

SENESCENCE IS A MAJOR MECHANISM OF CIRCULATING CD8+ T CELL DYSFUNCTION IN AGING HEAD AND NECK CANCER PATIENTS

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BACKGROUND

BACKGROUND: Impaired immuno-competence is a hallmark of aging. The aged immune system is plagued with dysfunctional lymphocytes rendering older adults more vulnerable to cancer. T cells experience two major but distinct mechanisms of dysfunction: senescence and exhaustion. In HNSCC, current immune-based therapies aim to augment endogenous anti-tumor T cell responses by targeting T cell exhaustion through immune checkpoint blockade (ICB) while no approved therapies currently exist for targeting T cell senescence. Despite concerns that age-related immune decline may impact the response to ICB, studies have shown that nivolumab is effective and safe in HNSCC patients ≥ 65 years old. Recent evidence suggests that the T cell response to ICB is largely due to the clonal replacement of tumor-specific T lymphocytes through recruitment of peripheral T cells into the tumor rather than through the reinvigoration of pre-existing tumor-infiltrating lymphocytes, thereby demonstrating a crucial role of the immune landscape of peripheral blood in the response to ICB. An improved understanding of how antitumor immunity is molded by aging can elucidate the potential for response to immunotherapies and guide development of new immune-based therapies for the increasingly older cancer population who are ineligible to receive chemotherapy past 70 years of age.

AIMS: We aimed to compare the levels of senescent/exhausted circulating CD8+ T cells and their immune checkpoint landscape in “young” and “old” HNSCC patients.

METHODS

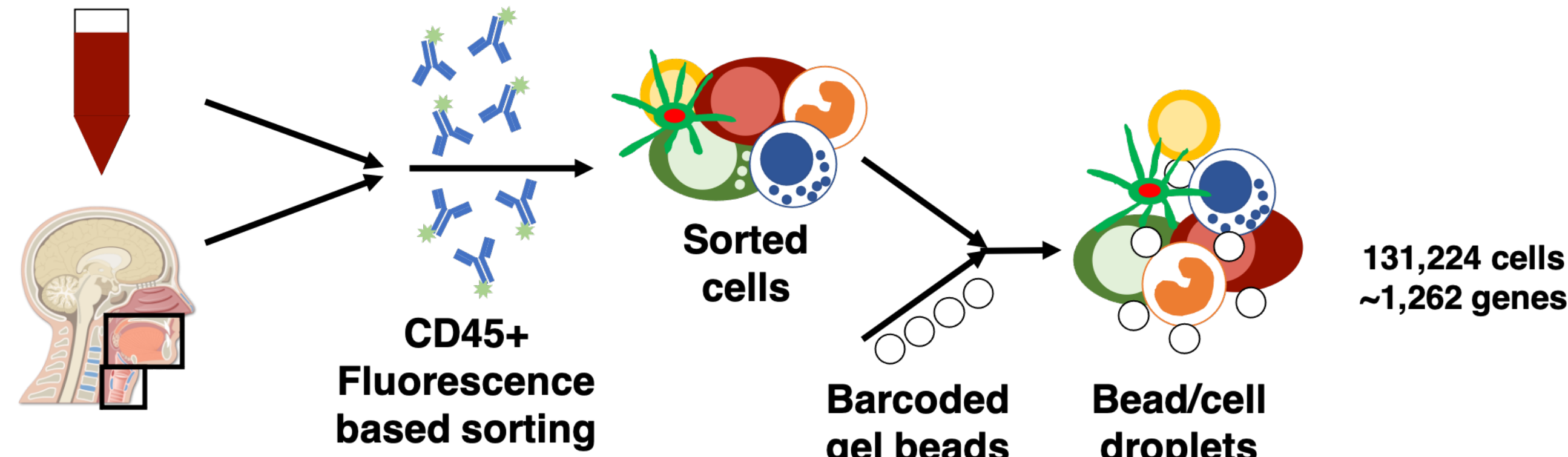


FIGURE 1 | Droplet-based scRNA-seq. Live CD45+ cells were sorted from PBMCs of n=12 “young” (mean age, min-max age; 48, 35-55) and n=10 “old” (73, 66-80) patients and single-cell suspensions prepared from healthy and head and neck cancer tissue samples and subjected to single cell RNA-sequencing.

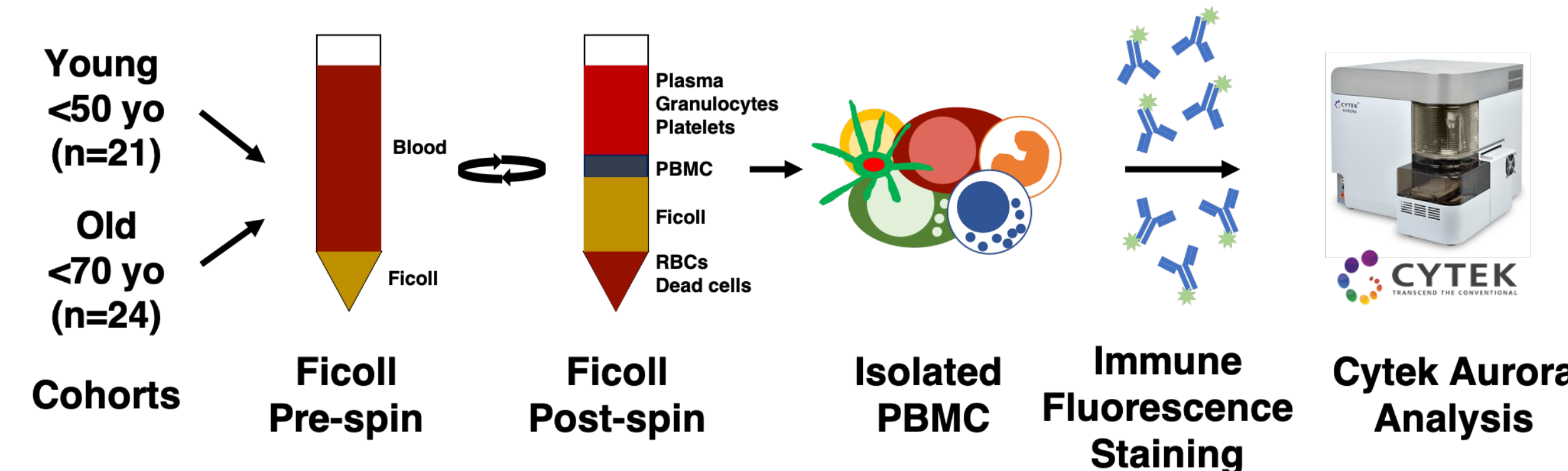


FIGURE 2 | Flow experiment schematic. PBMCs isolated from n=21 “young” (47, 36-50) and n=24 “old” (75, 71-86) HNSCC patients using a Ficoll gradient were analyzed at a single-cell level using the Cytex Aurora cytometer and stained for 18 different markers.

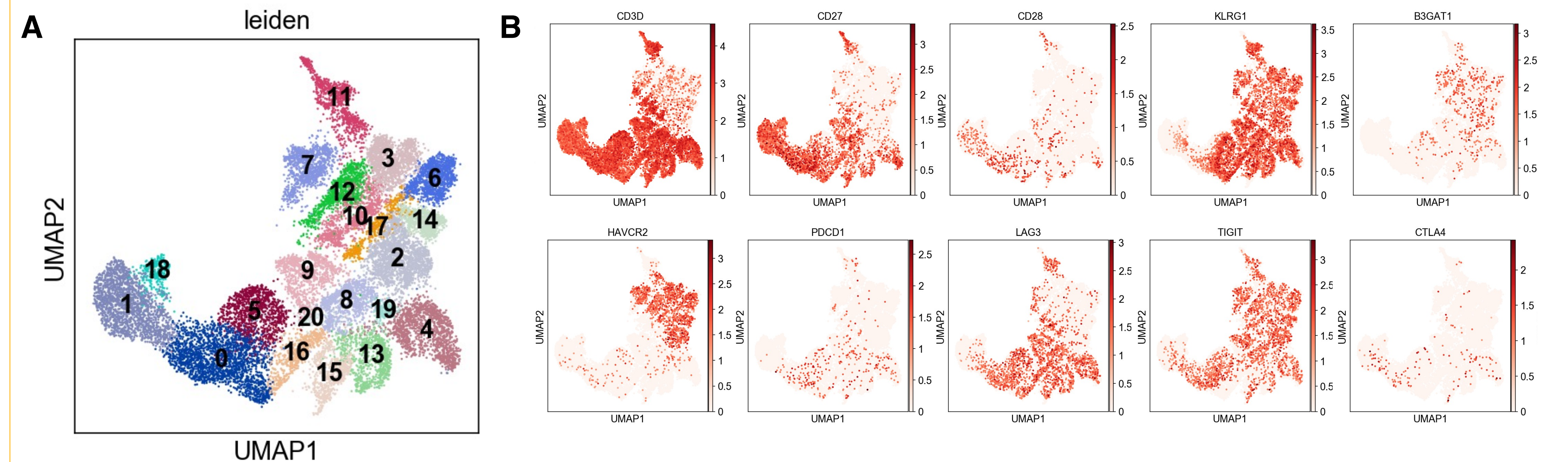


FIGURE 3 | Identification of senescent and exhausted CD8+ T cell populations via UMAP visualization of scRNA-seq data. A) UMAP visualization of all CD3+CD8+ peripheral blood lymphocytes identified 20 unique clusters. B) UMAP plots representing expression values of selected genes for immune checkpoint receptors (ICRs) and T cell senescence markers (CD27-, CD28-, KLRG1+, B3GAT1+). Based on the expression profiles of immune checkpoint receptors, senescence markers and CD3 expression, cells within clusters 7, 10, 12, 17 were identified as senescent T cells and cells within clusters 4, 5, 8, 9, 15, 20 have been identified as exhausted T cells

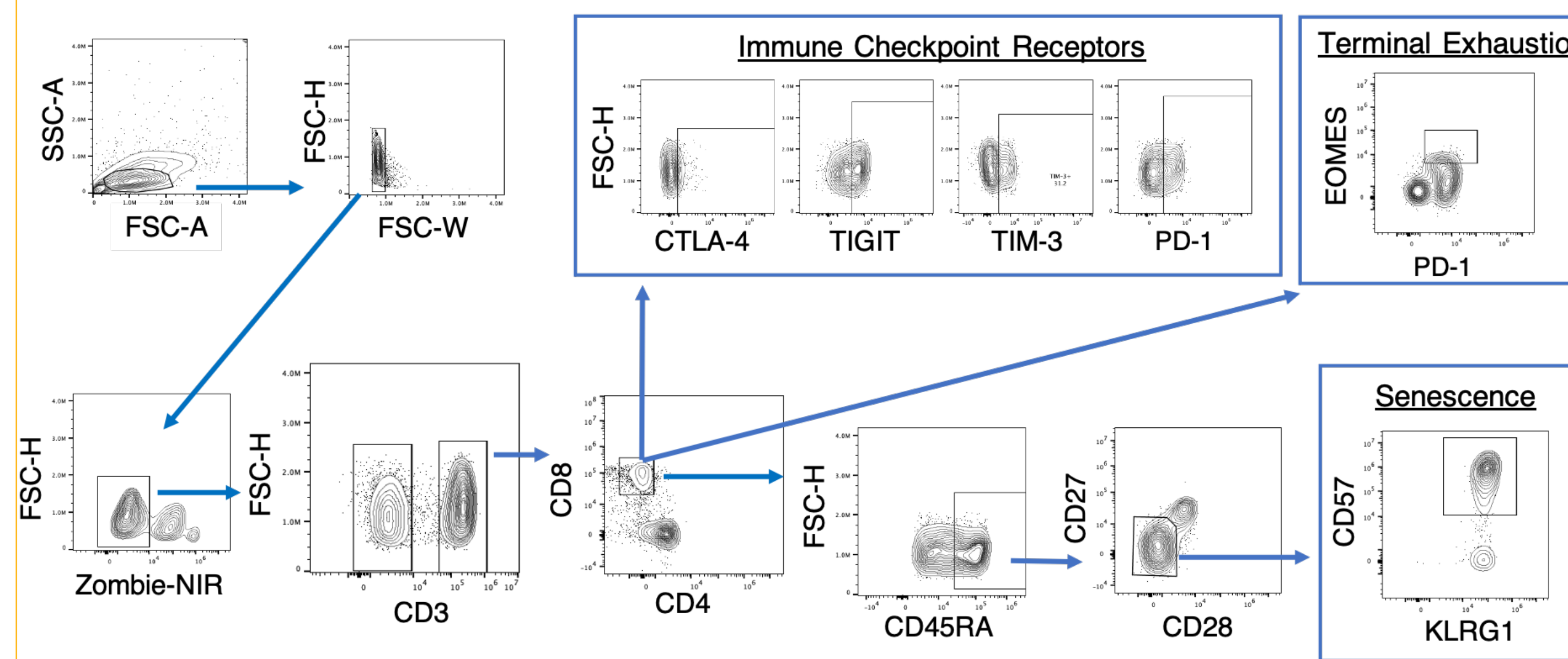


FIGURE 4 | Gating strategy for PBMC flow analysis. Lymphocytes were gated based on forward-scatter area (FSC-A) and side-scatter area (SSC-A). Single cells were separated based on forward scatter width (FSC-W) and a forward scatter height (FSC-H) gate. Zombie-NIR was used as a viability dye to isolate live cells. Live, singlet lymphocytes were initially gated broadly to CD3+ cells. Among the CD3+ cells, CD8+ cells were identified and analyzed for surface exhaustion markers using immune checkpoint receptors (PD-1, CTLA-4, TIM-3 and TIGIT), terminal exhaustion (EOMES+PD-1^{high}) and senescence (CD45RA+CD27-CD28-KLRG1+CD57+). CD8+ T cells were the primary populations of interest due to their physiologic roles as antitumor cells in the immune system.

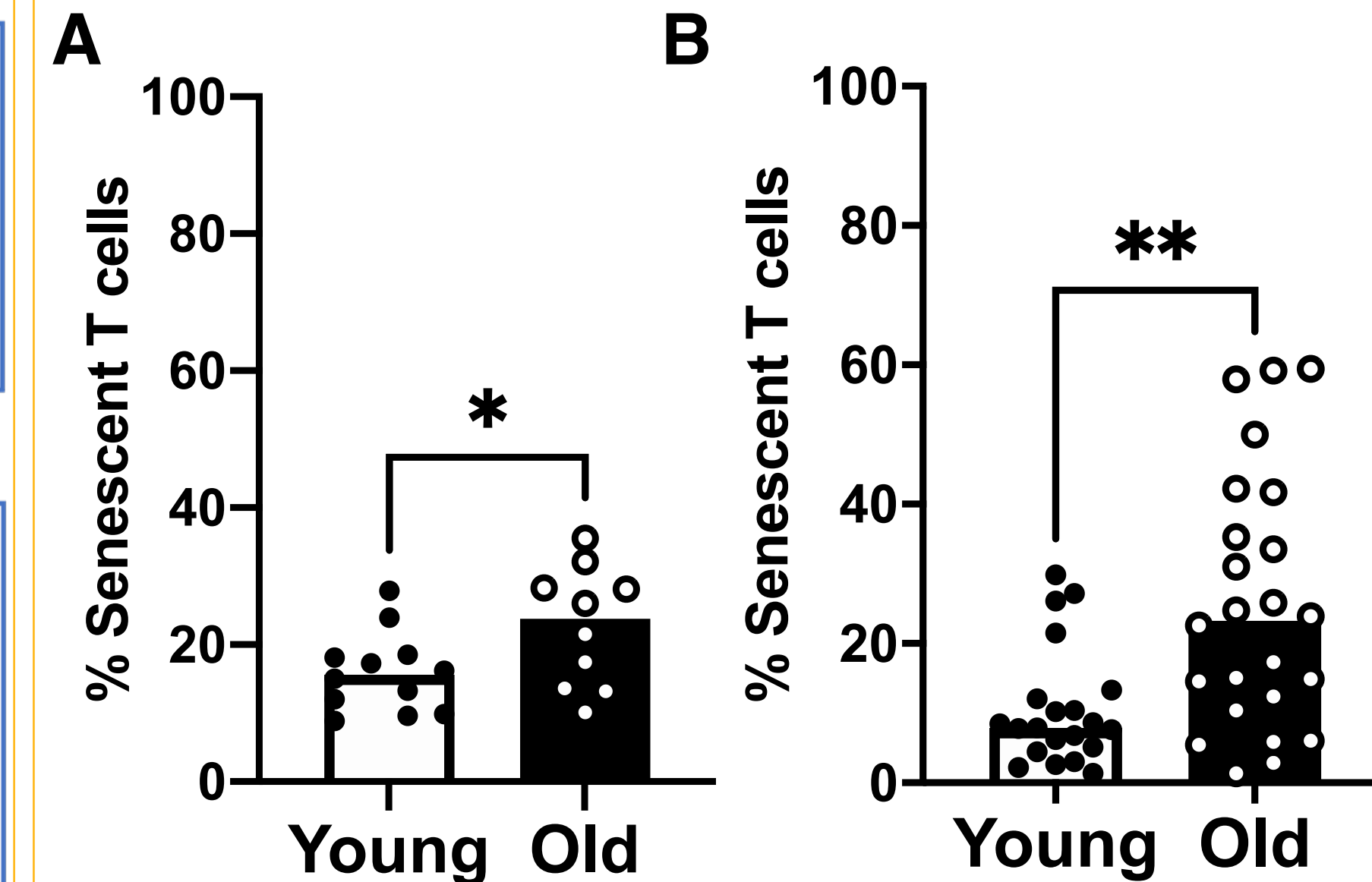


FIGURE 5 | Older HNSCC patients have higher levels of senescent circulating CD8+ T cells. A) CD8+ T cells identified as senescent in our scRNA-seq dataset were counted and the percentages of senescent cells in the total CD8+ T cell pool were plotted based on whether the cells came from “young” vs “old” patient samples. B) Flow cytometry data validated the proportions and trends we found in our scRNA-seq data. Each dot represents an individual patient sample. *p < 0.05 **p < 0.01 based on two-tailed t testing

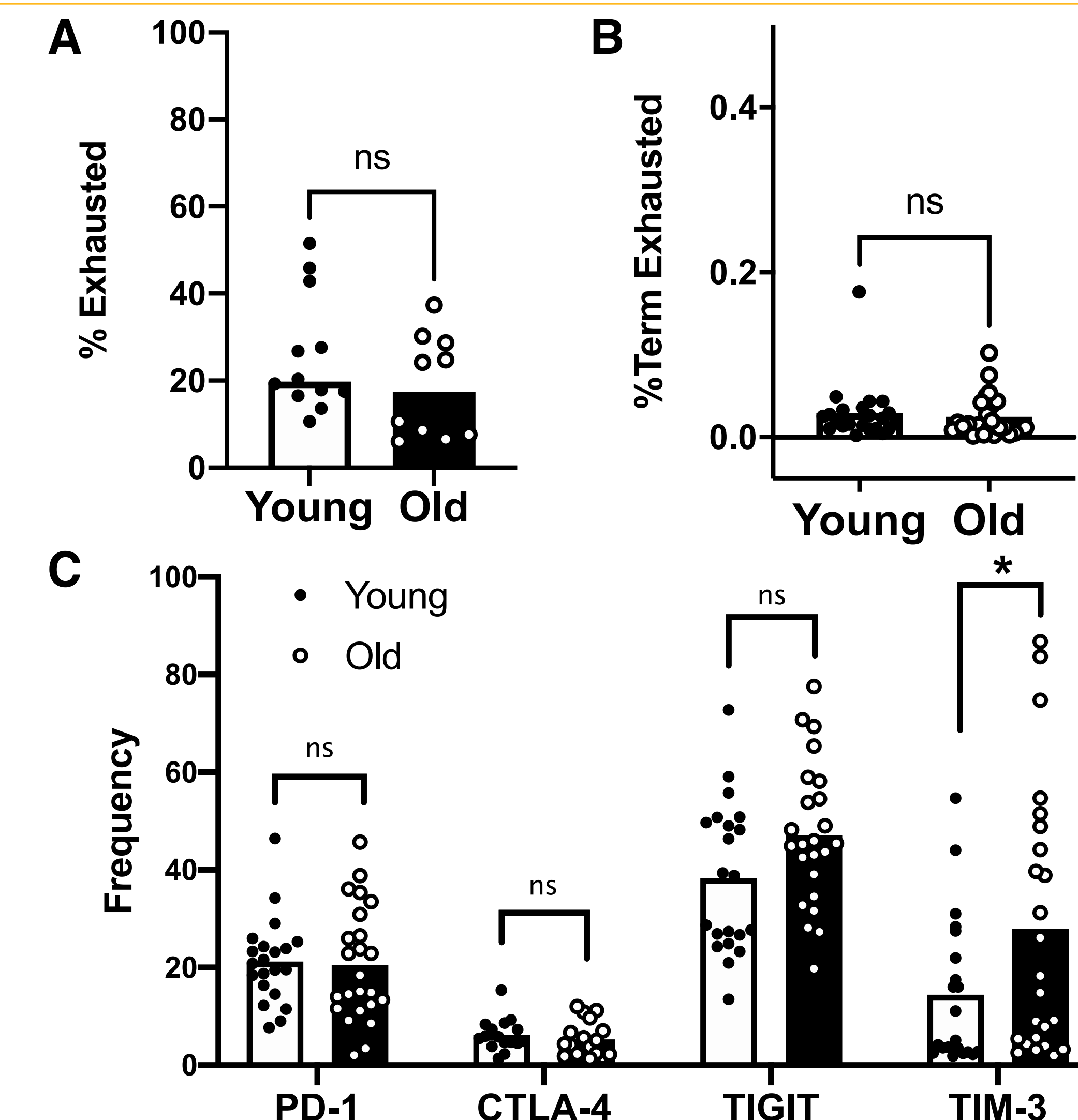


FIGURE 6 | Young and old HNSCC patient have similar levels of exhausted peripheral CD8+ T cells but old patients have an increase in cells expressing TIM-3. There is no significant difference between young and old HNSCC patients in levels of exhausted T cells based on scRNA-seq (A) and flow cytometric evaluation of EOMES+PD-1^{hi} expressing CD8+ T cells (B). C) ICR landscape of peripheral CD8+ T cells demonstrates that cells expressing TIM-3 are elevated in aging HNSCC patients *p < 0.05

DISCUSSION

Older HNC patients demonstrated features of a weakened immune system with higher levels of circulating CD8+ T cells with dysfunctional phenotypes. Transcriptional and immunologic signatures identified senescence rather than terminal exhaustion as the crucial dysfunctional state and TIM-3 as the major checkpoint receptor elevated in the aging HNSCC population. These data suggest that older HNSCC patients may benefit from PD-1+TIM-3 combination ICB and from emerging therapies targeting the reversal of T cell senescence. Validation of the impact of these findings in anti-PD-1 treated patients is ongoing

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