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Single-Center Experience with a Targeted Next Generation Sequencing Assay for Assessment of Relevant Somatic Alterations in Solid Tumors

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Abstract

Companion diagnostics rely on genomic testing of molecular alterations to enable effective cancer treatment. Here we report the clinical application and validation of the Oncomine Focus Assay (OFA), an integrated, commercially available next-generation sequencing (NGS) assay for the rapid and simultaneous detection of single nucleotide variants, short insertions and deletions, copy number variations, and gene rearrangements in 52 cancer genes with therapeutic relevance. Two independent patient cohorts were investigated to define the workflow, turnaround times, feasibility, and reliability of OFA targeted sequencing in clinical application and using archival material. Cohort I consisted of 59 diagnostic clinical samples from the daily routine submitted for molecular testing over a 4-month time period. Cohort II consisted of 39 archival melanoma samples that were up to 15 years old. Libraries were prepared from isolated nucleic acids and sequenced on the Ion Torrent PGM sequencer. Sequencing datasets were analyzed using the Ion Reporter software. Genomic alterations were identified and validated by orthogonal conventional assays including pyrosequencing and immunohistochemistry. Sequencing results of both cohorts, including archival formalin-fixed, paraffin-embedded material stored up to 15 years, were consistent with published variant frequencies. A concordance of 100% between established assays and OFA targeted NGS was observed. The OFA workflow enabled a turnaround of 3½ days. Taken together, OFA was found to be a convenient tool for fast, reliable, broadly applicable and cost-effective targeted NGS of tumor samples in routine diagnostics. Thus, OFA has strong potential to become an important asset for precision oncology.

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Introduction

Next-generation sequencing (NGS) is an emerging technology for molecular diagnostics. It enables the parallel identification of multiple genomic variants even from small tissue samples [1,2]. Scalable and cost-effective NGS solutions to reliably identify therapeutically relevant genomic driver alterations of tumors are the prerequisite for precision oncology. However, implementing multiplexed and comprehensive NGS assays into the clinical routine is challenging because data analysis and interpretation require specialty infrastructure and expertise [3]. In addition, most established routine tests cannot assess somatic copy number variations (SCNVs) and/or gene fusions, which presently guide treatment selection for several common cancers [4]. To make precision medicine approaches

Abbreviations: CLPv2, Ion AmpliSeq Colon and Lung Cancer Research Panel v2; CRC, colorectal cancer; DNA, deoxyribonucleic acid; FFPE, formalin-fixed, paraffin-embedded; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NSCLC, non–small cell lung cancer; NSCLC (adeno), non–small cell lung cancer, adenocarcinoma; NSCLC (squamous), non–small cell lung cancer, squamous cell carcinoma; NSCLC (pleo), non–small cell lung cancer, pleomorphic carcinoma; NSCLC (NOS), non–small cell lung cancer, not otherwise specified; NGS, next generation sequencing; OFA, Oncomine Focus Assay; pT, tumor stage as determined by pathological assessment; RNA, ribonucleic acid; SSM, superficially spreading melanoma; SCNV, somatic copy number variation

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available for all cancer patients, there is a need for fast, reliable, and cost-effective NGS systems that can detect all classes of currently clinically relevant genomic targets from routine formalin-fixed,paraffin-embedded (FFPE) tissues [5–7]. To address these challenges, targeted NGS solutions have been developed to identify recurrently altered oncogenes as well as tumor suppressors, genes with frequent high-level amplifications or deletions, and driving gene fusions in a variety of cancers [8]. However, this emerging approach has so far not been sufficiently evaluated on routine diagnostic FFPE material in terms of feasibility, reliability, cost, and capacity [6,9,10].

The Oncomine Focus Assay (OFA, Thermo Fisher Scientific, San Francisco, CA) is a targeted, multibiomarker NGS assay that enables fast simultaneous detection of hundreds of variants across 52 genes relevant to solid tumors [8,11]. These variants are treatable by on-market oncology drugs approved by the U.S. Food and Drug Administration as well as drugs that are part of the National Comprehensive Cancer Network guidelines or are currently listed in clinical trials [8,11]. The assay analyzes clinically relevant gene alterations including single nucleotide variants, short insertions and deletions, SCNVs, and gene fusions from DNA and RNA in a single workflow. It enables the detection of tumor-specific genomic alterations using low-input FFPE samples such as needle biopsies and fine needle aspirates and is compatible with benchtop Ion Torrent sequencers. The power of the OFA technology for identification of genetic alterations is underlined by the present application in the nationwide NCI–Molecular Analysis for Therapy Choice Trial [12]. Here we evaluated the performance and applicability of this novel targeted NGS assay for transfer into daily diagnostic practice.

Table 1. Demographic and Histopathological Features of the Prospective Clinical Samples (Cohort I)

Characteristic	*n* = 51	%
Gender		
Male	28	54.9
Female	23	45.1
Age		
Median	67	–
Range	31-85	–
Tumor entities (*n* = 51)		
Colorectal cancer	20	39.2
Non–small cell lung cancer	19	27.3
Thyroid cancer	3	5.9
Melanoma	2	3.9
Pancreatic cancer	2	3.9
Breast cancer	2	3.9
GIST	1	2.0
Nonmelanoma skin cancer	1	2.0
Erdheim Chester disease	1	2.0
Non–small cell lung cancer (*n* = 19)		
Adenocarcinoma	15	78.9
Squamous cell carcinoma	1	5.3
Pleomorphic carcinoma	1	5.3
Combined	1	5.3
NOS	1	5.3
Primary tumor/metastasis		
Primary tumor only	32	62.7
Metastasis only	13	25.5
Matched primary and metastasis	3	5.9
Two different primary tumors †	3	5.9
Nucleic acid analysis		
DNA and RNA	39	76.5
DNA only	12	23.5
Tumor cell content		
10%-30%	7	13.7
31%-70%	15	29.4
>70%	29	56.9

Combined small cell carcinoma with adenocarcinoma and sarcoma components.

† Two different, synchronous or metachronous primary tumors or different manifestations of the disease (for Erdheim Chester disease case) were analyzed.

Materials and Methods

Study Cohorts and Patient Selection

This study was carried out in accordance with the guidelines of the Cantonal Ethics Committee Basel (KEK-EKBBno. 326-12, 2016-01134, and 2016-01499). Cohort I consisted of 59 diagnostic FFPE tissue samples from 51 consecutive patients that were analyzed in the diagnostic routing during a 4-month time period (March-June 2016). All patients had a clinical indication for molecular testing and were informed about the purpose of the molecular analysis by the treating physician. The demographic and histopathological features of these prospectively collected samples are listed in Table 1. Cohort II consisted of 39 archival FFPE tissue samples from 39 patients with cutaneous malignant melanoma. Tissue blocks were up to 15 years old. Median follow-up was 19.5 months (range 0-62 months) for cases with lymph node or distant metastasis and 27 months (range 12-144 months) for cases without metastases. Patients in this cohort were characterized and described in a previous study (cohort 1 with 57 patients in Garg et al.). [13] Cases with insufficient material remaining on the tissue block were excluded from molecular analysis (*n* = 18). The clinicopathological features of cohort II are provided in Table 2.

Genomic Profiling of Samples by Targeted NGS

All samples in this study were analyzed using the commercially available OFA platform. The genes targeted in this panel are carefully selected biomarkers derived from expertly curated cancer genomics data [8]. The assay analyzes a maximum of six parallel samples per run for DNA and RNA. It can be used on FFPE samples (10 ng DNA and 10 ng DNase-treated RNA per reaction) and is compatible with benchtop Ion Torrent sequencers (Ion Personal Genome Machine, Ion Proton System, Ion S5 System, Thermo Fisher Scientific).

The percentage of tumor cells relative to other cells (e.g., stromal, inflammatory, and preexisting epithelial cells) was estimated on one hematoxylin and eosin (H&E) stained tumor section by a

Table 2. Demographic and Histopathological Features of 39 Archival Cutaneous Melanoma Samples (Cohort II)

Characteristic	*n* = 39	%
Gender		
Male	24	61.5
Female	15	38.5
Age		
Median	70	–
Range	31-91	–
Melanoma types		
Superficial spreading melanoma	24	61.5
Nodular melanoma	15	38.5
Metastases		
Melanoma with metastases	12	30.8
Melanoma without metastases	27	69.2
Tumor cell content		
10%-30%	9	23.1
31%-70%	7	17.9
>70%	23	59.0

The cohort consisted of cases with lymph node and/or distant metastases detected at the time of first diagnosis or during a median follow-up of 19.5 months (range 0-62 months) and of cases with no evidence of no lymph node and/or distant metastases at the time of first diagnosis or during a median follow-up of 27 months (range 12-144 months).