CANCER-SPECIFIC DIFFERENCES OF TERTIARY LYMPHOID STRUCTURES AND CELLULAR RESPONSES AGAINST FREQUENTLY EXPRESSED CANCER TESTIS ANTIGENS

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Background Tertiary lymphocyte structures (TLS) can be detected in the tumor microenvironment across a wide range of cancer types and are associated with increased patient survival and susceptibility to immune checkpoint inhibition. However, evidence for the functional significance of TLS on humoral and cellular immunity is scarce. In this study, we combine assessment of abundance and spatial distribution of TLS with expression levels of 10 tumor associated antigens (TAA) and functional analyses of T cell responses to these antigens.

Materials and Methods 52 treatment naïve cancer patients across 5 tumor types (NSCLC, CRC, RCC, HCC and BCA) were included. Presence and localization of TLS was assessed in immunohistochemical stainings (CD20) of whole section slides from FFPE embedded tumor samples. B cell clusters were quantified in the whole tumor region and in two different tumor margins (300 μm, 2000 μm). A panel of 30 cancer testis antigens was selected via GEPIA software (TCGA Database) and their expression in our cohort was determined using NanoString based RNA expression analysis of tumor samples and patient-matched healthy tissue. The 10 peptide pools with the largest cross-cancer overlap were selected based on our NanoString results. 2-color Fluorospot assays (IFN-γ and IL-2) were applied to assess the frequency of tumor-specific T-cell responses in patient PBMCs (triplicates for each TAA).

Results CD20 immunohistochemistry and enumeration of intra- and peritumoral TLS revealed different distribution patterns of TLS/mm2 with the largest proportion in the 300 μm margin (p < 0.01) in most of the cancer types. This effect was particularly observed in patients with non-small cell lung cancer (NSCLC). The 10 tumor antigens CEP55, CT83, GAGE1, IGF2BP3, MAGEA1, MAGEA3/6, PBK, PRAME, Survivin and TTK were selected as they showed the highest overlap across different cancer types and the most pronounced differential expression between tumor and matched normal tissue. While 31/52 (59.6%) patients showed an IFN-γ response against at least one of the tested CTAs, Survivin was the CTA presenting the highest frequency of responses (18/52 IFN-γ and 5/52 IL-2 responses). PBMCs of patients with NSCLC showed the highest frequency of T-cell responses (83.3% with at least one IFN-γ response).