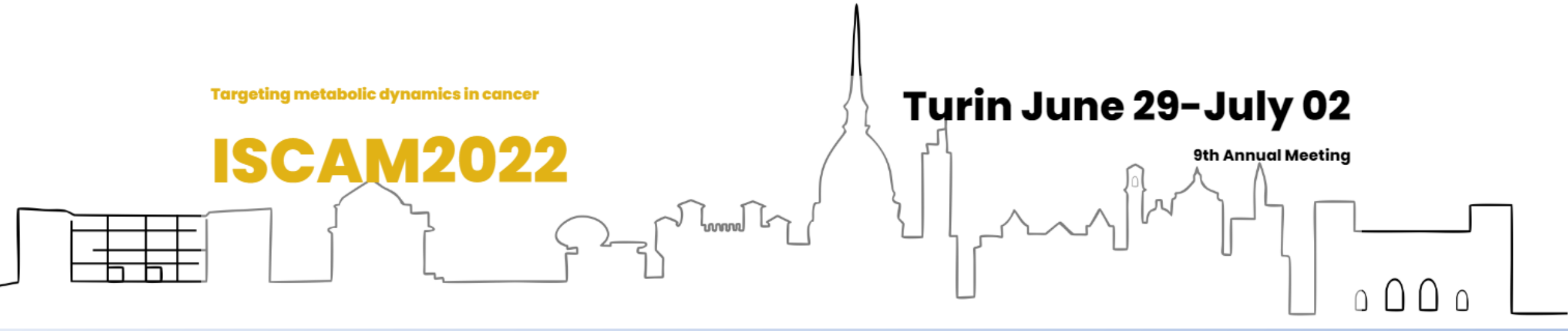


TARGETING ACIDOSIS TO IMPROVE IMMUNOTHERAPY IN A PANCREATIC DUCTAL ADENOCARCINOMA MODEL

Jardim-Perassi BV, Abrahams D, Ibrahim Hashim AA, Irrera P, Alkhouli MA, Sherlock TW, Whelan C, Beatty M, Longo DL, Uger MD, Böhler C, Pilon-Thomas SA, Gillies RJ

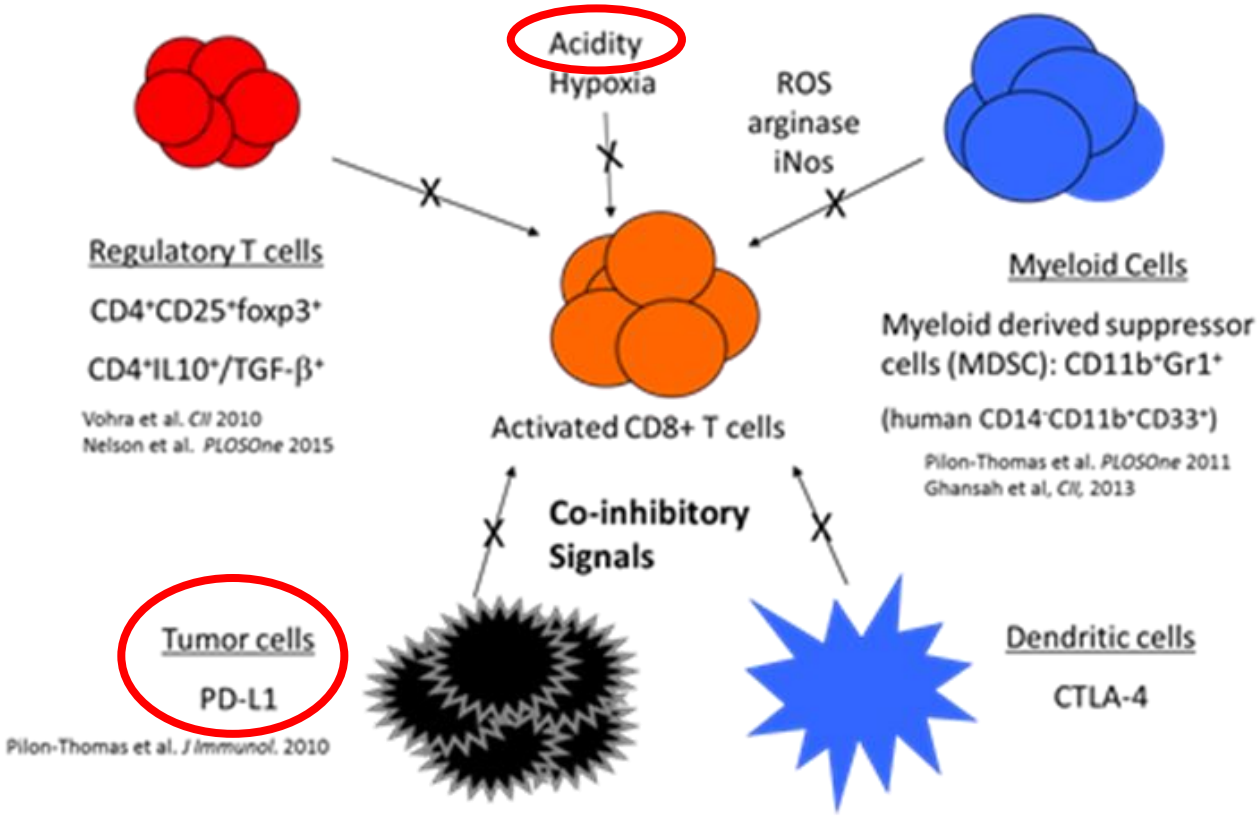
HelixBioPharmaCorp.

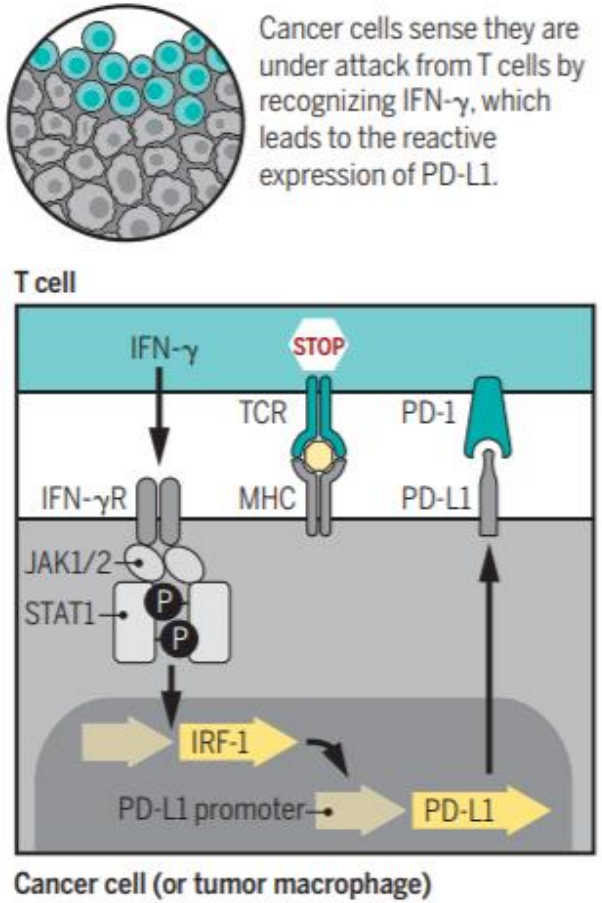
Presenting author: *Irrera Pietro, PhD*
pietro.irrera@moffitt.org

MOFFITT CANCER CENTER

Introduction

- Re-modeling of tumor microenvironment can inhibit immune cells and promote tumor growth
- Tumor cells alone can also evade T-cells function





Ribas, A., et al. Science 2018; Topalian et al. NEJM, 2012; Wolchok et al. NEJM, 2013

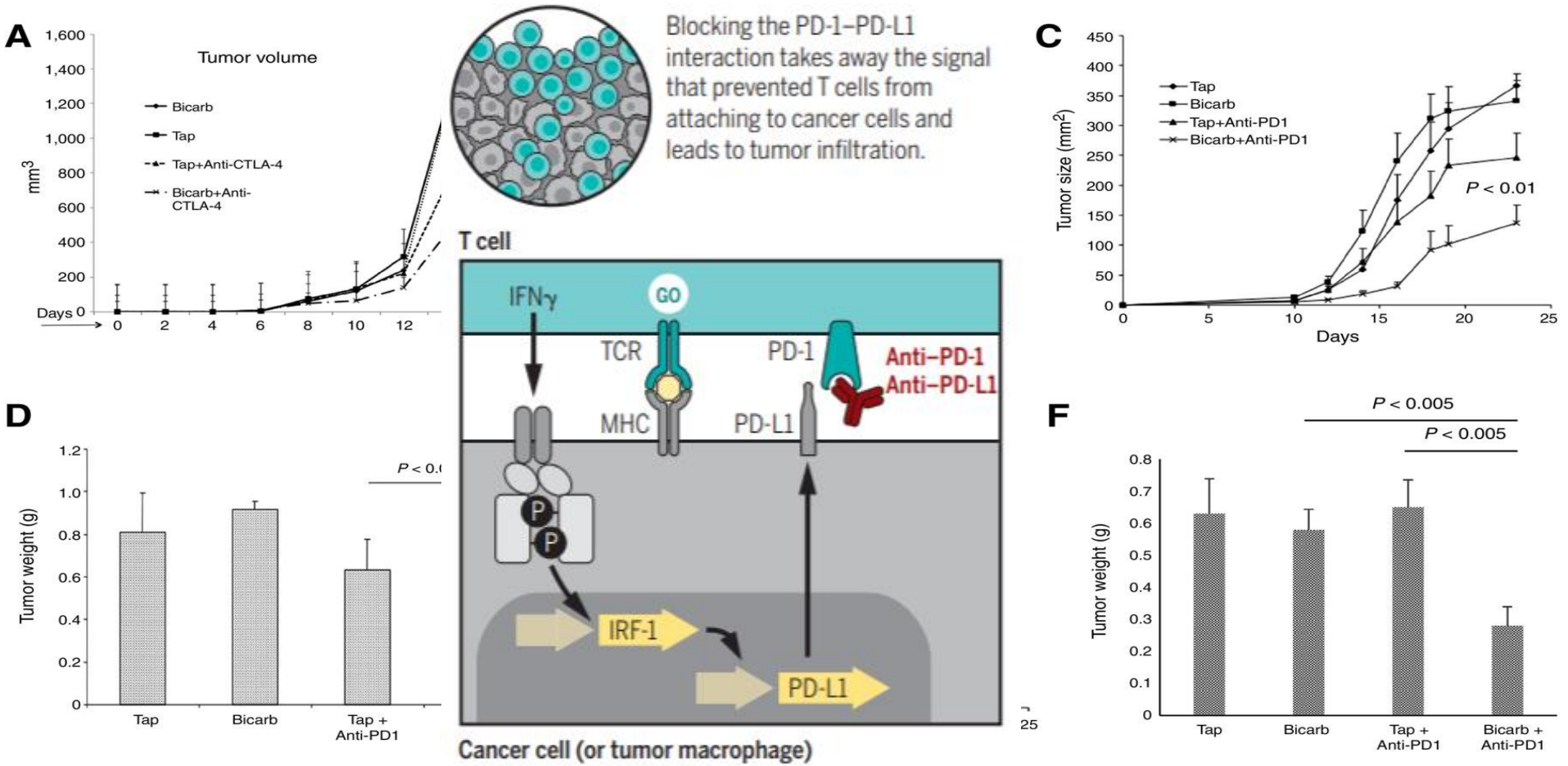
Microenvironment and Immunology

Cancer Research

➢ Immunotherapy with checkpoint blockade can lead to durable respo[illegible]nic, but response rates are still low (non-responders: ≈60%)

Neutralization of Tumor Acidity Improves Antitumor Responses to Immunotherapy

Shari Pilon-Thomas[1], Krithika N. Kodumudi[1], Asmaa E. El-Kenawi[2,3], Shonagh Russell[2], Amy M. Weber[1], Kimberly Luddy[2], Mehdi Damaghi[2], Jonathan W. Wojtkowiak[2], James J. Mulé[1], Arig Ibrahim-Hashim[2], and Robert J. Gillies[2]



➢ Challenging to translate clinically, as it requires ingestion of approximately 50 (920 mg) capsules per day

➢ Phase I/II clinical trials failed to reach endpoints due to poor patient compliance

Shari Pilon-Thomas et al. Cancer Res 2016; Wu et al. Nature Communication 2020

Hypothesis

The combination of buffer therapy with immunoblockade approach is promising and can increase success rate of treatment regimens

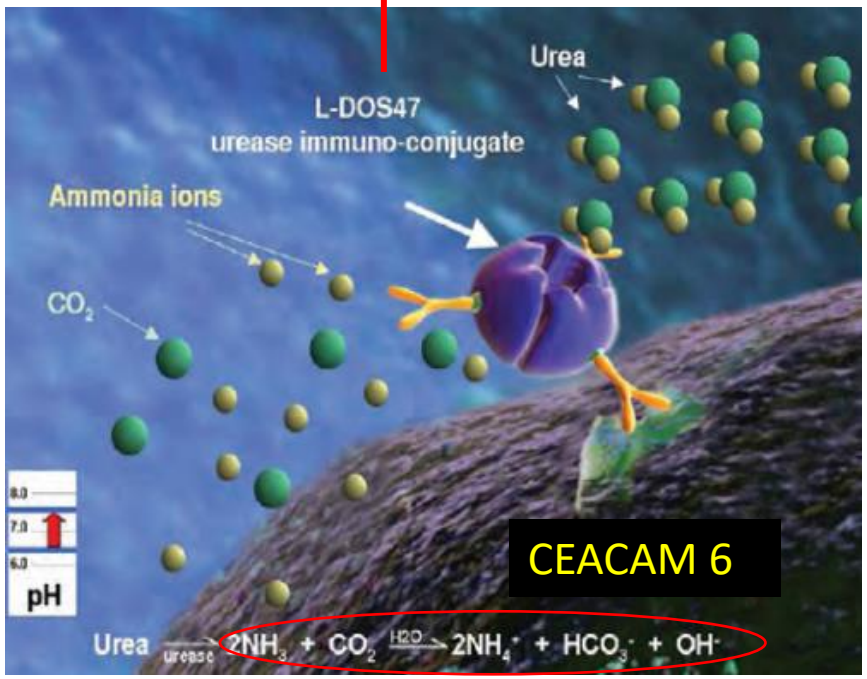
Aim of the work

Use an immuno-conjugate **urease** (L-DOS47) to increase NH_4^+ and OH^- production and consequently the pH in the tumor microenvironment, coupled with anti-PD1 treatment

L-DOS47

HelixBioPharmaCorp.

Immunoconjugate composed of AFAIKL2, a recombinant camelid single-domain antibody which recognizes CEACAM6 expressed in tumor cells, and the enzyme urease from Jack beans



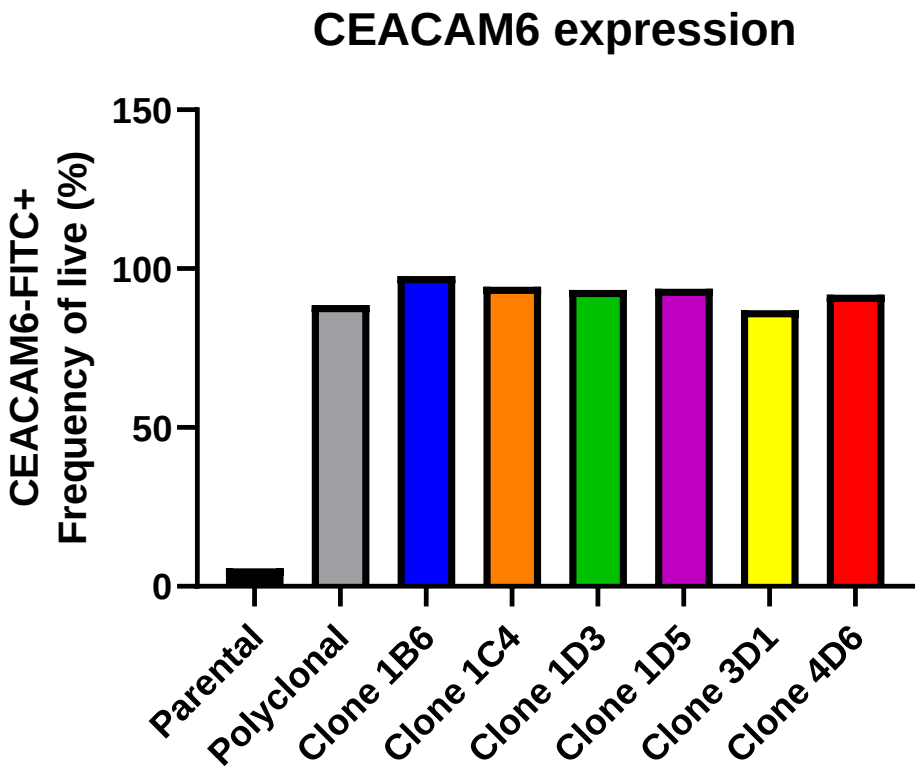
Selective binding of the AFAIKL2 antibody to CEACAM6 on tumor cells results in the accumulation of **urease, which converts the extracellular urea into ammonia,** which is cytotoxic and creates an alkaline environment

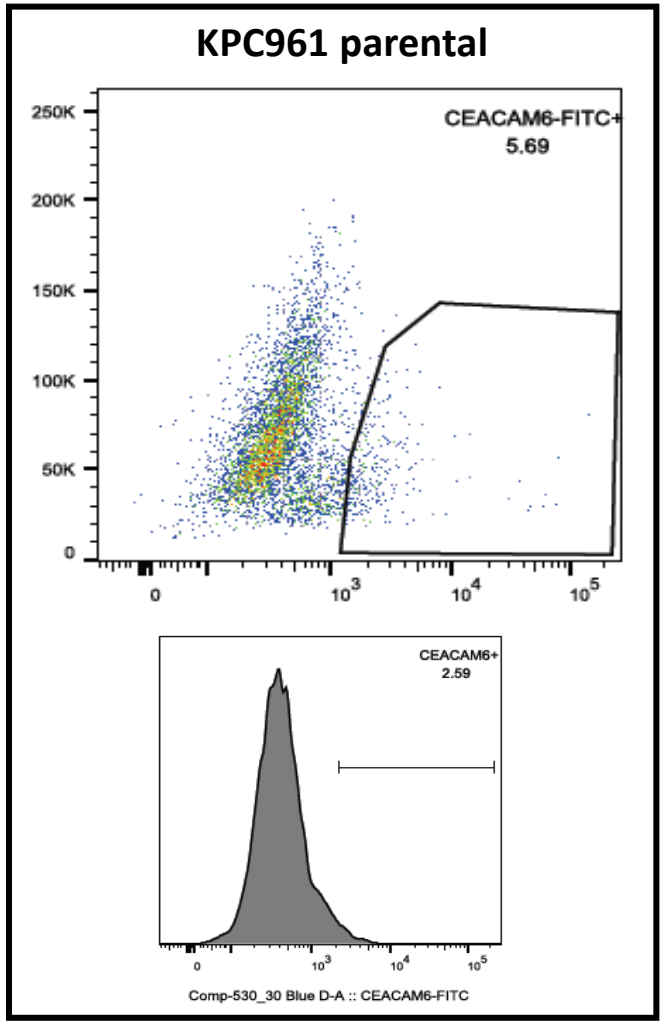
Produce OH^- → Tumor pH ↑

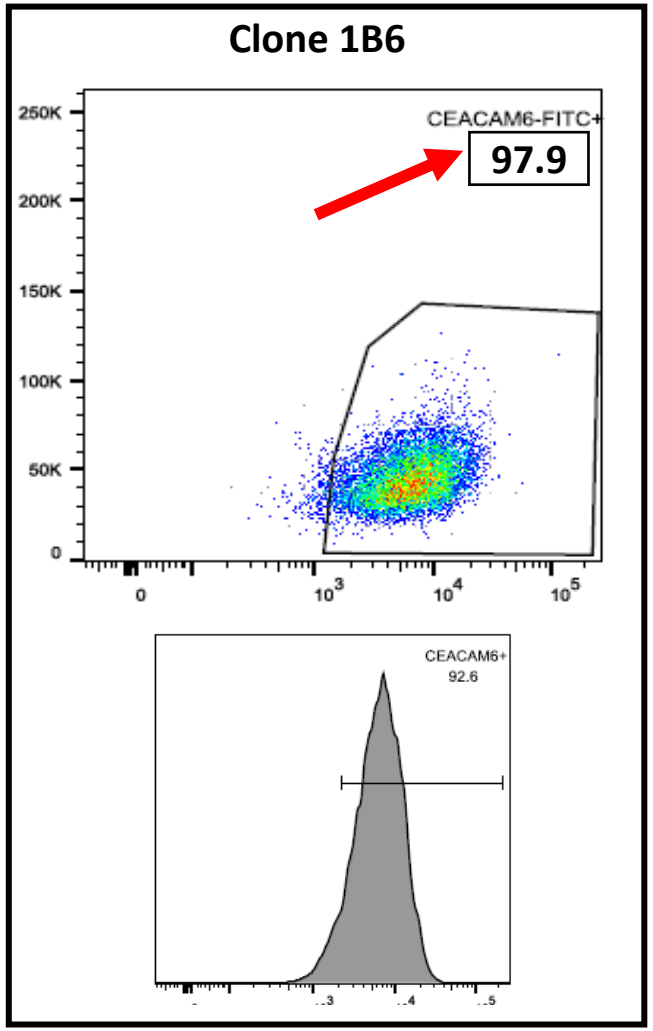
- **Mouse cells do not express CEACAM6 (Carcinoembryonic Antigen-Related Cell Adhesion Molecule 6)**
  → Mouse tumor models were retrovirally infected with human CEACAM6

Establishment of the tumor model: *induction of CEACAM6 expression*

- KPC961 murine pancreatic adenocarcinoma cells were infected with human CEACAM6 lentivirus and expressing clones were selected with puromycin
- **Clone 1B6** was selected for *in vivo* studies



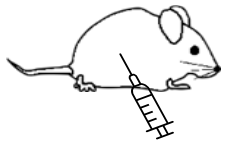




Experimental setup

DAY 0 — DAY 7 — DAY 14 — DAY 21 — DAY 28

Surgery for tumor cells inoculation



- Ultrasound (US) to check tumor volume and randomization into groups of equal tumor volumes (n=10 each).

(1) Control (no therapy)
(2) Bicarb (200mM) in drinking water
(3) Anti-PD1 (300ug IP) twice a week
(4) L-DOS47 (90 ug/kg IV) twice a week
(5) Bicarb + Anti-PD1
(6) L-DOS47 + Anti-PD1

US for tumor volume

US for tumor volume

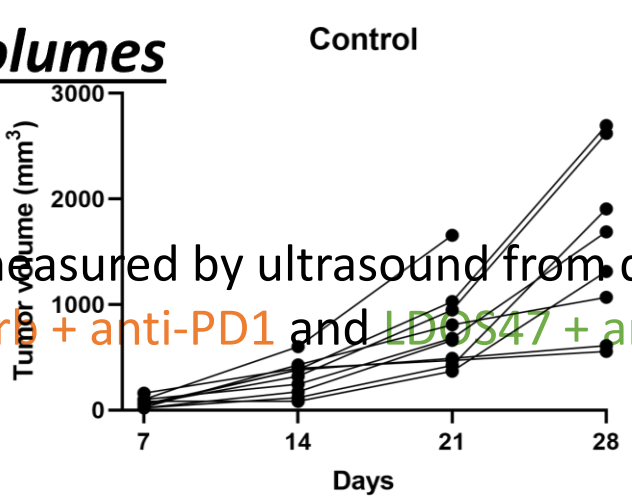
- US for tumor volumes
- Tumor collection for *ex vivo* analyses

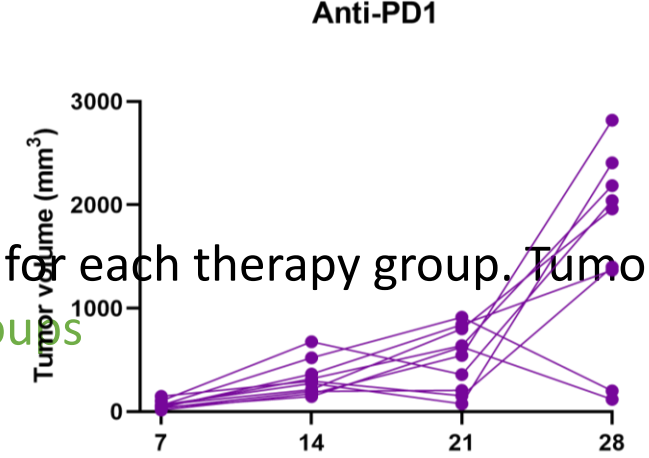
Some mice were euthanized before the study endpoint due to ascites or tumor burden.

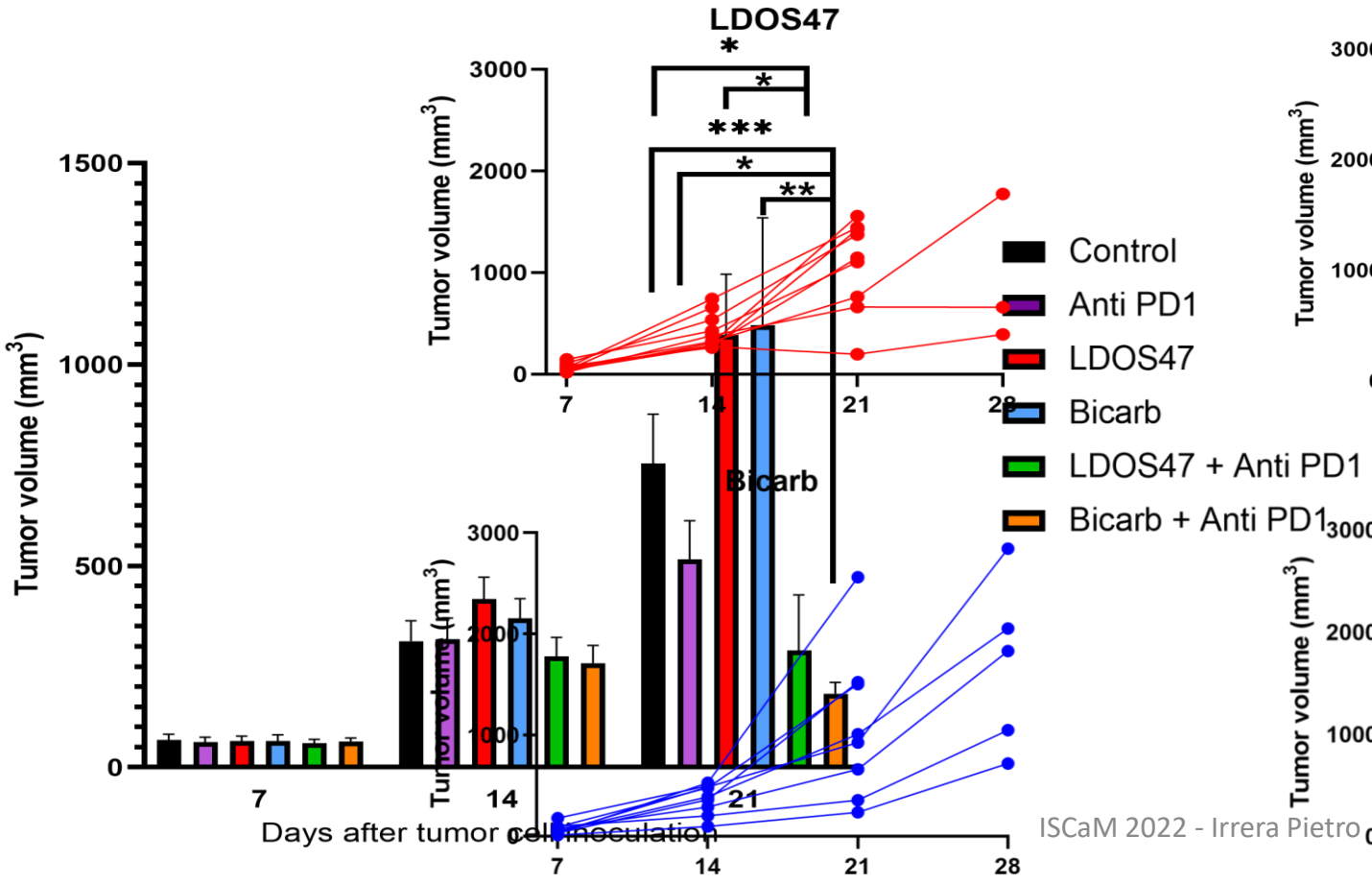
Chemical exchange saturation transfer-magnetic resonance imaging (CEST-MRI) was used to measure tumor extracellular pH (pHe) *in vivo.*

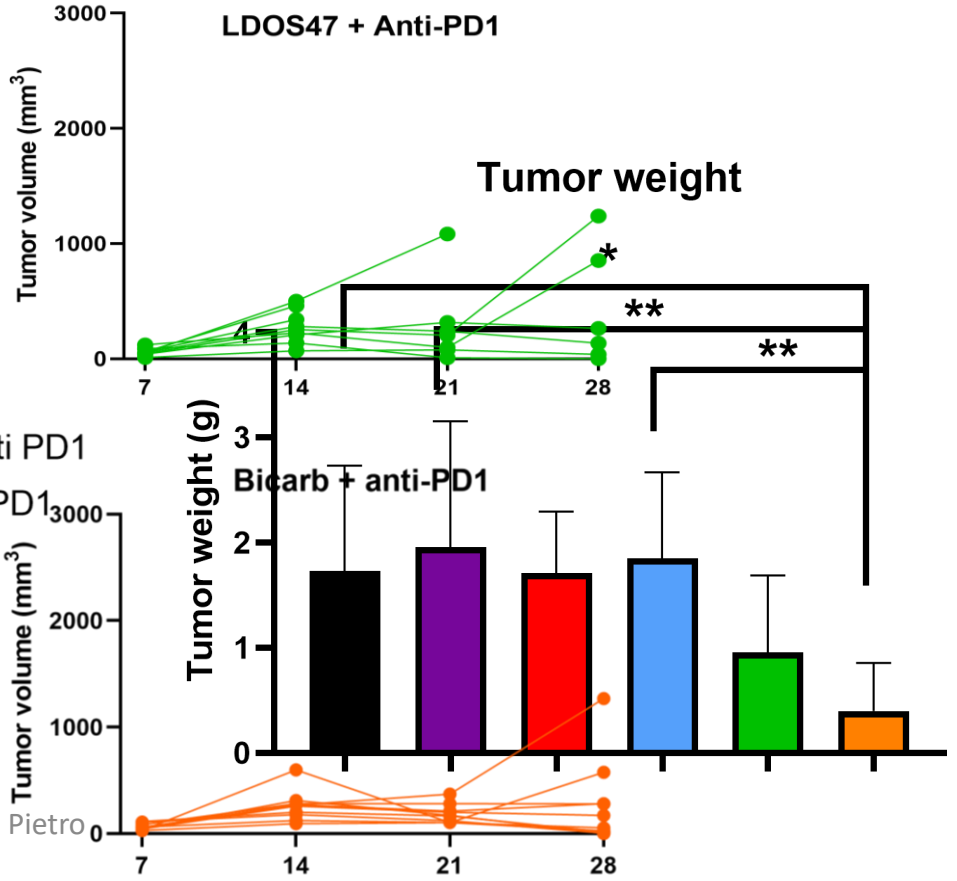
Results: *tumor volumes*

- Tumor volumes measured by ultrasound from day 7 to 28 for each therapy group. Tumor regression was observed for bicarb + anti-PD1 and LDOS47 + anti-PD1 groups





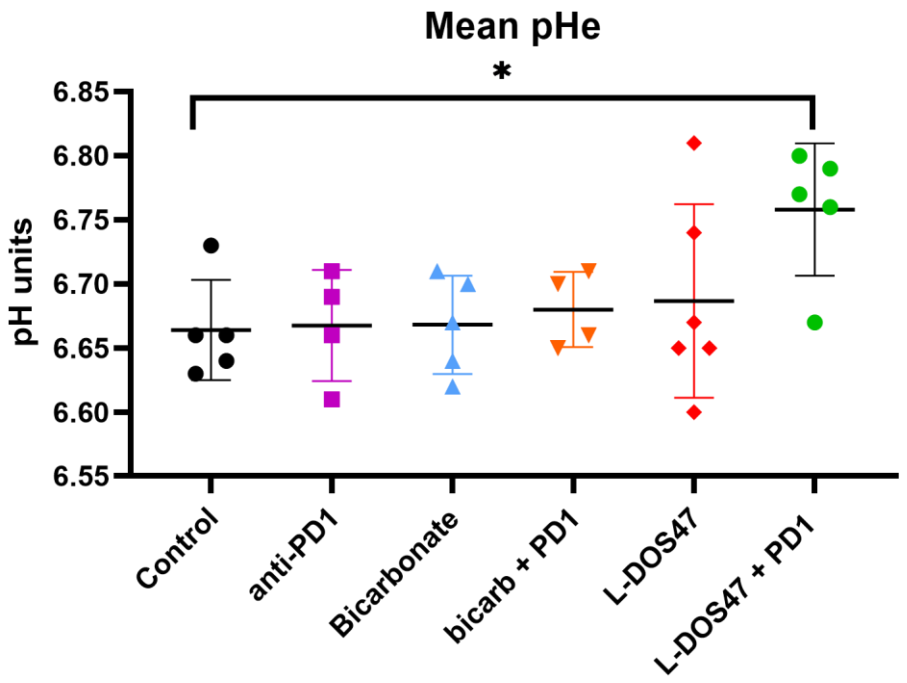


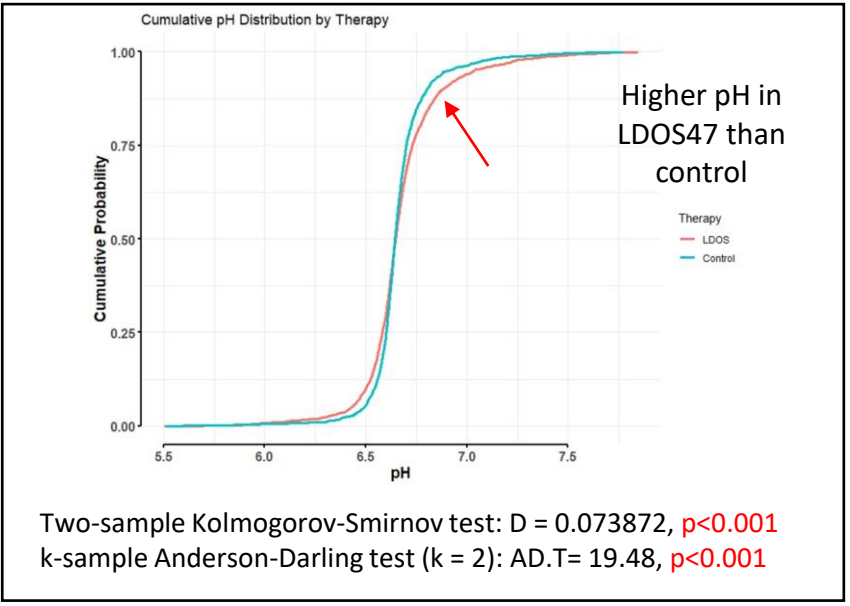


Results: *extracellular pH between groups*

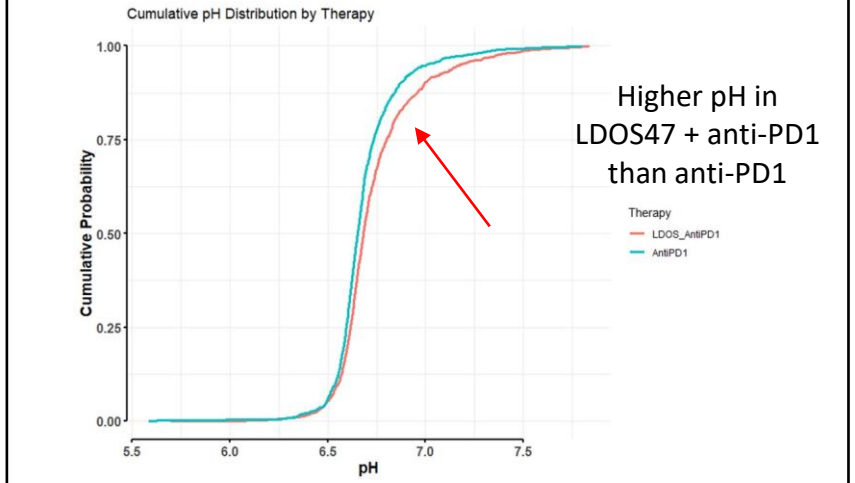
- **pH measurements** revealed a consistent alkalinization of the extracellular environment for the LDOS47 + anti-PD1 mice (**p value* 0.05, unpaired t-test)

- **Cumulative distribution function analyses** showed a clear tendency towards alkaline pH in LDOS47 + anti-PD1 mice

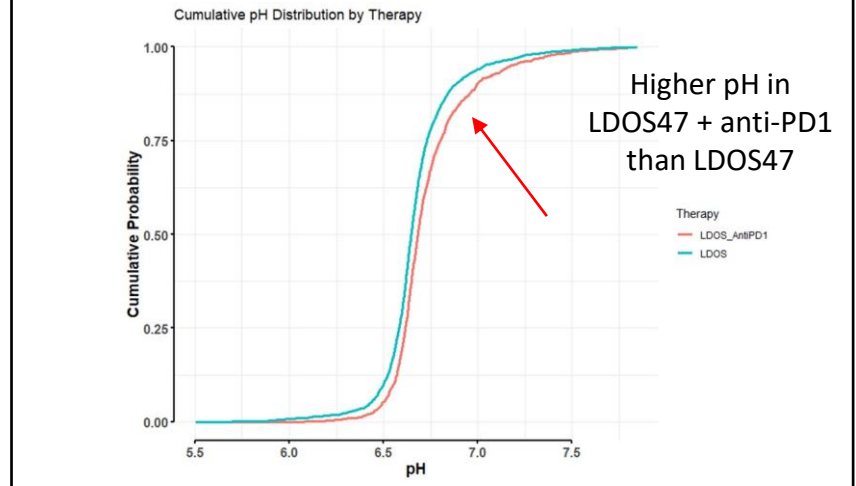




Two-sample Kolmogorov-Smirnov test: D = 0.073872, p<0.001
k-sample Anderson-Darling test (k = 2): AD.T= 19.48, p<0.001



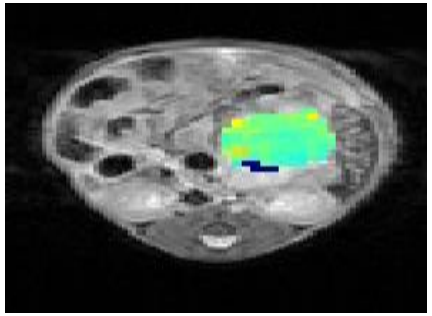
Two-sample Kolmogorov-Smirnov test: D = 0.13112, p<0.001
k-sample Anderson-Darling test (k = 2): AD.T= 28.71, p<0.001

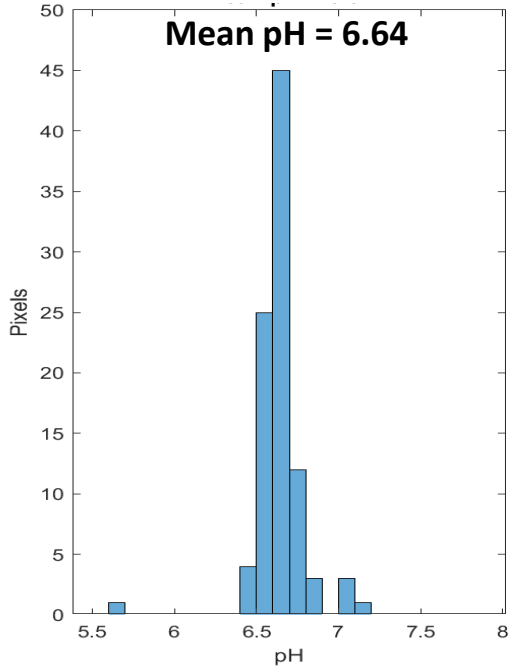


Two-sample Kolmogorov-Smirnov test: D = 0.13904, p<0.001
k-sample Anderson-Darling test (k = 2): AD.T= 51.38, p<0.001

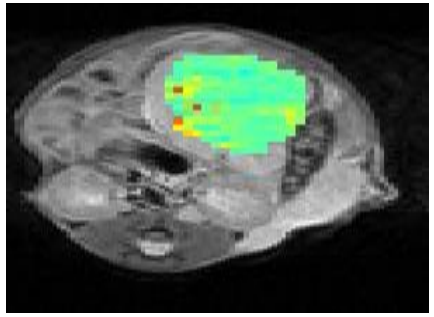
Results: *extracellular pH – representative pH-maps*

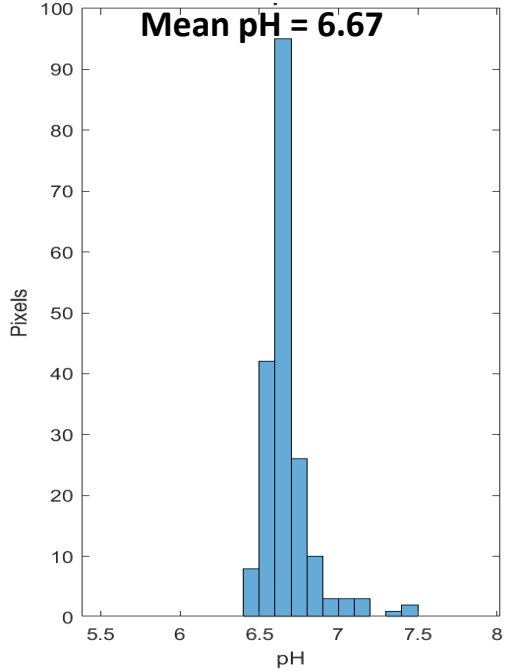
Control



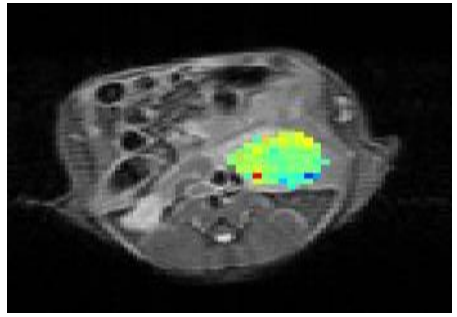


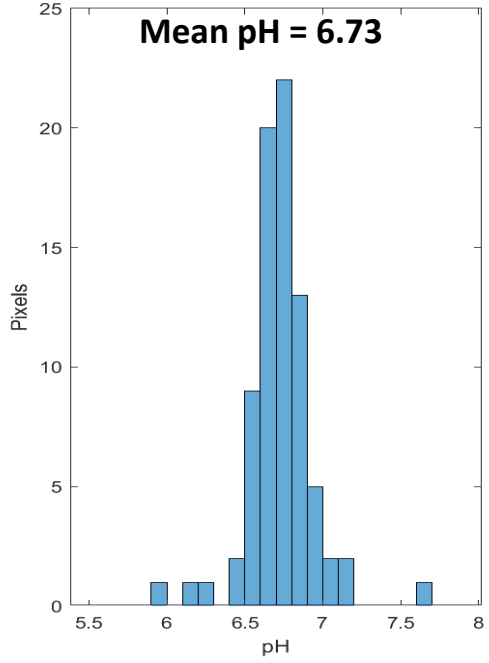
Anti-PD1



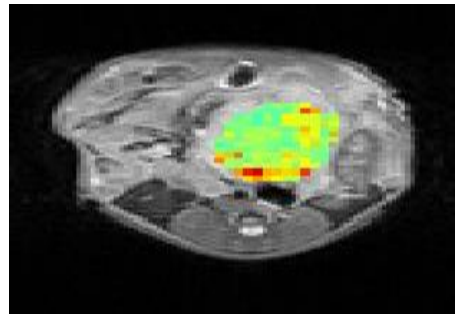


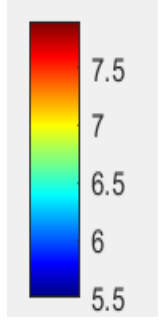
Bicarb + anti-PD1

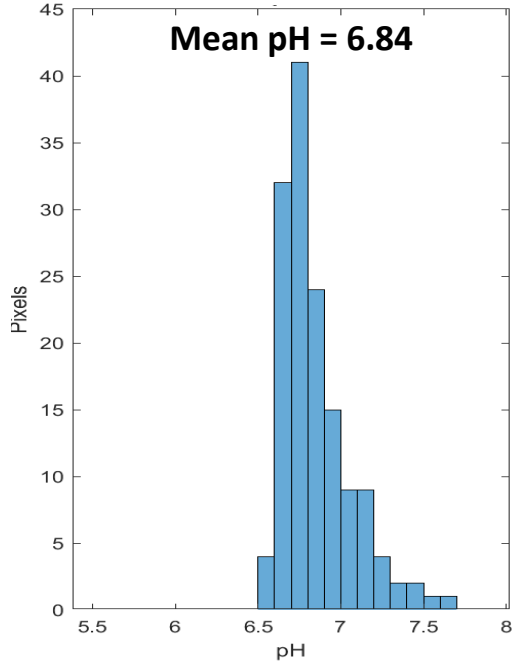




LDOS47 + anti-PD1







CONCLUSIONS

- L-DOS47 induces pH changes that can be detected with CEST-MRI pH imaging
- Tumor growth was strongly affected by the combination of buffer therapy with immunoblockade drugs
- Although the bicarb + anti-PD1 group showed lower tumor volumes and weights compared to L-DOS47 + anti-PD1, the latter provided a more consistent pH shift
- **Neutralizing tumor acidosis strengthens the response to immune checkpoint blockade in the PDAC model**
- Further studies are ongoing to depict the BD and PD of L-DOS47

Acknowledgement

Gillies Lab
Jardim-Perassi BV
Abrahams D
Ibrahim Hashim AA
Irrera P
Sherlock TW

Pilon-Thomas Lab
Alkhouli MA
Beatty M

Helix BioPharma
Uger MD
Böhler C

Collaborators
Whelan C
Longo DL

Thanks to the organizers and to all of you for your attention