SOP 14 Scoring of Chromogenic IHC

SOP 14.1 Scoring Tumor-infiltrating Lymphocytes (TILs)

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Materials

Name	Source/Vendor	Catalog#	LOT# (optional)
n/a			

Buffer and Media

n/a

Protocol

A) By eye, by two independent pathologists

1. Pathologist checks the morphology of the tissue based on an H&E staining, excludes any tissue with crush artifacts and necrosis, and marks it as “non-evaluable”

2. Once the sample passes quality control, pathologist identifies 3-5 representative fields of view in that tissue

3. In each field of view, pathologist counts ie: CD4 (+) cells within the borders of the invasive tumor, and excludes TILs outside of the tumor border.

4. The percentage (%) of TILs as percent of total cells is assessed and reported

5. Pathologists meet and reconcile their scores for each case

B) By Image Analysis Software (PerkinElmer)

1. The pathologist takes 3-5 representative pictures for each case at 200x magnification

2. Tissue classification of the images is performed, where tumor and stroma are distinguishably labeled.

3. The pathologist performs “cell segmentation” where the whole image is segmented into individual cells based on hematoxylin as a nuclear stain

4. The pathologist trains the software to identify ie: CD4 (+) and CD4 (-) cells on a cell-to-cell basis, based on expression and comparison to control tissues (tonsil).

5. Thresholds are established and then applied to the whole image by the pathologist.

6. A second pathologist checks the threshold, and either approves or disapproves it. In case of disproval, the two pathologists reconcile between them.

7. Scoring for positive and negative cells is performed in the tumor and in the tumor-stroma interface compartments

8. The results are exported in a excel sheet format and the information is reported in the form of: ie: number of CD4 (+) cell per unit area for both a) intratumoral and b) tumor-stroma interface regions.