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Multi-Site Analytical Validation of TruSight® Tumor 15 (TST15) Determining Robustness and Concordance

Introduction

Molecular testing to select targeted and conventional therapies for cancer patients is becoming standard practice. The College of American Pathologists, International Association for the Study of Lung Cancer, and the Association for Molecular Pathology have jointly created guidelines for molecular testing of non-small cell lung cancer for EGFR mutations and ALK fusions, while an expert panel have published recommendations for analyzing the *BRAF* gene in metastatic melanoma.[1,2] In addition, the French National Cancer Institute has developed national guidelines for the implementation of genomic biomarkers for testing relevant cancer-related genes across a broad spectrum of solid tumors.[3] Moreover, the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology strongly recommend evaluation of genes such as *KRAS, NRAS*, and BRAF in colorectal cancer. The guidelines also state that mutational testing needs to adhere to best laboratory practices including performance, provide clinically appropriate turnaround times and enable optimal utilization of tissue specimens.[4]

These guidelines call for a methodology that allows for simultaneous analysis of a panel of genes known to potentially harbor actionable genomic alterations. The methodology should also account for the fact that formalin-fixed, paraffin-embedded (FFPE) tumor samples can be small, sparse and may contain degraded and cross-linked DNA.

About TruSight Tumor 15

TruSight Tumor 15 (TST15) is a targeted cancer gene panel dedicated to detecting variants by covering the implicated coding regions of 15 genes associated with solid tumors and allows researchers to explore these genes in more detail. TST15 uses next-generation sequencing (NGS) technology to provide a comprehensive assessment of the most commonly mutated genes (Table 1) in 7 cancer types (Lung, Melanoma, Breast, Colon, Ovarian, Gastric, and Prostate). It detects low-frequency variants down to a 5% minor allele frequency (MAF) and requires only 20 ng of DNA isolated from FFPE tumor tissue. TST15 allows for parallel sequencing of multiple cancer-related genes without the need to perform several sequential tests.

Objectives

The objective of this study was to test the analytical validity of TruSight Tumor 15 (TST15) by measuring:

1. Analytical robustness of TST15 when used in a broad array of samples in multiple laboratories
2. The concordance between previously detected variants and TST15 reported variants
3. The improved variant assessment by TST15, detecting previously unreported variants

Table 1. TruSight Tumor 15 (TST15) Gene Panel Content

Gene Region Content (with Target) covered by TruSight Tumor 15 with potential disease states		
AKT1 Exon 3* ; E17K Breast	**GNA11** Exon 5*; Q209L Melanoma	**NRAS** Exons 2* , 3* (partial), 4 Codons 12, 13, 59, 61, 117, 146 Colon
BRAF Exon 15* (partial); V600E/K/R/M Melanoma, Colon, Lung	**GNAQ** Exon 5* (partial); Q209L Melanoma	**PDGFRA** Exons 12, 14, 18 Gastric, Melanoma
EGFR Focal Amplification, Exons 12* (partial), 18, 19, 20; G719A, G719X; Exon 21 (L858R), L861Q, S7681, T790M Lung	**KIT** Exons 8, 9, 10, 11, 13, 14, 17, 18 Gastric, Melanoma	**PIK3CA** Exons 9, 20 Lung, Breast, Prostate
ERBB2 Focal Amplification, p.E770_A771insAYVM Exons 14* (partial), 17, 18, 19, 20* (partial), 21* (partial), 24, 26 Breast, Lung	**KRAS** Exon 2* (partial), 3* (partial), 4 Colon, Gastric, Lung	**RET** Exon 16 (M918T) Lung
FOXL2 Exon 1* (partial); C134W Ovary	**MET** Focal Amplification Lung, Colon, Gastric	**TP53** Full coding sequence Lung, Melanoma, Ovary, Colon

* = Coverage of these exons is only partial and targets specific hotspots.

Samples and Methods

Seven labs from Europe participated in the study:

- Genolytic GmbH, Leipzig, Germany
- State Hospital Mistelbach, Austria
- Paul Brousse hospital, Villejuif, France
- GD Hospital, Luxembourg
- Provincia di Chieti Hospital, Italy
- Hospital Clinico San Carlos, Madrid, Spain
- Aarhus University Hospital, Denmark

Each lab selected 8 unique FFPE samples from numerous tumor types that had previously been characterized by either Sanger sequencing, pyrosequencing, quantitative polymerase chain reaction (qPCR), or other NGS panels. FFPE sections and DNA extraction were performed in accordance with the TruSight Tumor 15 workflow instructions, which recommend tissue sections of at least 140mm2 thickness containing a minimum of 30% tumor cells. The TruSight Tumor 15 kit uses an amplicon-based, multiplexed PCR approach to generate libraries. To avoid primer dimers, 2 distinct primer pools are prepared and sequenced as separate libraries.

As part of the workflow, eight samples are pooled to run on a MiSeq® System with a 2 × 150 bp sequence run configuration.

The TST15 sample report includes the following: For each identified variant, the amino acid change, the variant type (SNVs, Insertions, and Deletions), the nucleotide change, variant frequency, and transcript. The report also specifies “no calls” for indeterminate results.

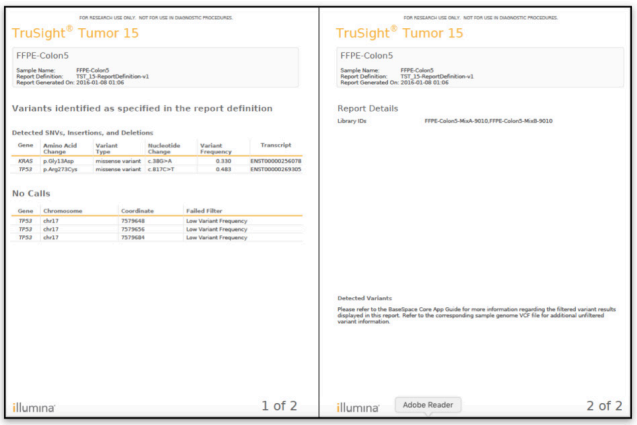

Figure 1: Sample Report

Results

From the 7 participating labs, a total of 115 sample reports were submitted, of which 16 were excluded from the current analysis, as they were negative controls. The 99 remaining samples comprised 6 tumor types (colon, melanoma, lung, bladder, GIST, and breast) with 119 established gene variants (Table 2).

1. Analytical robustness

All 99 samples were analyzed with TST15 in the local participating labs and results were generated. The average DNA concentration was 40.3 ng/microliter (range 1-282, unreported for 19 samples) and the average reported tumor cell percentage was over 50% (range 20-100%). When DNA quality was measured by qPCR, the deltaCq values ranged from 3.2 to 7.6. Previous methodology for sequencing included other NGS panels (16 samples), PCR (33 samples), Pyrosequencing (16 samples), and ‘other’ (34 samples).

2. Concordance

Comparing TST15 NGS results with the results of the previous characterization used by the labs showed a concordance rate of 100% (119 out of 119 gene variants).

Table 2. TST15 assessment of reported variants; Twenty samples were submitted with 2 variants (“second reported variant”)

Gene with reported variant	Number of variants	Second reported variant	TST15 concordant	Tumor type(s)
Total	**n=119**	**n=20**	**139/139**	
BRAF	13	0	13/13	Colon n=5 Melanoma n=5 Not provided n=3
EGFR	23	0	23/23	Lung n=23
PI3KCA	14	9	14/14 9/9	Colon n=11 Bladder n=2 Lung, n=1
GNAQ	1	0	1/1	Melanoma (uveal)
ERBB2	1	0	1/1	Lung
KIT	7	0	1/1	GIST
KRAS	34	0	34*/34	Colon n=31 Lung n=3
NRAS	13	1	13/13 1/1	Colon n=11 Melanoma n=1 Lung n=1
PDGFRA	3	1	3/3 1/1	GIST
TP53	10	9	10/10 9/9	Colon n=6 Lung n=2 Breast n=1 Bladder n=1

* One sample is a colon cancer sample with a K117N KRAS variant as originally reported by pyrosequencing. TST15 reported a different variant, K117Y. After careful re-examination of the original pyrosequencing results, the TST15 variant calling turned out to be correct.

3. Variant detection rate improvement by TST15

TST15 identified an additional 42 variants that were previously unreported (Table 3). Out of the 33 samples, 12 were assessed using methodology defined as "other", 13 with PCR, 7 with pyrosequencing, and 1 with Sanger technology.

Table 3. Newly identified variants by TST15 (samples can overlap)

Gene variant	Number of variants	Tumor type (s)
TP53	31 in 24 samples	colon, lung, unknown
PIK3CA	6	colon, lung
KRAS	3	colon
AKT	1	colon
PDGFRA	1	GIST

Potential relevance of the newly identified variants by TST15

The most common gene in which previously undetected variants were reported was TP53. Thirty-one TP53 mutations were detected in 24 subjects (Table 3) with the most common mutations affecting the arginine 175 residue (R175H) and the arginine 273 residue (R273C). Single TP53 variants (besides the "primary" mutation for which the sample was originally submitted) were present in 18 samples. Three tumor samples were assessed to have 2 variants, and one sample was found to have five TP53 alterations. TheTP53 tumor suppressor gene is commonly mutated in many types of cancer and drug development activities are underway to address it.

A previously undetected *KRAS* variant was assessed in one colon cancer sample, affecting the alanine 146 residue. *KRAS* mutations are well recognized negative predictive biomarkers since they indicate a lack of response to anti-EGFR antibodies in metastatic colorectal cancer.[3] Two additional KRAS variants were found in two samples: One colon sample had a KRAS variant affecting the lysine 117 residue (K117Y) as well as a *PIK3CA* variant (E545K). Another colon sample had a variant affecting the 12 glycine residue (G12D), an AKT1 variant affecting the glutamic acid 17 residue (E17K) and a single TP53 mutation. Even though preclinical studies have repeatedly demonstrated increased AKT activation as a result of genomic alteration of upstream mediators, its role in predicting drug sensitivity to PI3K, mTOR and AKT inhibitors is less clear.

One tumor sample was found to bear a *PDGFRA* variant affecting the valine 658 residue (V658A). This variant has been reported as likely pathogenic for certain types of cancer, however no clinical application is known to date [5].

In *PIK3CA*, 8 previously undetected variants were found, most commonly affecting the glutamic acid 545 residue (E545K). This variant was found in 6 colon cancer samples, of which one is the earlier described sample with a co-occurring *KRAS* variant. The E545K variant was also found in a lung cancer sample along with a *TP53* variant. Activating mutations of *PI3KCA* are considered oncogenic in a variety of cancer types. The location and type of PIK3CA mutations appear to have some association with specific disease etiology, where different mutations are enriched in specific cancer subtypes [6,7].

Summary

This study showed excellent analytical robustness of TST15 used in 7 laboratories from participating hospitals throughout Europe. The concordance was 100% between previously measured variants and TST15 reported variants. Importantly, TST15 shows improved variant detection with the identification of an additional 42 previously unreported variants. For some of these variants, clinical significance has been reported [5,6,7] and may eventually help with the development of alternative therapeutic approaches. For other variants, our findings may help in understanding the molecular landscape of cancers and contribute to the worldwide ongoing effort to determine whether certain variants are potentially actionable variants.

This study shows how TST15 provides high sensitivity even when used in and by local labs. The system provides guidelines for sample input, including tissue requirements and DNA extraction methods. TST15 may be considered a streamlined NGS technology assay with a standardized workflow describing pre-analytic, analytic, and post-analytic processes.

With the continuing discovery of new cancer biomarkers, researchers will need to implement more comprehensive molecular testing methodologies. Sequential single gene assays are elaborate, use inappropriately large amounts of scarce tissue samples and are less cost effective. Methods such as NGS provide simultaneous information on multiple genes with high quality performance, appropriate turnaround times, and optimal use of tissue specimens.

Learn More

For more information about targeted sequencing with solid tumors, visit www.illumina.com/OncologyPanel.

References

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