The excluded phenotype has been previously described by the presence of abundant immune cells that do not penetrate the parenchyma of these tumors but instead are retained in the stroma that surrounds nests of tumor cells. These features support a pre-existing anti-tumor immune-related response, but the details of this mechanism are not well elucidated.

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 GEP approved assay, Immune Report Card™ (IRC), we routinely perform CD8 and PD-L1 immunohistochemistry (IHC) to identify the PD-L1 excluded phenotype.

Methods

300 formalin-fixed, paraffin-embedded (FFPE) metastatic cutaneous melanoma samples were evaluated for the excluded phenotype. Medical and electronic pharmacy records were reviewed to identify individuals with an excluded phenotype and receiving FDA-approved checkpoint inhibitors (n = 11) for which response by RECIST v1.1 was available.

Immunohistochemical studies. The expression of PD-L1 on the surface of tumor cells was assessed in all samples by means of the Dako Omnis platform (Agilent, Santa Clara, CA) and the 28-8 pharmDx antibody. Expression levels, and were scored as per published guidelines. A tumor was considered PD-L1 positive if ≥1% of viable tumor cells exhibited complete circumferential or partial linear plasma membrane staining at any intensity.

Additional serially sectioned tissue was evaluated for lymphocyte infiltration using the anti-CD8 antibody C8/1448 (Agilent, Santa Clara, CA) and assigned a qualitative score of non-infiltrated, infiltrated, or excluded.

- Non-infiltrated referred to a sparse number of CD8+ T-cells that infiltrate nests of neoplastic cells and with less than 5% of the tumor showing an infiltrating pattern.
- Infiltrated represents frequent CD8+ T-cells that infiltrate nests of neoplastic cells in an overlapping fashion at least focally and in more than 5% of the tumor.
- Excluded represents restriction of more than 95% of all CD8+ T-cells in a tumor to the periphery or interstitial stromal areas and not actively invading nests or groups of neoplastic cells.

Mutational burden. DNA was extracted from each sample and processed for whole-exome DNA-seq. Mutational burden was evaluated by targeted capture and sequencing of 409 cancer-related genes on samples that met validated quality control (QC) thresholds. Somatic mutation calling was conducted using Ion Torrent Suite software’s variant caller plugin. Mutational burden cutoff was derived from a reference population whereby the median MuB was 3.53 mutations per megabase DNA. This value was used as a baseline and a high MuB was defined as 2x this median value.

Results

Prevalence of Excluded Phenotype in 300 Metastatic Melanoma

The excluded phenotype represents 11% of melanoma with an even distribution of T-cell excluded (14%; 7%) and PD-L1 excluded (20%; 4%).

For Every Patient

- Providing a precisely defined nomenclature and method of testing for the immune-excluded phenotype is important for both clinical and research purposes
- Excluded phenotype represents an estimated 11% of cutaneous metastatic melanoma
- Two types of immune-excluded phenotype exist: T-cell and PD-L1 excluded
- Contrary to current understanding of the “immune-excluded” phenotype the excluded phenotype in melanoma does respond to checkpoint inhibition therapy
- Not surprisingly the PD-L1 excluded phenotype has a higher response rate than the T-cell excluded phenotype, although the numbers to support this conclusion are limited