THE MICROBIOME-DERIVED METABOLITE TMAO DRIVES IMMUNE ACTIVATION AND BOOSTS RESPONSE TO IMMUNE CHECKPOINT BLOCKADE IN PANCREATIC CANCER

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Background Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal cancer with poor prognosis. Although pancreatic tumors exhibit prominent leukocyte infiltrates, immunotherapy has so far failed to improve clinical outcomes in patients with PDAC. The immunotherapy responses can be improved through strategies that shift the PDAC tumor microenvironment (TME) from an immunosuppressive state to a more immune-activated state. The composition of the gut microbiome controls innate and adaptive immunity and has emerged as a key regulator of tumor growth and the success of immune checkpoint blockade (ICB) therapy. However, the underlying mechanisms remain unclear.

Methods The main objectives of this study were to elucidate how the gut microbe-derived metabolite trimethylamine N-oxide (TMAO) influences the host immune responses in PDAC TME, to determine if TMAO would synergize with immune checkpoint therapy to reduce tumor growth, and to assess whether there is a clinical correlation between TMAO production and survival in cancer patients. The overall design did employ some in vitro approaches but relied on in vivo mouse model systems, including orthotopic tumor implantation, to achieve the first two objectives; to test for a clinical correlation, we assessed datasets of bacterial species generated by three other groups. We employed flow cytometry and multomic approaches to determine the impact of TMAO on phenotype of immune infiltrates in the TME.

Results Using a non-targeted, LC-MS/MS-based metabolomic screen, we identified that TMAO enhanced anti-tumor immunity to PDAC. Delivery of TMAO intraperitoneally or via a dietary choline supplement to orthotopic PDAC bearing mice reduced tumor growth and was associated with an immunostimulatory tumor-associated macrophage (TAM) phenotype and activated effector T cell response in the tumor microenvironment. Mechanistically, TMAO potentiated the type-I interferon (IFN) pathway and conferred anti-tumor effects in a type-I IFN-dependent manner. Notably, delivering TMAO-primed macrophages intravenously produced similar anti-tumor effects. Combining TMAO with ICB (anti-PD1 and/or anti-Tim3) in a mouse model of PDAC significantly reduced tumor burden and improved survival beyond TMAO or ICB alone. Finally, the levels of trimethylamine (TMA)-producing bacteria and of the CutC gene (an enzyme that generates TMA, the TMAO precursor) expression correlated with improved survival and response to anti-PD1 in cancer patients.

Conclusions Our study demonstrated that the microbial metabolite TMAO enabled TAMs to become immunogenic and promote effector T cell activity, transformed the TME to an immune activated state, and rendered PDAC sensitive to checkpoint immunotherapy, suggesting that strategies that alter levels of TMAO could be a promising clinical intervention to manage PDAC.

Ethics Approval Mouse experiments were performed following National Institutes of Health (NIH) guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of The Wistar Institute.