TITLE CD39 AND CD73 EXPRESSION ON T CELLS AND TUMOR CELLS IN BLADDER CANCER **CODE** S.02

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ABSTRACT

Introduction: Emerging evidence suggests that tumors generate adenosine in tumor microenvironment (TME), inhibiting effector function of multiple immune cell subsets, thereby allowing neoplastic growth. This is dependent on the adenosinergic pathway (AP), in which CD73 and, more recently CD39, seem to play a key role. We aim to quantify and characterize the phenotype of different subpopulations of T cells (CD4+, CD8+ and Treg) at tumor microenvironment, in surrounding non-malignant tissue and in peripheral blood and, in parallel, evaluate the expression of CD39 and CD73 in urothelial bladder cancer (BC) cells. This is part of a larger study aiming to trace an immunologically-based signature of the AP in BC, with therapeutic and prognostic purposes.

Methods: We conducted a study with 24 patients with histological confirmed urothelial carcinoma of the bladder, with indication for surgery – transurethral resection of the bladder or radical cystectomy. Peripheral blood, tumor and normal-appearing matching tissue were sampled and analyzed by flow cytometry, with a FACSCanto (R) II cytometer. A systemic functional evaluation of the immune and adenosinergic systems, with regard to the subpopulations of T cells and adenosinergic pathway (CD39; CD73) was performed.

Results: Compared to the normal matching bladder tissue, the immunophenotype of BC tissue was characterized by a specific profile of T cell infiltration: increased CD4+ (44.7 vs. 32.3%) and decreased CD8+ (52.3 vs. 66.1%) T cells. Most notably, BC exhibited a marked increase of regulatory T cells (CD4+, CD25+bright, CD127+dim)(18.6 vs. 6.4%, p= 0.008). The majority of T cells, particularly in tumor and normal tissues, had the CD39+/CD73- phenotype. We found an evident increase on the expression of CD39 in all subpopulations of T cells, (CD4+, CD8+ and Treg) either with an activated phenotype (HLA-DR+ and/or CD25+) or not, reaching a mean factor of 20.0 x, when comparing tumor microenvironment to peripheral blood, and 8.6 x compared to normal matching tissue. There was a significant correlation between the percentage of CD4+ Treg cells and the expression of CD39, not only in peripheral blood (p=0.005), but also in normal tissue (p=0.005) and tumor tissue (p=0.018). The same correlation occurred for CD8+ T cells, but only in tumor tissue (p=0.012). In turn, CD73 expression is mostly associated with tumor cells, as 53.6 ± 13.2% of tumor cells express this enzyme.

Conclusion: Our results point to an immunosuppressive tumor microenvironment in bladder cancer, with a decreased infiltration of cytotoxic T cells and an increase of Treg subpopulations, which seems to be associated with an amplified activity of the adenosinergic pathway, where T cells (expressing CD39) and tumor cells (expressing CD73) apparently play a complementary role.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE PERIPHERAL LYMPHOCYTE SUBPOPULATIONS IN PROSTATE CANCER - DATA FROM AN ANIMAL MODEL **CODE** S.03

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ABSTRACT

Introduction: Prostate cancer (PCa) is one of the most common cancers among men worldwide. The presence of immune cells in human cancer raises a fundamental question in oncology. The interaction between immune system and PCa is an important field for translational research. This work aimed to characterize the peripheral lymphocyte subpopulations in a PCa animal model.

Methods: Twenty-five male Wistar Unilever rats (Rattus norvegicus) with twelve weeks of age were randomly divided into two groups: Control (n=10) and Induced (n=15). All procedures were approved by the Portuguese Competent Authority (DGAV no. 021326). Prostate lesions were induced through the administration of flutamide (50 mg/kg, TCI Chemicals, USA), testosterone propionate (100 mg/kg, TCI Chemicals, USA) and N-methyl-N-nitrosourea (30 mg/kg, Sigma Chemical Co., Spain), and crystalline testosterone implants. Animals were humanely sacrificed at 61 weeks of age. Peripheral blood of all animals was collected by intracardiac puncture and transferred into tubes containing EDTA salt as an anticoagulant for flow cytometry analysis. The following conjugated monoclonal antibodies were used: cyCD3-BV421, CD3-FITC, CD25-APC, CD45-BV510, CD127-PE, CD161-FITC, CD4-PE/Cy7, CD45RA-APC/Cy7, OX-82-PE and CD8a-PerCP. The flow cytometry immunophenotyping was performed in a BD FACSCantoTM II cytometer (BD Biosciences, USA) and data were analysed with InfinicytTM, flow cytometry software 1.7 version. Statistical analysis was performed using SPSS 25. The differences were considered statistically significant at p0.05). Similarly, CD8+ lymphocyte population was higher in control group than in induced group (9.56±0.74 vs 6.38±0.32) (p<0.05). Inversely, the population of regulatory T cells (TRegs) (2.99±0.46 vs 4.630±0.35), the TRegs/CD8 ratio (0.35±0.09 vs 0.45±0.08) and the TRegs/Natural Killer ratio (0.52±0.05 vs 1.03±0.13) were higher in induced group when compared with control one (p<0.05).

Conclusion: The population of Tregs increased in induced animals, while the population of NK decreased in these animals, which is in accordance with data previously published by other authors reporting the increase of Tregs and decrease of NK cells in animals with cancer. The characterization of these immune system subpopulation can be important for other studies such as preclinical cancer models.

CONFLICT OF INTEREST No potencial conflict of interest to report.

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