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Comprehensive Genomic Profiling of solid tumor patients with the OncoDEEP assay for broad analysis in clinical diagnostics

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Introduction

With the fast-growing number of recommended and required genomic biomarkers small gene panels have become vastly insufficient for most tumor types. Comprehensive Genomic Profiling (CGP) is amenable to screen for subtle nucleotide variants (SNVs and indels) in several hundred of cancer-related genes. Moreover, CGP can provide information on copy number variations (CNVs), gene fusions and tumor-agnostic genomic biomarkers including microsatellite instability (MSI), tumor mutation burden (TMB) and homologous recombination deficiency (HRD) for optimal clinical patient management with diagnostic, prognostic and therapeutic value in a wide variety of solid tumors. Only few CGP panels have been diagnostically validated in the clinic. Here, we report on an extensive multicentric comparative analysis of the novel CGP assay OncoDEEP from OncoDNA, with the diagnostically validated TSO500 assay (Illumina) [1].

Table 1. Comparison of the number of genes for variant calling and the ability of biomarker detection for the TSO500 and OncoDEEP assays

Detection at DNA level		
	TSO500	**OncoDEEP**
Total size	1.9 Mb	1.8 Mb
	# genes	
SNVs and indels	523	638
CNV	59 *(514 [&])*	614
LOH	0 *(514 [&] *)*	41
	pan-tumor biomarkers	
MSI	Yes	Yes
TMB	Yes	Yes
HRD	Yes*	Yes

[&] in current kit using DRAGEN analysis

** in current kit as an add-on to the assay*

Detection at RNA level		
	TSO500	**OncoDEEP**
	# driver genes	
Fusions	55	11 *(13°)*
Splice variants	3	9

° in current kit

Materials and Methods

Both assays were performed as described in the user guides.
In total, 234 diagnostic DNA and RNA samples with known TSO500 data were analyzed with the OncoDEEP assay. In addition, reference DNA and RNA samples resp., were analysed for exon skipping and gene fusion detection by most laboratories. The diagnostic samples included more than 20 tumor types, representative of the real life situation in the NGS diagnostic centers. Pooled libraries of both assays were sequenced on a NextSeq500/550 or NovaSeq6000 instrument (Illumina). The major differences between both assays are listed in Table 2.

Table 2. Comparison of TSO500 and OncoDEEP assay features

	TSO500 (Illumina)	**OncoDEEP (OncoDNA)**
	Pre-analytics	
Recommended input	DNA: 80 ng *(40 ng)* RNA: 40 ng	DNA: 100 ng *(40 ng)* RNA: 200 ng dried *(80 ng)*
	Library prep	
DNA Fragmentation method	Shearing	Enzymatic
Use of UMIs	Yes	No
Normalisation	With beads	Quantification and dilution
	Hybridization capture	
Pooling before hyb	No	Yes (8 samples)
# Hybridization steps	2	1
	Sequencing on a NextSeq550	
Read length	101 bp	74 bp
#Samples per run	8; DNA + RNA	24; DNA + RNA
Flowcell NextSeq550Dx	HO v2.5 -300 cycles	HO v2.5 -150 cycles
	Data analysis	
Secundary analysis	TSO500 local app *(DRAGEN)*	OncoKDM
Tertiary analysis	*(ICI as an add-on)*	OncoKDM
	Hands-on-time and cost	
Hands-on-time	5 h	4 h
Cost/sample	€€€	€€

Italic text between brackets indicate the current changes.

Results

General comments OncoDEEP:

. The mean **coverage** of the samples is **more uniform** (**Figure 1**) thereby allowing to pool 2- to 3-times more samples per seq run.

. More samples failed the sequencing **QC metrics** (mean coverage, uniformity of coverage).

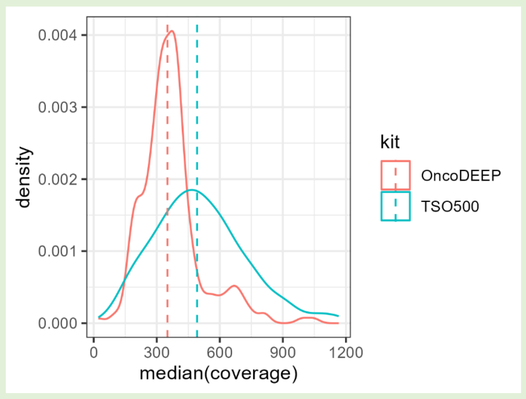

Figure 1. *Distribution of the median coverage for TSO500 (blue) and OncoDEEP (red) (SD 217 vs 145) showing the higher capture uniformity*

Variant detection with OncoDEEP:

90% concordance for **SNP** and **indel** detection (**Figure 2A**)
with high correlation of VAFs (R^2 = 0,9371)

Missed variants (**Figure 2B**) were due to:

- *VAF did not reach the 5% threshold (7%)*
- *Insufficient coverage (<80) at the variant position (52%)*
- *Insufficient number (<20) of variant reads (32%)*
- *Unknown reason based on Bam file (9%)*

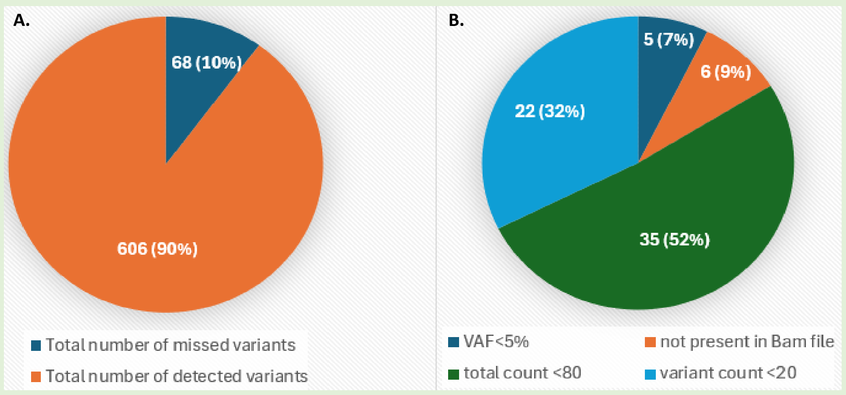

Figure 2. A. *Of the 676 TSO500 (Likely) Pathogenic SNV and indel variants 90% were also detected with the OncoDEEP assay (orange).* ***B.*** *Most (84%) missed variants were due to insufficient coverage at the variant position.*

Amplifications (33 with fold change >6) were all concordant
LOH *could not be assessed (was not yet validated for TSO500)*

Gene **fusions** (43) and **exon skipping** (11) events were concordant in 47 cases (87%). Reasons for discordance were:

- 3 cases: the reciprocal fusion was detected
- *1 case: MYO18A::ROS1 while this was MYO18A::GOPC in TSO500*
- *3 cases: the reason was unknown*

Pan-cancer **biomarkers**:

- Concordance of **MSI** (162 samples) was 98.8% (**Figure 3**)
- Concordance of **TMB** (175 samples) was 94.9% (**Figure 4**)
- Concordance of **HRD** (22 samples) was 90,9% (more samples required)
  - *Discordant calls for biomarkers mostly had values close to the thresholds*

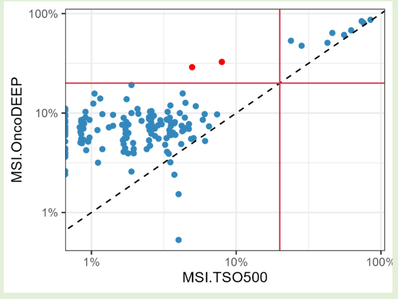

Figure 3. *MSI ratio plot (log) for samples analyzed with TSO500 and OncoDEEP. Only 2 discordant calls (red) were present.*

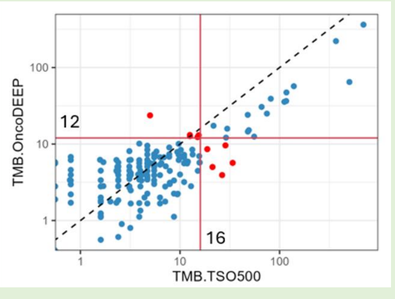

Figure 4. *TMB values (log) for TSO500 (Thr 16 mut/Mb) and OncoDEEP (Thr 12 mut/Mb revealed 9 discordant calls (red).*

Conclusions OncoDEEP CGP assay

- Economic targeting capture provides a uniform selection of the targeted regions in a single hybridisation step.
- Pooling of 24 samples for sequencing can result in insufficient coverage of low quality samples.
- The assay includes variant classification and interpretation via OncoKDM, which also generates the reports.
- The OncoDEEP assay can efficiently detect somatic variants and CNVs in a broad range of tumor tissue types.
- Gene fusion detection is efficient but is curently only possible for 13 diagnostic driver genes.
- Pan-cancer biomarker analysis is highly concordant but values close to the thresholds can result in a discordant call.
- Successful analytical validation for precision, sensitivity, specificity, limit-of-detection and input amount, was performed.
- The OncoDEEP assay can reliably be implemented in clinical cancer diagnostics.

Reference

[1] Froyen et al. Diagnostic Validation of a Comprehensive Targeted Panel (TSO500) for Broad Mutational and Biomarker Analysis in Solid Tumors. Cancers 2022

AMP Association for Molecular Pathology