MSI status in each sample was determined using the Promega MSI Analysis System v1.1.2 on ABI 3500x Genetic Analyzer. The system is a fluorescent PCR-based assay to detect the presence of five (5) mononucleotide repeat markers (BAT25, BAT26, NR21, NR24, and NR27) and two pentanucleotide repeat markers (Penta C and Penta D). Two (2) ng DNA from each sample, measured by Qubit, were used per library. TMB expression was compared with the MSI status. Two samples were classified as MSI-H, 22 samples as MSI-L/MMRd, and five samples as MSS. The correlation between the MSI status and the TMB level in each sample was also evaluated. In order to fully determine the value of TMB as a predictive biomarker for immunotherapy, a standardization of worklfows and data analysis pipelines to assess TMB is still needed. In this study we evaluated the performance of a commercially available targeted NGS panel and workflow for TMB analysis. The correlation between the MSI status and the TMB level in the same sample was also evaluated.

Materials and Methods

Samples

A set of 24 FFPE tumor samples from cancers that occur in the gastrointestinal tract (esophagus, stomach, large intestine, the reproductive system (ovaries and uterus) and lung were analyzed (Table 1). DNA was extracted using either the Roche Whole Nucleic Acid Isolation Kit, or Promega Maxwell CSC DNA FFPE kit on the Maxwell CSC Nucleic Acid Isolation System or Promega Maxwell CSC DNA FFPE kit on the Maxwell CSC Nucleic Acid Isolation System.

Targeted NGS Analysis

A preliminary assessment of the performance of a targeted panel compared to Whole Exome Sequencing (WES) for TMB assessment was conducted using the Ion Comprehensive Cancer Panel. This panel targets 400 genes and is similar in content to the OncorMx™ Tumor Mutation Load Research Assay (see below). When performing TMB analysis using the IonPlex Panel system, exome- and exons-skip (exon) single nucleotide variants (SNVs) in coding regions were included in the total count. Variants were then removed that were present with a frequency below 10%: 2) those found in dbSNP (germline mutations) and the COSMIC database; 3) those with a wild-type frequency of 0.5% or less; and 4) those that failed in inclusion using the Integrative Genomics Viewer (IGV).

Comparison of the THF workflow and the MMD workflow

The TMB was measured by counting the somatic SNVs per Mb that 1) were reproducibly detected in each duplicate sample; 2) were exonic; 3) had a mutant allele frequency above 10% and below 90%; and 4) were not found in dbSNP (germline mutations) or the COSMIC database.

Results

WES vs Targeted NGS for TMB Analysis

In a separate experiment, a set of seven FFPE samples was evaluated and TMB assigned using WES and the CCP09 target gene panel. The CCP040 panel data was analyzed using a WES filtering method (See Materials and Methods). A good correlation was observed (Figure 2), demonstrating the feasibility of using a targeted panel for reproducible TMB analysis results. Other panels provide advantages over WES when performing clinical sample analysis including lower DNA input requirements, lower cost and faster TAT.

Conclusion

We hypothesized that duplicate analysis could eliminate false positive results due to poor DNA quality. Therefore we evaluated both in duplicate library preparations while minimizing the additional cost. To demonstrate the feasibility of this approach, an in-silico analysis was first performed where SNVs of the aligned reads in the bam files generated by the IonPlex workflow were randomly removed to mimic the 50% drop in mean depth when using the MMD workflow. These "full" bam files were then re-analyzed in IGV using the same setting in the THF OncorMx Mutation load workflow, except that the "trim HBp" filter for calling a SNV was reduced from 60 to 30. The results indicate that TMB result from "full" bam file vs "full" bam file is comparable to those obtained from a "trimmed" bam file vs "trimmed" bam file with a Pearson correlation coefficient of 0.9484.

In silico TMB analysis - MMD workflow

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Materials and Methods continued

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