345 TARGETING ACIDITY IN BLADDER CANCER TO ENHANCE EFFICACY OF ADOPTIVE CELL THERAPY (ACT) WITH TUMOR REACTIVE T CELLS

[1]Sarah Bazargan*, [2]Jamie Blauvelt, [2]Amy Hall, [2]Matthew Beatty, [2]Michael Poch, [2]Katarzyna A Rejniak, [3]Shari Pilon-Thomas. *[1]Moffitt Cancer Center/University of South Florida, Ormond Beach, FL, USA; [2]Moffitt Cancer Center, Tampa, FL, USA; [3]H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA*

Background Tumor acidity attenuates the functionality and anti-tumor responses of T cells.[1] Our preliminary data shows that the average pH of urine from bladder cancer patients is 6.57 and in mice bearing orthotopic MB49-OVA bladder tumors, the pH is 6.5. We predict that neutralizing the acidity within the bladder microenvironment with sodium bicarbonate water may augment anti-tumor responses of T cells adoptively transferred into the bladder.

Methods For *in vitro* studies, OT-I T cells were stimulated with cognate peptide for 48–72 hours in pH 7.4 and 6.6 media. Proliferation of T cells was assessed through thymidine incorporation and dilution of cell trace violet. Reactivity of OT-I T cells to cognate peptide was measured via intracellular IFN-gamma. T cell migration was evaluated in response to CXCL10. The pH of the urine from mice was measured using dipsticks. The pH of urine from MB49-OVA-bearing mice was monitored with and without continuous 200 mM sodium bicarbonate water and tumor volumes were monitored via ultrasound.

Results OT-I T cells stimulated in pH 6.6 media had an average count per minute (c.p.m.) of 13,637 while T cells stimulated in 7.4 media had an average c.p.m of 48,651 ($p<0.0001$). The percentage of $CD8^+$ T cells producing IFN-gamma was 11.90% in the 7.4 condition and 3.36% in the 6.6 condition ($p=0.0461$). For T cell migration, T cells at pH 7.4 demonstrated a 13.5-fold increase in migration compared to T cells at pH 6.6 in response to 1000 ng/mL CXCL10 ($p=0.0025$). Chronic administration of 200 mM bicarbonate in water in mice bearing orthotopic MB49-OVA tumors showed an increase in urinary pH within 2 hours and a stabilization to a pH of 7.08 within one week. Returning mice to tap water resulted in urine pH normalization back to 6.5 within 24 hours. Administration of chronic bicarbonate water at the time of orthotopic tumor implantation results in average tumor volumes of 153.915 mm^3 in the tap water group and 94.048 mm^3 in the chronic bicarbonate group 20 days after tumor instillation.

Conclusions Urine from bladder cancer patients and tumor-bearing mice is acidic. T cells stimulated in acidic conditions are less responsive to stimulation. Administration of sodium bicarbonate in water reverses this acidity, we predict that neutralization of acidity within the bladder tumor microenvironment will enhance anti-tumor responses of T cells adoptively transferred into the bladder.

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Ethics Approval This study was approved by the Advarra IRB; approval number IRB# 00000971 and the University of South Florida IACUC, approval number R IS000011476

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