Pharmacodynamics of targeted urease and checkpoint blockade using CEST and ^{31}P MRSI. Is there a role for hyperpolarized ^{13}C & ^{15}N?

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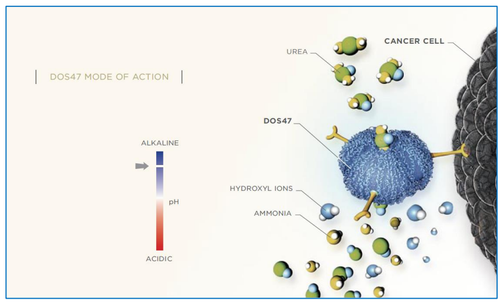
Introduction

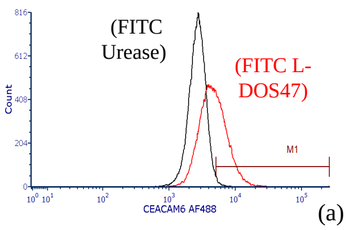


There is strong evidence that the tumor micro-environment of solid tumors is acidic, which inhibits the efficacy of chemo-, radio-, and immuno-therapies.

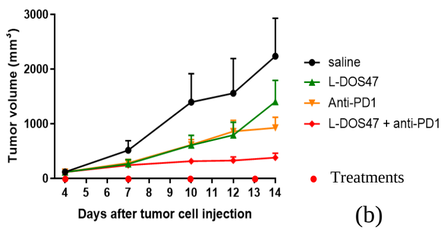
Acidosis can be directly neutralized with a CEACAM6-targeted urease, L-DOS47 (Helix Biopharma). CEACAM6 is highly expressed in lung and GI cancers, including pancreatic cancer. L-DOS47 was well-tolerated and dose escalated in a phase I/II trial of NSCLC (NCT02309892) and will soon be tested in pancreatic cancer. Urease converts endogenous non-ionized urea into 1X CO_2 and 2X NH_3, which rapidly ionize to HCO_3^- and NH_4^+, thus consuming a net H^+ in the process and directly raising the local pH. The ability to measure tumor pH in order to determine PK in vivo, would be useful as a biomarker to be used for personalized medicine.

Combination of L-DOS47 with anti-PD1

Panc02 murine pancreatic adenocarcinoma cells were infected with human CEACAM6 lenti virus to provide the expression of the drug target. C57BL/6 mice were injected with CEACAM6-Panc02 cells (1 million cells/mouse) in the right flank subcutaneously. Treatments started 4 days after tumor inoculation and all mice were sacrificed on day 15, after receiving 4 doses of drug(s).



(a)

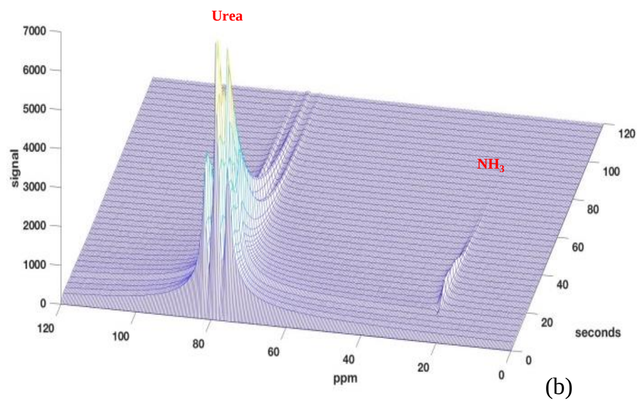


(b)

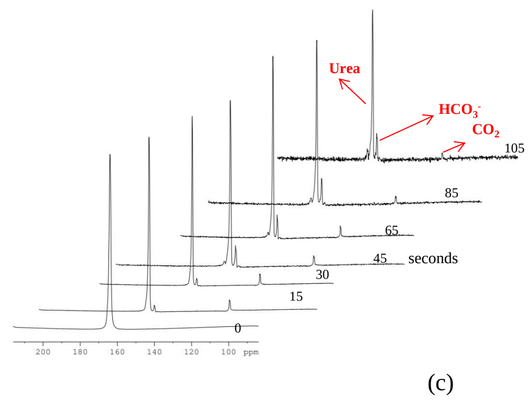
Figure 1: (a) CEACAM6 expression for Panc02 cells was verified with flow cytometry. (b) Average tumor volumes with SEM are given for all groups (5 mice/group).

Hyperpolarized ^{13}C & ^{15}N experiments

(a)



(b)



(c)

Figure 2: (a) A 10 cc syringe containing 1 mL of urease solution was placed in an animal bed and 3 cc of hyperpolarized sample was injected into the magnet with a connection line from out of the magnet, in order not to move the bed during the scan. (b) Conversion of HP ^{15}N Urea to NH_3/NH_4^+. A ^{15}N / ^{1}H 30 mm Doty coil was used to acquire spectra. (c) Conversion of HP ^{13}C Urea to CO_2/HCO_3^-. A ^{13}C / ^{1}H 30 mm Doty coil was used to acquire spectra.

In vivo pH measurements

BxPC3 human pancreatic adenocarcinoma cell line which naturally expresses human CEACAM6 antigen and lenti-virus infected Panc02 murine pancreatic adenocarcinoma cells were used for pH measurement experiments. 5 million BxPC3 cells/ mouse or 1 million CEACAM6-Panc02 cells were injected to the right flanks of NSG or C57BL/6 mice. Once sizes reached ~800 mm^3 and ~500 mm^3 for BxPC3 and Panc02 tumors respectively, pH measurements were started.

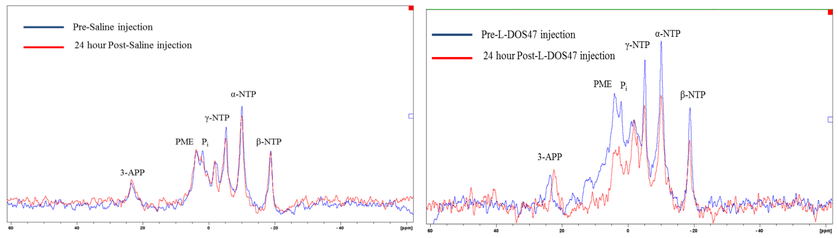
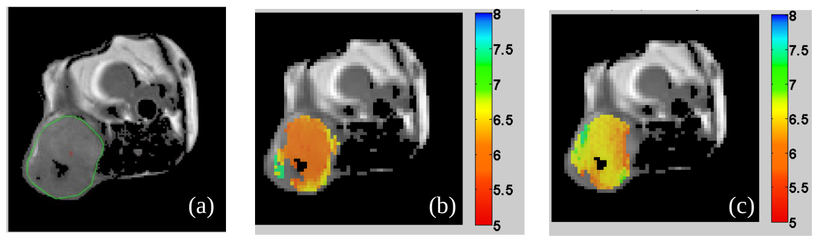


Figure 3: ^{31}P MRS of 3-aminopropylphosphate (3-APP) [1] with an 8 mm Doty surface coil. BxPC3 SC tumor bearing mice were injected with 200 µl saline/ 90 µg/kg L-DOS47 iv. pHs were measured before and 24 h after treatment by injecting 350 µl of 3-APP ip prior to imaging. 24 hours after injection of L-DOS47, the pH of the tumor had increased by 0.55 units.



(a) (b) (c)

Figure 4: CEST MRI of iopamidol for pH imaging [2] of a CEACAM6-Panc02 SC tumor. (a) T2 weighted image, (b) CEST MRI before L-DOS47 injection, (c) ~30 minutes after 90 µg/ kg L-DOS47 injection. The increase in mean pH is 0.38 units. L-DOS47 was administered iv. Iopamidol was administered SC, next to the tumor.

Conclusions

In this study, pH increases in tumors induced by L-DOS47 were observed in vivo with two different imaging techniques. For the first time, urease activity in vitro was shown using HP ^{13}C and ^{15}N Urea samples, which directly confirms the mechanism of action of L-DOS47. It was also shown in vivo that increasing tumor pH helps to control tumor growth when combined with anti-PD1 treatment.

References

1. Gillies, R.J., Liu, Z., and Bhujwalla, Z.: '31P-MRS measurements of extracellular pH of tumors using 3-aminopropylphosphonate', Am J Physiol, 1994, 267, (1 Pt 1), pp. C195-203.
2. Longo, D.L., Dastru, W., Digilio, G., Keupp, J., Langereis, S., Lanzardo, S., Prestigio, S., Steinbach, O., Terreno, E., Uggeri, F., and Aime, S.: 'Iopamidol as a Responsive MRI-Chemical Exchange Saturation Transfer Contrast Agent for pH Mapping of Kidneys: In Vivo Studies in Mice at 7 T', Magn Reson Med, 2011, 65, (1), pp. 202-211.