Evaluation of a tumor-only pan-cancer targeted semi-conductor based next-generation sequencing (NGS) test for microsatellite instability in FFPE samples

P88

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INTRODUCTION

Comprehensive genomic profiling using next-generation sequencing (NGS) has become an essential tool to support routine clinical research in oncology. Advent of cancer immunotherapies also requires assessment of immune checkpoint inhibitor biomarkers such as microsatellite instability (MSI) and tumor mutational burden (TMB).

MSI arises from defects in the mismatch repair (MMR) system and is associated with hypermutability of short DNA sequence repeats, microsatellite locations, throughout the genome. Such defects are commonly observed in colorectal, gastric and endometrial cancers and have been shown to be predictive of response to immunotherapy treatment. Traditionally MSI testing has been done using limited biomarker tests such as PCR/fragment analysis or immunohistochemistry (IHC) that require high sample input, matched tumor/normal pair and are time consuming.

We developed an NGS based Oncomine Comprehensive Assay optimized for formalin-fixed paraffin-embedded (FFPE) tissues. The assay addresses biomarkers for targeted and immune checkpoint therapies and allows the user to evaluate MSI status among multiple other capabilities.

MATERIALS AND METHODS

The performance of our RUO NGS based MSI approach was tested in the context of a large Ion AmpliSeq™ panel composed of more than 13,000 amplicons covering 500+ genes. The content includes a diverse set of microsatellite markers targeting MSI locations comprised of mono- and di-nucleotide repeats that range from 7 to 34 bp and cover different coding and non-coding regions of the human genome.

Oncomine Comprehensive Assay Plus™ (OCA Plus) uses Ion AmpliSeq™ technology and automated templating on the Ion Chef™ system. Sequencing was carried out on the Ion 550™ chip using the Ion GeneStudio™ S5 system. An automated alignment and MSI score and status reporting workflow is provided within the Ion Reporter™ Software. Streamlined access to decision support software is achieved through Oncomine Reporter™.

In-sample standards were designed and incorporated as internal references utilized by the analysis pipeline to ensure the robustness of results to any possible variations to sample prep or run conditions.

A novel algorithm was developed that leverages the unique signal processing properties inherent in semi-conductor sequencing. The test provides results for individual microsatellites and generates a total MSI score and status for the sample of interest. The system does not require a matching normal tissue.

We have evaluated the panel performance using a set of 199 unique samples including 125 FFPE samples from different tissue types such as colorectal, gastric and endometrial cancer. The FFPE samples include 24 MSI-High samples. Truth was provided by the supplying vendor for part of the samples and evaluated using orthogonal Capillary Electrophoresis based methods for the rest.

DNA samples used for evaluation were purchased from Discovery Life Sciences, BioIVT, BioChain Institute, Conversant Bio or obtained by agreements with Horizon Discovery and OmniSeq LLC.

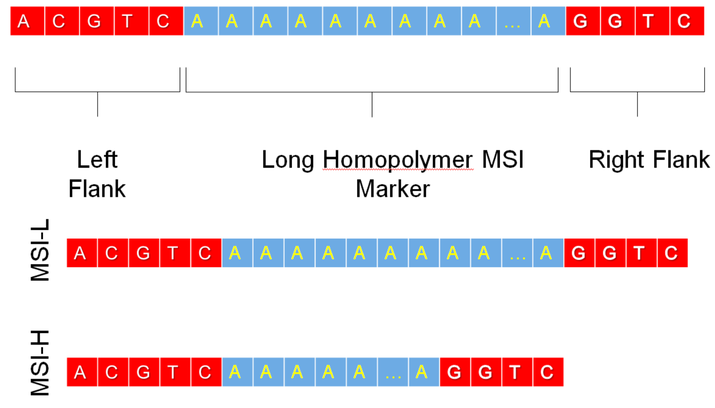

Figure 1: Identification of unstable MSI markers is achieved by identifying the change in homopolymer length

RESULTS

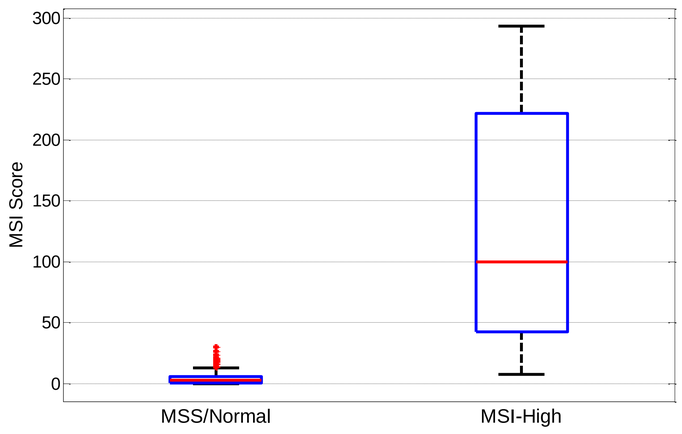

Figure 2: Distribution of generated MSI Scores for MSS/Normal and MSI-High samples. For MSI-High samples the scores are dependent on the tumor purity, tissue type and FFPE sample quality.

	FFPE	Cell line	Total
Total Samples(N)	125	74.0	199.0
True Positive (TP)	22.2	1.0	23.2
False Positives (FP)	1.2	1.0	2.2
True Negatives (TN)	99.8	72.0	171.8
False Negartive (FN)	1.8	0.0	1.8
Sensitivity	92.36%	100.00%	92.67%
Specificity	98.84%	98.63%	98.75%

Table 1: Sensitivity and specificity for MSI classification of FFPE and cell line samples.

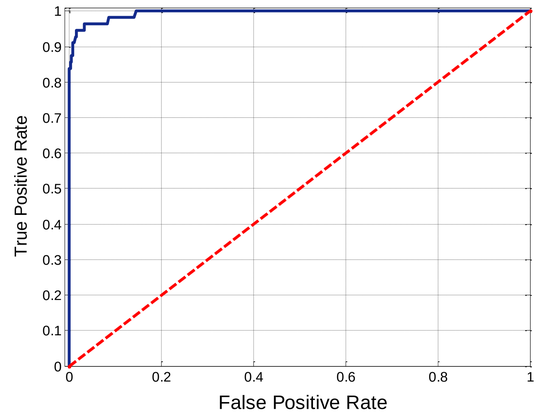

Figure 3: ROC (Receiver Operating Characteristic) curve for MSI Classification, AUC (Area under curve) is .9945

In MSI-High samples, Long homopolymers tend to become shorter, we expect a Tumor purity of at least 15% in order to accurately determine MSI score. False negative samples were determined to be of a lower tumor purity. The wide distribution in MSI Score results from significant variation in Tumor purity in addition to variation in cancer type. ROC curve analysis for MSI status classification yielded an area under the ROC curve (AUC) values of .9945 when distinguishing MSI-H from MSS/Normal status. The Assay also provides the ability to monitor variations in MMR genes and that added information can collaborate Microsatellite instability observations.

CONCLUSION

A targeted NGS assay, Oncomine Comprehensive Assay Plus ™, was developed to support a wide range of Oncomine diagnostics. Currently, the assay provides MSI, TMB and variant calling across 500 genes in addition to copy number variation analysis.
The Assay, running on the Ion 550™ chip, allows for running four samples concurrently. An associated Bioinformatics pipeline was developed to assign MSI status to tumor samples with great precision. The accuracy of the system was verified using orthogonal tests and MSI status can be assigned using tumor-only samples.

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