Improving Survival in Pancreatic Cancer Using Doxorubicin in Combination with L-DOS47

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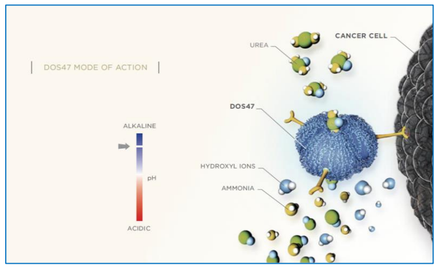
Introduction



There is strong evidence that the tumor micro-environment of solid tumors is acidic, which inhibits the efficacy of chemo-, radio-, and immuno-therapies. Specifically, acidic pH inhibits the activity of weakly basic drugs, such as doxorubicin. In order to test whether neutralization of tumor acidity will improve survival in a pancreatic cancer model treated with doxorubicin, we tested this in combination with L-DOS47, a novel therapy comprised of jack bean urease conjugated to an antibody that targets cell surface CEACAM6. The urease component of L-DOS47 raises the pH of the tumor microenvironment by converting endogenous urea into 2 NH_3 and 1 CO_2. We chose pancreatic cancer as our model as it is known to be acidic, and one of the most lethal cancers. Our findings demonstrate that raising tumor pH can improve responses to chemotherapy, with the potential for clinical use.

CEACAM6 murine tumor model

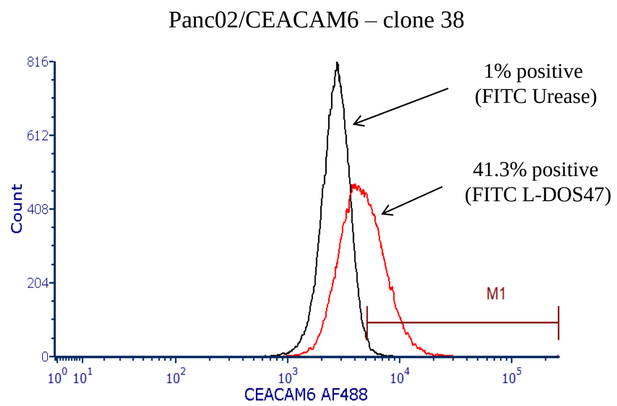


Figure 1: The isogenic Panc02 pancreatic cancer cell line was engineered to express CEACAM6 with lentivirus, followed by puromycin selection to generate a series of clones with differential CEACAM6 expression. Binding of L-DOS47 to clone 38 cells showed a single positive population.

In vivo pH measurements

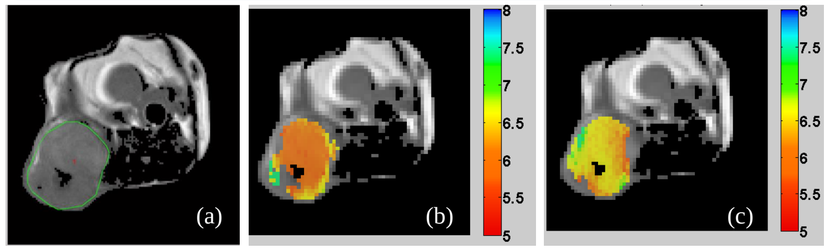


Figure 2: CEST MRI of iopamidol for pH imaging [1] of a Panc02 clone 38 SC tumor. (a) T2 weighted image, (b) CEST MRI before L-DOS47 injection, (c) ~30 minutes after 90 µg/ kg L-DOS47 injection. The difference in mean pHs is 0.38 units. L-DOS47 was administered iv. Iopamidol was administered SC, next to the tumor.

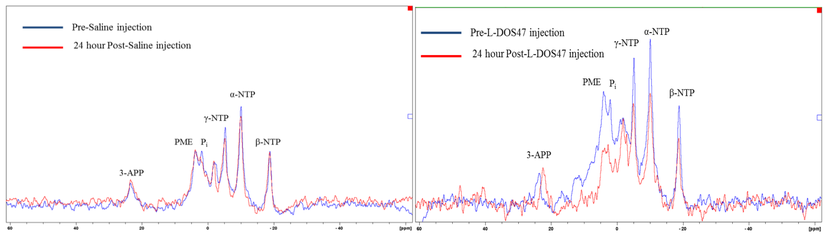
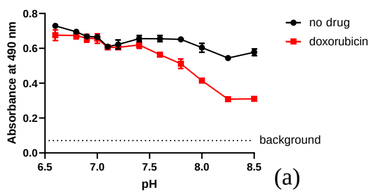
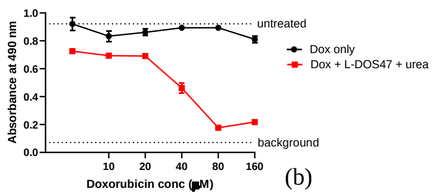


Figure 3: pH determination of a BxPC3 SC pancreatic tumor by ^{31}P magnetic resonance spectroscopy of 3-aminopropylphosphate (3-APP) [2] with an 8 mm Doty surface coil. Mice were injected with 90 µg/kg L-DOS47/ 200 µl saline iv and pHs were checked before and 24 h after treatment by injecting 350 µl 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.

In vitro experiments





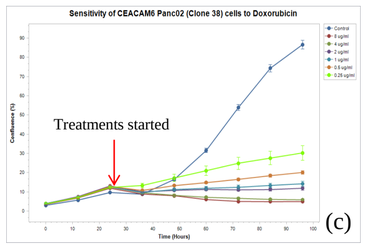


Figure 4: (a) Viability of A549 cells treated with 100 µM doxorubicin in different pH buffers. Doxorubicin activity increases as pH increases. (b) Viability of A549 cells that were initially incubated for 2 hours with L-DOS47, washed, and then combined with urea and Doxorubicin. L-DOS47 increases Doxorubicin activity. (c) Sensitivity of Panc02 clone 38 cells to Doxorubicin.

In vivo efficacy

1 x 10^6 CEACAM6 Panc02 Clone 38 cells were injected subcutaneously in the right flank of C57BL/6 mice. Four days later, tumor sizes were measured with calipers and mice were forcibly randomized into 3 groups (10 mice/ group). 1) Doxorubicin (2.5 mg/kg) only; 2) L-DOS47 (90 µg/kg) 4 hours before Doxorubicin (2.5 mg/kg); and L-DOS47 (90 µg/kg) 24 hours before Doxorubicin (2.5 mg/kg).

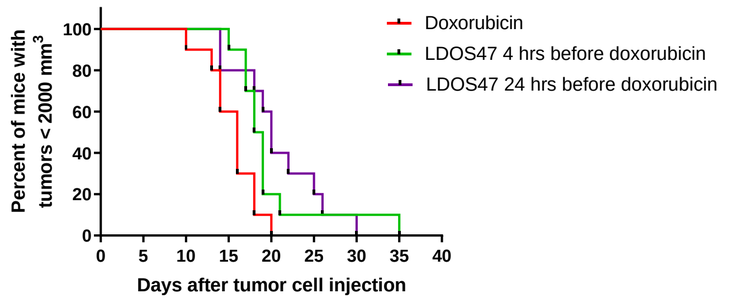


Figure 5: There is a statistically significant difference among the three groups. $p<0.0075$ using three tests (Log-rank Mantel-Cox test, Log-rank test for trend, Gehan-Breslow-Wilcoxon test)

Conclusion

In this study we have observed that neutralizing tumor pH can improve the effect of chemotherapy *in vivo*. This is consistent with prior observations where neutralization with bicarbonate improved the response to chemotherapy via reversal of an ion-trapping effect [3]. The current work is significant, however, as L-DOS47 can be used clinically, whereas bicarbonate cannot.

References

1. 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.
2. 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.
3. Raghunand, N., Mahoney, B.P., and Gillies, R.J.: 'Tumor acidity, ion trapping and chemotherapeutics. II. pH-dependent partition coefficients predict importance of ion trapping on pharmacokinetics of weakly basic chemotherapeutic agents', Biochem Pharmacol, 2003, 66, (7), pp. 1219-1229.