NKG2A AND HLA-E DEFINE A NOVEL MECHANISM OF RESISTANCE TO IMMUNOTHERAPY WITH M. BOVIS BCG IN NON-MUSCLE-INVASIVE BLADDER CANCER PATIENTS

¹Amir Horowitz*, ¹Jorge Daza, ¹Y Alice Wang, ¹Daniel Ranti, ¹Berengere Salome, ¹Elliot Merritt, ¹Julie-Ann Cavallo-Fleming, ²Everado Hegewisch-Sollosa, ²Emily Mace, ¹Adam Farkas, ¹Sanjana Shroff, ¹Michelle Tran, ¹Jingjing Qi, ¹Manishkumar Patel, ¹Daniel Geanon, ¹Geoffrey Kelly, ¹Ronaldo de Real, ¹Brian Lee, ¹Seunghye Kim-Schulze, ¹Tin Htwe Thin, ¹Monica Garcia-Barros, ¹Kristin Beaumont, ¹Ying-Chih Wang, ¹Li Wang, ³Dominic LaRoche, ³Yong Lee, ¹Robert Sebra, ¹Rachel Brody, ¹Reza Mehrizin, ¹Jun Zhu, ¹Anna Tocheva, ¹Benjamin Hopkins, ¹Peter Wiklund, ¹Matthew Galsky, ¹Nina Bhardwaj, ¹John Sfakianos. ¹Icahn School of Medicine at Mount Sinai, New York City, NY, USA; ²Columbia University, New York, NY, USA; ³HTG Molecular Diagnostics, Tucson, AZ, USA

Background 75% of diagnosed bladder tumors are non-muscle-invasive (NMIBC)[1, 2]. Most require intravesical instillation of M.bovis Bacillus Calmette-Guérin (BCG). Recurrence after immunotherapy occurs in ~50% patients. Development of treatments for BCG-resistant disease has lagged partly because few studies have attempted to understand the relationship between timing of tumor recurrence, reasoning for the recurrence, and the state of immune system at the time of recurrence. Immune exhaustion is observed following microbial infections, cancers and chronic inflammation [3–5]. Natural Killer (NK) cells are among the earliest responders[6–8] and undergo a similar program of exhaustion as T cells[9]. HLA-E strongly inhibits NKG2A-expressing NK and CD8+T cells and is commonly upregulated on tumors[10]. We evaluated the potential restorative capacity of NKG2A and PD-L1-blockade for reinvigorating NK and CD8+T cell antitumor functions in BCG-resistant bladder cancer.

Methods mRNA analysis of 2,892 genes was performed on tumor tissue of NMIBC patients before and after BCG therapy (n=35). Immunostaining (serial-IHC, immunofluorescence, imaging-mass cytometry) was performed on consecutive tissue sections. Single-cell-RNA-sequencing (scRNAseq) was performed on fresh bladder tumors (NMIBC, n=4; MIBC, n=9). OLink Proteomics ("Inflammation" panel) was performed longitudinally on plasma/urine from a prospective cohort of NMIBC patients. Patient tumors (n=3) were expanded as organoids and co-cultured with autologous tumor-derived NK and CD8+T cells in presence/absence of anti-PD-L1/NKG2A antibodies.

Results We demonstrate a robust local TME and systemic response to BCG that correlates with chronic inflammation and adaptive resistance rather than with preventing tumor recurrence. This resistance is mediated through IFN- γ -production by tumor-infiltrating NKG2A+NK and NKG2A+PD-1+CD8+T cells and results in increased HLA-E and PD-L1 on recurring tumors. Co-culture of treatment-naïve NMIBC tumors with recombinant IFN-gamma directly enhanced expression of PD-L1 and HLA-E. Longitudinal analysis of plasma before and during BCG immunotherapy revealed an inflammatory signature, including but not limited to IFN-gamma, that is maintained throughout treatment. Immunostaining and scRNAseq of NMIBC specimens revealed highly enriched infiltration by NKG2A+NK and NKG2A+CD8+T cells in HLA-EbrightPD-L1+ tumors and were spatially organized relative to tumors in a manner suggesting direct inhibition. Tumor-derived NK and CD8+T cells from BCG-resistant patients were co-cultured with autologous tumor organoids. Preliminary analyses demonstrated an improved anti-tumor response in presence of NKG2A/PD-L1-blockade.

Conclusions Our data support a model of BCG-resistance that points to a novel checkpoint axis that contributes to BCG-

resistance: HLA-E/NKG2A. New insights into this axis in NMIBC and how it is altered with repeated BCG exposure will enable us to explore combination therapies (PD-L1/NKG2A-blockade) that may reduce BCG-resistance and provide durable response.

REFERENCES

1. Eidinger D, Morales A: Discussion paper: treatment of superficial bladder cancer in man. *Ann N Y Acad Sci* 1976, 277:239–240.
2. Morales A, Eidinger D, Bruce AW: Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. *J Urol* 1976, 116:180–183.
3. Blank CU, Haining WN, Held W, Hogan PG, Kallies A, Lugli E, Lynn RC, Philip M, Rao A, Restifo NP et al: Defining 'T cell exhaustion'. *Nat Rev Immunol* 2019, 19:665–674.
4. Hashimoto M, Kamphorst AO, Im SJ, Kissick HT, Pillai RN, Ramalingam SS, Araki K, Ahmed R: CD8 T Cell Exhaustion in Chronic Infection and Cancer: Opportunities for Interventions. *Annu Rev Med* 2018, 69:301–318.
5. McLane LM, Abdel-Hakeem MS, Wherry EJ: CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. *Annu Rev Immunol* 2019, 37:457–495.
6. Lanier LL: NK cell receptors. *Annu Rev Immunol* 1998, 16:359–393.
7. Biron CA, Gazzinelli RT: Effects of IL-12 on immune responses to microbial infections: a key mediator in regulating disease outcome. *Curr Opin Immunol* 1995, 7:485–496.
8. Welsh RM, Jr.: Cytotoxic cells induced during lymphocytic choriomeningitis virus infection of mice. I. Characterization of natural killer cell induction. *J Exp Med* 1978, 148:163–181.
9. da Silva IP, Gallois A, Jimenez-Baranda S, Khan S, Anderson AC, Kuchroo VK, Osman I, Bhardwaj N: Reversal of NK-cell exhaustion in advanced melanoma by Tim-3 blockade. *Cancer Immunol Res* 2014, 2:410–422.
10. van Hall T, Andre P, Horowitz A, Ruan DF, Borst L, Zerbib R, Narni-Mancinelli E, van der Burg SH, Vivier E: Monalizumab: inhibiting the novel immune checkpoint NKG2A. *J Immunother Cancer* 2019, 7:263.

Ethics Approval Primary urothelial bladder cancer tumor tissue was obtained after obtaining informed consent in the context of an Institutional Review Board (IRB)-approved genitourinary cancer clinical database and specimen collection protocol (IRB #10-1180) at the Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai (New York, NY).

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.621>