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NEUTRALIZING ACIDOSIS WITH ADC L-DOS47 UREASE IMMUNOCONJUGATE ENHANCES RESPONSE TO ANTI-PD1 CHECKPOINT BLOCKADE IN A PRECLINICAL ORTHOTOPIC MODEL OF PANCREATIC ADENOCARCINOMA

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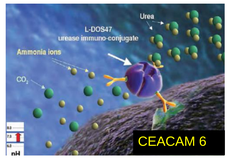
BACKGROUND

- Acidosis in the tumor microenvironment is an important immunosuppressive mechanism that leads to tumor growth [1]
- We have shown that neutralization of tumor pH using sodium bicarbonate improves responses to immune checkpoint blockade (ICB) in pre-clinical models [2]
- Phase I/IIa clinical trials with bicarbonate failed due to poor patient compliance [3]

Our aim is to test clinically translatable agents to neutralize tumor acidity and improve cancer outcomes in response to ICB

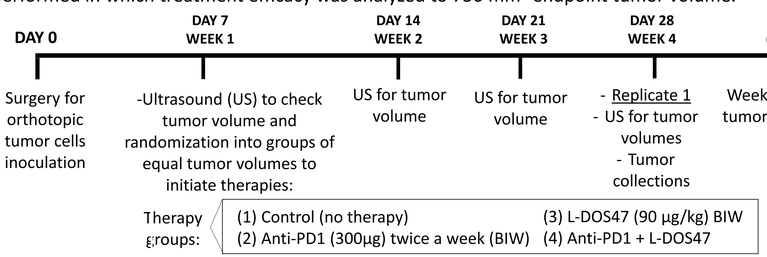


- L-DOS47 is a targeted urease anti-carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6) immunoconjugate designed to directly neutralize tumor acidity that has been well-tolerated in phase I/IIa clinical trials [4,5]
- **An unconventional antibody-drug conjugate (ADC), L-DOS47** binds to the surface of tumor cells expressing CEACAM6 where its urease moiety cleaves endogenous urea into two NH_4^+ and one CO_2 raising extracellular tumor pH [6]
- CEACAM6 is highly expressed in lung and gastrointestinal cancers, including pancreatic ductal adenocarcinoma (PDAC)

Here we demonstrate L-DOS47 function and efficacy in combination with anti-PD1 ICB targeting pancreatic cancer

METHODS

Immunocompetent B6.129 mice were inoculated orthotopically with murine pancreatic KPC961 cells transduced to express human CEACAM6; clone 1B6 was selected for its high CEACAM6 expression. Three biological replicates were performed in which treatment efficacy was analyzed to 750 mm^3 endpoint tumor volume.



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RESULTS

IHC screening of PDAC tissue microarrays confirms high CEACAM6 expression and L-DOS47 binding

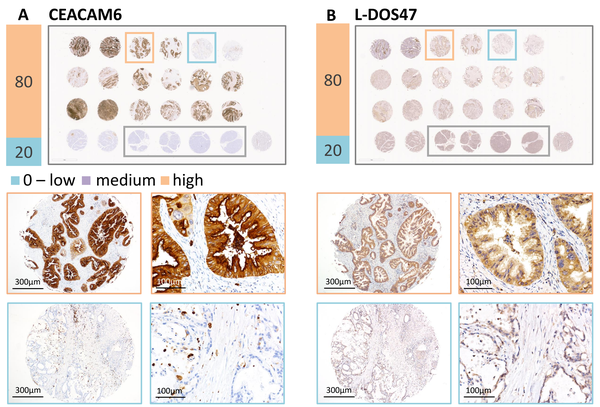


Figure 1A. Examples of PDAC cases with high (orange boxes) and low (blue boxes) CEACAM6 expression and **B.** L-DOS47 binding on a TMA comprising duplicate cores from ten cancer cases and two cancer adjacent tissues (grey boxes, TMA from TissueArray.com). PS2+ scoring [7] is shown in the bar charts.

Tumor extracellular pH (pHe) increases in acidic PDAC tumors in response to L-DOS47

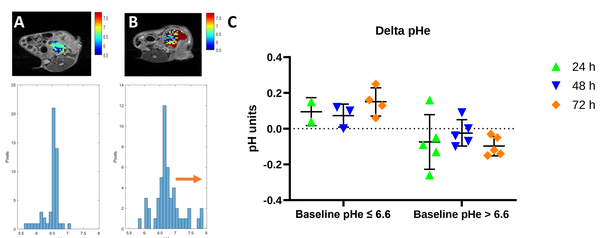
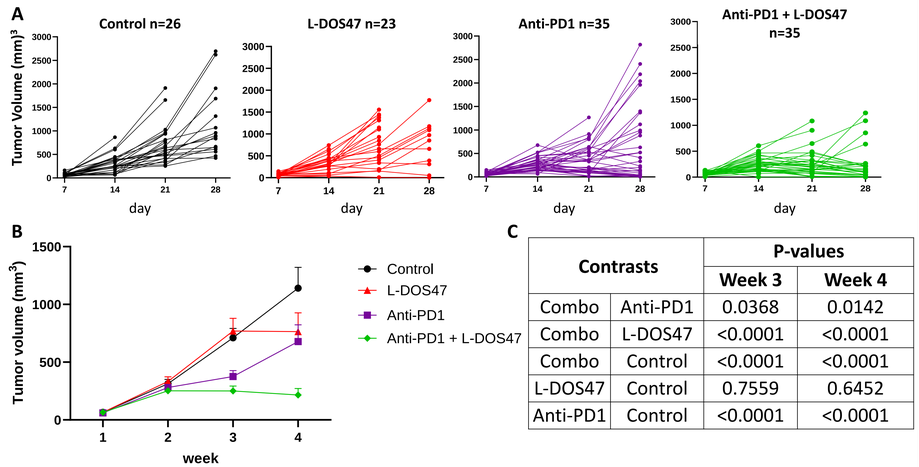


Figure 2. Chemical exchange saturation transfer-magnetic resonance imaging (CEST-MRI) [8] of tumor pHe pre- and post-L-DOS47. Representative pHe maps and histograms show pHe pixel distributions in tumors **A.** at baseline (pre-dose) and **B.** 72h post-L-DOS47 **C.** Delta pHe was calculated by subtracting the mean tumor pHe post-L-DOS47 from pre-L-DOS47 baseline tumor pHe. Responses segregated around baseline pHe 6.6.

Anti-PD1 combination therapy with L-DOS47 significantly reduced tumor growth compared with anti-PD1 alone



C

Contrasts		P-values	
		Week 3	Week 4
Combo	Anti-PD1	0.0368	0.0142
Combo	L-DOS47	<0.0001	<0.0001
Combo	Control	<0.0001	<0.0001
L-DOS47	Control	0.7559	0.6452
Anti-PD1	Control	<0.0001	<0.0001

Figure 3A. Tumor volumes for each mouse within each therapy group for combined replicates measured weekly by ultrasound up to 4 weeks. The number of mice/group is indicated. **B.** Mean tumor volumes ± SEM for combined replicates. **C.** Table of P-values comparing outcomes between treatment groups. A linear mixed effects model was employed in which the endpoint tumor volume was fixed at 750 mm^3; Kenward-roger degrees of freedom were applied.

SUMMARY

- ✓ CEACAM6 is highly expressed on PDAC tumors to which L-DOS47 binds
- ✓ L-DOS47 administration increases pHe of acidic tumors (baseline ≤ pH 6.6)
- ✓ Combining L-DOS47 with anti-PD1 significantly reduces tumor growth compared to anti-PD1 alone

CONCLUSION

These studies provide strong evidence that the unconventional ADC L-DOS47, by neutralizing acidic tumor pH, significantly improves responses to anti-PD1 immunotherapy.