Tumor Mutational Burden (TMB): Assessment of Inter- and Intra-tumor heterogeneity

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As an emerging biomarker, tumor mutational burden (TMB) is associated with response to immune checkpoint inhibitors. The tumor tissue selected for molecular analysis could be critical for accurate TMB assessment and therapeutic selection. We evaluated TMB and the tumor specimen tested to determine the extent tissue selection and specimen characteristics affect TMB analysis.

Methods

Following anatomical pathologist review, DNA was isolated from resection or needle core biopsy FFPE specimens collected from 36 cancer patients. Two to six specimens per patient, including primary, recurrent and metastatic cases with neoplastic content, percent necrosis and tissue amounts representative of typical clinical samples were procured. TMB was evaluated by performing targeted NGS using a 409 gene panel from 30ng of DNA (Figure 1). TMB was determined and reported as non-synonymous mutations per megabase DNA (Muts/Mb).

Speckmens

Figure 1: TMB NGS Workflow

Nine patients had multiple specimens from the same surgical event (Figure 2) for which a subset of tumors were evaluated for intra-tumor (spatial) heterogeneity. An additional 27 patients had specimens from multiple surgical events and anatomical sites spanning up to seven years (Figure 3). In total, 98 specimens were evaluated for TMB for 36 inter-patient comparisons (Figure 4) representing inter-tumor, intra-tumor and intra/inter-tumor comparisons (Figure 5). The percentage of common variant overlap within tumors was calculated for each subject. For each patient, the heterogeneity of TMB values within tumors (spatial), between tumors (spatial and temporal), and across collection times was assessed (Figures 6 and 7). Over representation analysis (v-test) was performed to determine association of TMB variation with specimen characteristics including collection time, necrosis, tumor amount (mm²), neoplastic content and PMR (Figure 8).

Results and Conclusions

• Inter- and intra-tumor TMB variation occurs, but in <15% of patients tested
• Spatial and temporal differences in specific mutations that make up TMB is common
• Sample selection and pre-analytical pathologist review is critical for TMB evaluation
• Clinical interpretation of TMB will need to factor in multi-site sequencing, influence of treatment and neo-antigenicity

Figure 2: Tumors from 9 patients collected at a single surgical event with TMB evaluated within a tumor (Site 1) and between tumors (sites 1-4).

Figure 3: Tumors from 27 patients collected from multiple surgical events and anatomical sites with TMB evaluated across several timepoints.

Figure 4: Comparisons for 36 patients evaluating intra-tumor and inter-tumor TMB heterogeneity.

Table 1: Tumor samples from 36 patients evaluated for TMB. Patient’s with red arrows (→) are represented in figure 5. Specimen characteristics were determined by microscopic anatomical pathologist review.

Results

Figure 6: TMB variation between inter-tumor and intra-tumor specimens across all specimens.

Figure 7: TMB variation between tumors within a patient (circles) across time.

Figure 8: TMB variation within a patient is most influenced by tissue amount.