadenant (A2AR antagonist), pembro = pembrolizumab (anti-PD-1), atezo = atezolizumab (anti-PD-L1), bev = bevacizumab (anti-VEGF), chemo = chemotherapy, nivo = nivolumab (anti-PD-1), PD = progressive disease, SD = stable disease, PR = partial response.

- Waterfall plot of best tumor response seen in Phase 1 portion of the study for evaluable patients. Treatment regimens are indicated.
- Responses were compared to best response of most recent therapy prior to receiving mupadolimab.
- Tumor regression was observed in some patients who had progressive disease as best response to most recent prior therapy, which often included an anti-PD-(L)1 (see yellow highlighted PDs).
- Enrollment in expansion cohorts of NSCLC and HNSCC is ongoing.

CONCLUSIONS

- Mupadolimab is a humanized anti-CD73 antibody that blocks adenosine production.
- Mupadolimab has immunomodulatory activities:
  - It induces B cell activation and differentiation into plasmablasts that is adenosine independent
  - It augments T cell function by blocking adenosine production
- In humanized mice receiving SARS-CoV-2 and influenza antigens, mupadolimab enhances humoral anti-viral responses, suggesting its potential application as an anti-viral therapy or vaccine adjuvant.
- The enhancement of humoral immunity by mupadolimab is being explored as a potential immunotherapy for various cancers, including virally associated malignancies.
- CD73 expression is high in NSCLC; expression can be membrane, cytoplasm and/or stromal. HNSCC expression of CD73 appears to be exclusively stromal.
- In a Phase 1 study, doses of mupadolimab 12 mg/kg or greater are well tolerated, and demonstrate full occupancy of the target.
- Mupadolimab binding to CD73 results in transient reductions in circulating B and T cells and is likely a result of redistribution of lymphocytes in to peripheral lymphoid tissues.
- In this Phase 1 study, tumor regression was observed in patients with HNSCC and NSCLC treated with mupadolimab or combinations, including patients who were refractory (progressive disease as best response) to their most recent prior regimen, which often contained an anti-PD-(L)1.

REFERENCES

1. Luke JJ, et al. Presented at the ASCO Annual Meeting; May 30-Jun 3, 2019; Chicago, IL, USA. Abstr 2505.
2. Silber, et al. *J Clin Invest.* 1975;56(5):1324-7.
3. Luke JJ, et al. Presented at the SITC Annual Meeting; Nov 6-Nov 10, 2019; National Harbor, MD, USA. Abstr O40.
4. Miller RA, et al. *medRxiv.* 2021; doi: https://doi.org/10.1101/2021.09.13.21263406

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