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# Characterizing the Immune Landscape of Non-Muscle-Invasive Bladder Cancer

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**ABSTRACT**

**Introduction:**

Non-muscle-invasive bladder cancer (NMIBC) accounts for approximately 70–75% of newly diagnosed bladder cancers and is characterized by substantial biological heterogeneity, leading to variable risks of recurrence and progression. The current standard of care for patients with high-grade non-muscle-invasive bladder cancer (NMIBC) consists of complete transurethral resection of the tumor followed by intravesical Bacillus Calmette–Guérin (BCG) immunotherapy. Current clinicopathological risk models have limited prognostic accuracy: at present, no clinically validated biomarkers are available to reliably identify, before treatment initiation, which patients are likely to benefit from BCG therapy, and which are destined to fail treatment. This translational study aims to characterize the immune infiltrate in NMIBC and evaluate its association with clinicopathological features and clinical outcomes to improve risk stratification and support personalized treatment strategies.

**Methods:** Advanced flow cytometry and computational cytometry have been used to perform deeply T cell Immunocore in terms of checkpoint expression, frequency of CD4+ and CD8+ T regulatory and effector cells.

**Results:** The cytometric profiling of infiltrating T CD8+ cells is associated with higher frequencies of CD8+CD28- T cells in samples derived from patients who have progressed. These cells are characterized by dysfunctional immunophenotyping, as high expression of molecules related to functional exhaustion (including PD-1, CD39) and progressive loss of effector markers (as CD57, granzyme). Moreover, we also found that the increase of CD8+CD28− T cells has been associated with the increase of CD8+CD28−CD127−CD39 Treg cells, suggesting that T cell exhaustion at the tumor site may be associated with the acquisition of regulatory properties.

**Conclusions:** Overall, the data stress that a progressive deterioration of intratumoral immune response, characterized by loss of memory T cells and functional exhaustion of effector T cell

subsets, is a relevant pathogenic mechanism of tumor immune escape. Based on this, the prognostic valence of the CD8+ T cell tumor infiltrate may depend not only on the amount of the whole CD8+ T cell infiltrate but also on the frequency of CD8+ Treg within the whole CD8+ T cell infiltrate. CD8+ Treg frequency could represent a new prognostic biomarker with predictive capacity on immunotherapy outcome in high grade NMIBC.