848 PREDICTIONS OF ENDOGENOUS NEOANTIGENS FROM VARIED GENOMIC SOURCES IN A SYNGENEIC MURINE CANCER MODEL

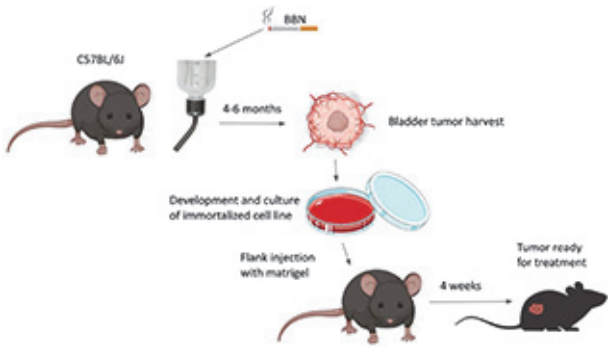
[1]Wolfgang Beckabir*, [1]Misha Fini, [1]Steven Vensko, [1]Jessica Alley, [1]Maria Sambade, [1]Andrew Truong, [1]Mi Zhou, [1]William Kim, [2]Benjamin Vincent. *[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [2]University of North Carolina School of Medicine, Chapel Hill, NC, USA*

Background Current immune checkpoint inhibitor immunotherapy relies on the presence of a pool of pre-existing neoantigen-specific T cells that can be unsuppressed and leveraged to eliminate cancer cells. However, in many patients in many cancers, the pre-existing T cell repertoire is unable to eliminate the tumor even after immune checkpoint therapy, suggesting that a targeted neoantigen vaccination approach could improve response to immune checkpoint inhibitor therapy. Murine antigen models exist to investigate neoantigen vaccine approaches, but most current murine neoantigen models rely on OVA or viral antigens that might have very different immunogenic properties to human neoantigens. Here, we investigate the landscape of predicted neoantigens in BBN963, a syngeneic murine bladder cancer model with endogenous neoantigens generated through mouse exposure to the tobacco carcinogen BBN in their drinking water (figure 1).

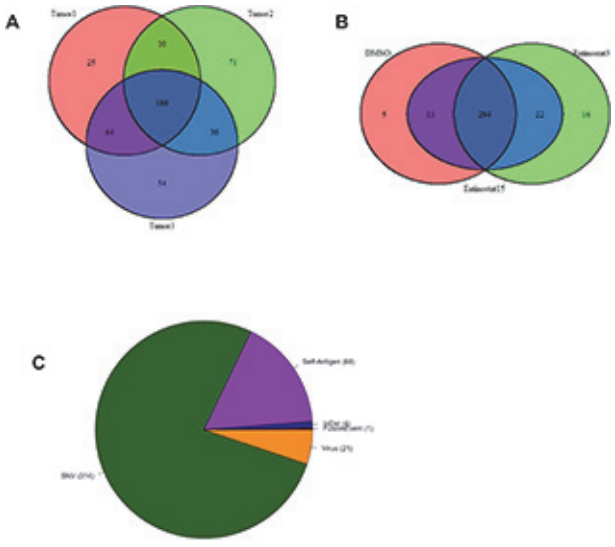
Methods We used LENS to predict neoantigens in the BBN963 cell line. We performed RNAseq on the BBN963 cell line treated for 72 hours with DMSO (control) or entinostat, an HDAC inhibitor that upregulates expression of some predicted BBN963 neoantigens. We performed DNAseq on BBN963 tumors after 4 weeks of treating the mice with normal or entinostat chow to assess for immunoediting at the DNA level.[1] From the list of LENS-predicted BBN963 neoantigens we assessed features of antigens with of strong immunogenic potential (strong binding affinity, low agretopicity, high binding stability, low baseline expression level), upregulated with entinostat in vitro, and immunoedited with entinostat treatment in vivo.

Results From LENS, we predicted 422 antigens from a variety of genomic sources (SNV, self-antigen, fusion event, virus, and indel). 147 of these antigens were immunoedited in vivo. A high degree of overlap existed between the neoantigens predicted by LENS from three biological replicate tumors (figure 2). Furthermore, different neoantigens are expressed by BBN963 cells in culture depending on concentration of entinostat.

Conclusions We present an endogenous neoantigen murine cancer model containing over 400 predicted neoantigens from multiple genomic sources. Some of these neoantigens have strong immunogenicity features. The BBN963 model will be invaluable in future investigation of tumor-immune evolution, immunoediting, neoantigen-specific CD8 T cell responses, and neoantigen vaccination (figure 3).



Abstract 848 Figure 1 Generation of BBN963 endogenous neoantigen murine cancer model. A C57BL/6J mice were treated with BBN orally in drinking water for 4–6 months. The tumor from one female mouse was harvested and used to generate the immortalized BBN963 cancer cell line. To establish subcutaneous tumors, BBN963 is cultured for one week in vitro and then implanted with matrigel by flank injection. After 4 weeks of growth in the recipient mouse, the mouse and tumor are ready for treatment by entinostat, anti-PD-1, and/or neoantigen vaccination

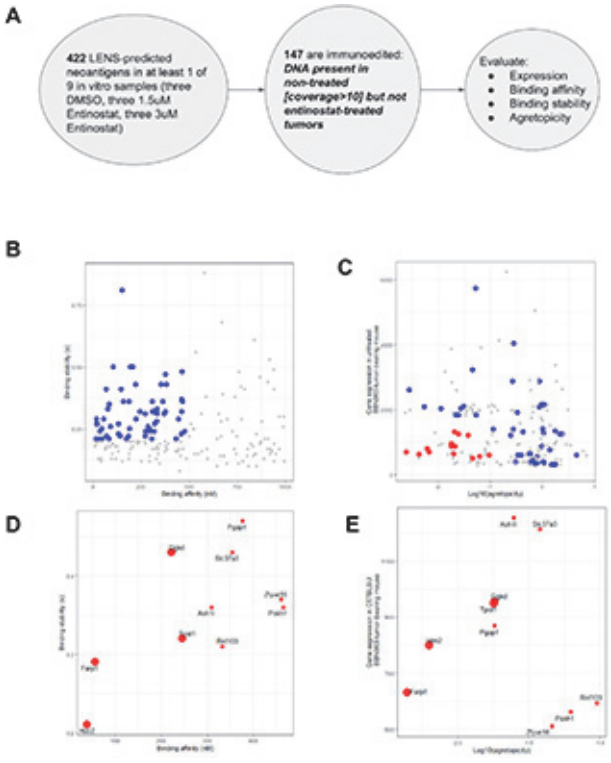


Abstract 848 Figure 2 LENS prediction of BBN963 neoantigens. We assessed overlap in the neoantigens predicted by LENS from three separate BBN963 tumors (A). From in vitro culture with DMSO, 1.5 uM entinostat, and 3uM entinostat, we assessed overlap in the neoantigens present in each treatment group (B). Neoantigens from multiple genomic sources were predicted from the in vitro culture BBN963 cells (C)

REFERENCE

1. Andrew S Truong, Mi Zhou, Bhavani Krishnan, Takanobu Utsumi, Ujjawal Manocha, Kyle G Stewart, Wolfgang Beck, Tracy L Rose, Matthew I Milowsky, Xiaping He, Christof C Smith, Lisa M Bixby, Charles M Perou, Sara E Wobker, Sean T Bailey, Benjamin G Vincent, William Y Kim. Entinostat induces antitumor immune responses through immune editing of tumor neoantigens. *J. Clin. Invest.* 2021;**131**.

J Immunother Cancer: first published as 10.1136/jitc-2023-SITC2023.0848 on 2 November 2023. Downloaded from http://jitc.bmj.com/ on September 14, 2024 by guest. Protected by copyright.



Abstract 848 Figure 3 Immunogenicity potential of LENS-predicted BBN963 neoantigens. LENS-predicted SNV neoantigens from cell culture RNAseq and DNAseq were filtered based on immunoediting in vivo and characterized based on immunogenicity features (A). Among the 147 immunoedited SNV neoantigens, we assessed binding stability and binding affinity (B), agretopicity from LENS, and gene expression in untreated mouse tumors (C). We assessed the features of the neoantigens with strong binding affinity, high binding stability, low agretopicity, and low baseline gene expression (D-E)

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0848

J Immunother Cancer: first published as 10.1136/jitc-2023-SITC2023.0848 on 2 November 2023. Downloaded from http://jitc.bmj.com/ on September 14, 2024 by guest. Protected by copyright.