# PD-1 EXPRESSION CORRELATES WITH FAVORABLE PROGNOSIS IN HIGH-GRADE SEROUS OVARIAN CANCER AND IS SELECTIVELY EXPRESSED ON INTRAEPITHELIAL ($CD103^+$) TUMOR-INFILTRATING CD8 T CELLS

**John R. Webb**[a,b], **Katy Milne**[a] **and Brad H. Nelson**[a,b,c]

[a]Trev and Joyce Deeley Research Centre, British Columbia Cancer Agency, Victoria, Canada, [b]Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada, [c]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.

**Rationale:** PD-1 is an important immunoregulatory molecule in cancer, but the mechanisms that regulate its expression *in vivo* are poorly understood. We recently reported that $\alpha_E(CD103)\beta_7$ specifically demarcates 'intraepithelial' $CD8^+$ tumor-infiltrating lymphocytes (ep-CD8 TIL) in ovarian cancer. $\alpha_E(CD103)\beta_7$ is a TGF-β-regulated integrin that mediates retention of lymphocytes in peripheral tissues by binding to E-cadherin expressed on epithelial cells. We hypothesized that $\alpha_E(CD103)\beta_7$-mediated retention of ep-CD8 TIL within tumor epithelium might also lead to PD-1 expression by facilitating chronic exposure to tumor antigen.

**Methods:** PD-1+ TIL were enumerated in a large cohort of ovarian tumors (N = 489) with known $CD103^+$ TIL content. Multi-parametric immunohistochemistry and flow cytometry were used to assess the intratumoral location and functional phenotype of TIL in prospectively collected specimens.

**Results:** PD-$1^+$ cells were present in 38.5% of high-grade serous carcinomas (HGSC) but were less prevalent in other histological subtypes. PD-1 expression strongly associated with increased survival in HGSC (hazard ratio=0.4864; *P*=0.0007). PD-1 expression was largely restricted to the CD3 T cell compartment and was expressed on a variable proportion of both CD4 and CD8 T cells. There was a high degree of PD-1 and CD103 co-expression within the CD8 TIL compartment, and PD-$1^+CD103^+$ CD8 TIL were preferentially localized to intraepithelial regions of the tumor. PD-$1^+CD103^+$ CD8 TIL appeared quiescent when assessed directly *ex vivo*, yet were capable of robust cytokine production after stimulation *in vitro* with PMA/ionomycin. Moreover, they showed negligible expression of additional exhaustion-associated markers including TIM-3, CTLA-4 and LAG-3.

**Conclusion:** As hypothesized, the vast majority of $CD8^+CD103^+$ TIL express PD-1, consistent with prolonged exposure to tumor antigens. Nonetheless, they remain functionally competent and prognostically favorable, indicating they are not terminally exhausted. We speculate that, after standard treatment, $PD\text{-}1^+CD103^+$ CD8 TIL might regain anti-tumor activity in vivo, an effect that could potentially be augmented by immune modulation.