Evaluation of programmed death-ligand 1 expression in canine lymphomas using flow cytometry

UBIALI ALESSANDRA*, MORETTI PIERANGELO*, RIONDATO FULVIO§, SINI FEDERICA§, DE MARIA RAFFAELLA§, STEFANELLO DAMIANO*, COMAZZI STEFANO*, ARESU LUCA§, MARCONATO LAURA°, MARTINI VALERIA*
*Department of Veterinary Medicine and Animal Sciences, University of Milan, Italy
§Department of Veterinary Sciences, University of Turin, Italy
°Department of Veterinary Medical Sciences, University of Bologna, Italy

Immune checkpoint molecules, including Programmed Death-Ligand 1 (PD-L1) play a key role in cancer immunotherapy. PD-L1 protein has been retrieved on the surface and within several different canine cancer cells. Increased amounts of mRNA encoding for PD-L1 by RNA-scope have been associated with worse prognosis in dogs with Diffuse Large B-Cell Lymphoma. Flow cytometry (FC) may represent an advantageous technique to detect PD-L1 expression in dogs with lymphoma compared to other techniques, because it can be performed at the same time and session of diagnosis analysis without any further sampling needed than those of diagnostic purpose. The aim of this study was to assess the membrane expression of PD-L1 of different canine lymphoma subtypes by means of FC. Surface expression of PD-L1 on neoplastic cells was assessed on surplus material from nodal samples obtained for diagnostic purpose from 46 dogs with diagnosis of lymphoma based on cytology and FC. Additional data recorded included: patient signalment, FC phenotype, cytologic (updated Kiel) and WHO histologic subtype. PD-L1 expression was calculated as the ratio between median fluorescence intensity (MFI) of neoplastic cells stained with anti-PD-L1 antibody and an isotypic control. MFI-ratio was arbitrarily considered negative if ≤ 1, low-positive if >1 and <1,2, positive if $\geq$1,2 and <1,7 and highly-positive if $\geq$1,7.
Thirty-three (71,7%) dogs had B-cell lymphoma (BCL), while 13 (28,3%) had T-cell lymphoma (TCL). Seventeen (37%) dogs were negative (11 BCL and 6 TCL), 11 (23,9%) low-positive (6 BCL and 5 TCL), 12 (26,1%) positive (10 BCL and 2 TCL) and 6 (13%) highly-positive (all BCL). Median MFI-ratio of positive samples was 1,41 (1,01 – 2,69), 1,12 (1,02-1,49) for TCL and 1,47 (1,01-2,68) for BCL. No difference in the prevalence of PD-L1 positivity was detected between neither FC phenotypes nor cytologic and histologic subtypes. However, positive BCL had significantly higher levels of protein expression compared to TCL. This is in line with the literature, thereby confirming FC as a reliable technique for the assessment of PD-L1 expression in canine lymphomas. Statistical power of analysis resulted >80% when comparing expression of PD-L1 between positive BCL and

TCL, but dropped when PD-L1 negative samples were included. Considering that, the lack of significance might also depend on the limited number of samples evaluated. Lastly, the significant difference of PD-L1 expression between B and T phenotype was quantitative rather than qualitative, suggesting that TCL might express PD-L1 as well but with a lower intensity.