Absence of tumor infiltrating lymphocytes in the tumor proper, referred to as the immune-excluded phenotype, has been directly described in the peer-reviewed literature for colorectal cancer, but indirectly for melanoma and other solid tumors in the field of immunotherapy discussions. As part of our clinical immune cell analysis using a New York State CLEP approved assay, Immune Report Card™ (IRC), we routinely perform CD8 and CD3 immunohistochemistry (IHC) to determine the infiltrating versus the non-infiltrating (excluded) phenotype for all tumor types.

For all tumor types tested including carcinoma, sarcoma, and melanoma the immune-excluded phenotype is defined as a restriction of more than 95% of all CD8+ T-cells present in a tumor tissue section to the periphery or interstitial stromal areas and not actively invading nest or groups of neoplastic cells.

Minimally Infiltrating – Sparse number of CD8+ T-cells that infiltrate nests of neoplastic cells and represent less than 5% of the tumor

Moderately Infiltrating – Frequent CD8+ T-cells that infiltrate nests of neoplastic cells in an overlapping fashion at least focally and represent less than 50% of the tumor cells

Strongly Infiltrating – Frequent CD8+ T-cells that infiltrate nests of neoplastic cells in an overlapping fashion diffusely and represent more than 50% of the tumor cells

Non-Infiltrating (Excluded) – Restriction of more than 95% of all CD8+ T-cells present in a tumor to the periphery or interstitial stromal areas and not actively invading nest or groups of neoplastic cells

Tumors are also defined as inflamed or non-inflamed based upon RNA-seq analysis of CD8 from the same tissue section and comparison to a reference population of several hundred prior analyzed samples. Tumors in upper 75th percentile of rank for CD8 gene expression are considered inflamed, while those in the lower 25th percentile are considered non-inflamed. RNA-seq analysis of CD8 had been previously calibrated against quantitative image analysis using the Aperio platform. A total of 306 consecutive samples tested, including 16 colorectal cancer and 290 of other tumor type, were evaluated for the excluded infiltrate phenotype.

In a subset of excluded cases the restricted T-cells show high expression of PD-L1 and negative staining of the adjacent neoplastic cells.

While the excluded phenotype was identified most commonly in colorectal cancer, it was also seen in multiple other tumor types. Most notable was the prevalence of the excluded phenotype in endometrial adenocarcinomas and head and neck (H&N) cancer.

• The non-infiltrating, or excluded, phenotype was identified in all tumor types evaluated.

• In tumor types with more than 10 cases examined the highest rate of non-infiltrating, or excluded, phenotype was colorectal cancer with H&N and endometrial cancer being very similar.

• Providing a precisely defined nomenclature and method of testing for the immune-excluded phenotype in inflamed and non-inflamed tumors is important for both clinical and research purposes.