Development a Customized Panel with Clinical Application Potential for Transcriptome Analyses in Selected Cancer types using FFPE Specimens

Abstract No 2107

Vittorio D'Urso[1], Dorothee Foernzler[1#], Dennis O'Rourke[2], Danyi Wang[2], Jorge F Sanchez-Garcia[2], Heidi Giese[2], Thomas Clarke IV[4], XiaoZhe (Janet) Wang[3], Andreas Machl[3], Ti Cai[3], Juergen Scheuenpflug[5], Zheng Feng[2*]

1 Clinical Genomics, Clinical Biomarkers & Technologies, Clinical Measurement Sciences, Global Research & Development, Merck KGaA, Darmstadt, Germany
2 Clinical Genomics, Clinical Biomarkers & Technologies, Clinical Measurement Sciences, Global Research & Development, EMD Serono, Billerica, MA, USA, A business of Merck KGaA, Darmstadt, Germany
3 Companion Diagnostics & Biomarker Strategies, Clinical Measurement Sciences, Global Research & Development, EMD Serono, Billerica, MA, USA, A business of Merck KGaA, Darmstadt, Germany
4 Data Science, Clinical Measurement Sciences, Global Research & Development, EMD Serono, Billerica, MA, USA, A business of Merck KGaA, Darmstadt, Germany
5 Clinical Measurement Sciences, Global Research & Development, Merck KGaA, Darmstadt, Germany

INTRODUCTION

Tumor transcriptome profiling has conquered an important place in biomarker development for cancer diagnostics, prognosis and therapeutic selection due to the fact that RNA expression plays an essential role in numerous tumor biology processes and that RNA expression of thousands of genes can be monitored in parallel in a variety of clinical samples. Over the last years, RNA-sequencing (RNA-seq) has established itself as the gold standard for quantitative transcriptional profiling. Due to its high analytical sensitivity and wide dynamic range, even small variations in gene expression can be detected. HTG EdgeSeq is a Next Generation Sequencing (NGS) based RNA Sequencing (RNA Seq) platform using a quantitative nuclease protection chemistry that enables extraction-free quantitation of mRNA/miRNAs with significantly reduced FFPE sample requirements compared to standard RNA-Seq, which makes it an attractive option for potential clinical applications. A customized gene panel was designed and developed for detecting gene signatures.

METHODS

The customized gene panel consists of 1327 genes, including 900 genes in signaling pathways in oncology & immuno-oncology, including Nature Kill (NK) cells, neutrophils, Plasmacytoid dendritic cells (pDCs), DCs, monocytes, Tregs, B cells, tumor associated macrophages (TAMs), TGFβ, EMT, interferon-γ signature, IL-17, β-catenin among others. and 15 house-keeping genes. The custom panel was evaluated using in silico probe design and feasibility testing. Validation studies included data comparison with The Cancer Genome Atlas (TCGA), reproducibility and repeatability testing and precision studies in six cancer indications: Non-Small-Cell Lung Cancer (NSCLC), Head and Neck Squamous Cell Carcinoma (HNSCC), Hepatocellular Carcinoma (HCC), Colorectal Cancer (CRC), gastric cancer (OC), ovarian cancer (GC)

RESULTS

(A)

NSCLC, Sample 1	Run1: Inst1, day1			Run2: Inst2, day1			Run3: Inst1, day2			Run4: Inst2, day2		
Sample Input [mm^2]	25	12.5	6.25	25	12.5	6.25	25	12.5	6.25	25	12.5	6.25
3.125	0.7881	0.7925	0.8031	0.8413	0.8265	0.8258	0.7874	0.8316	0.8283	0.9001	0.9042	0.9025
6.25	0.9666	0.9789		0.9633	0.9197		0.9087	0.9531		0.9691	0.9735	
12.5	0.9817			0.9647			0.9417			0.9846		

NSCLC, Sample 2	Run1: Inst1, day1			Run2: Inst2, day1			Run3: Inst1, day2			Run4: Inst2, day2		
Sample Input [mm^2]	25	12.5	6.25	25	12.5	6.25	25	12.5	6.25	25	12.5	6.25
3.125	0.9429	0.9515	0.9481	0.8863	0.899	0.9156	0.8929	0.9092	0.9018	0.7146	0.7239	0.7426
6.25	0.9747	0.9754		0.9635	0.9683		0.9592	0.9636		0.9598	0.9605	
12.5	0.9845			0.984			0.9762			0.9837		

(B)

NSCLC FFPE Sample 1, (R^2 values)						
Sample Input	Run 1 vs 2 2 processors	Run 1 vs 3 2 days	Run 1 vs 4 2 processors+days	Run 2 vs 3 2 processors+days	Run 2 vs 4 2 days	Run 3 vs 4 2 processors
25mm^2	0.992	0.9547	0.9779	0.9631	0.9775	0.9385
12.5mm^2	0.9296	0.9736	0.9788	0.9351	0.9309	0.9828
6.25mm^2	0.9769	0.9447	0.9679	0.9353	0.9601	0.9539
3.125mm^2	0.7504	0.7204	0.7763	0.7697	0.8173	0.8042
NSCLC FFPE Sample 2, (R^2 values)						
25mm^2	0.9899	0.9868	0.9872	0.9837	0.9854	0.9884
12.5mm^2	0.9852	0.9772	0.9796	0.9791	0.9805	0.9767
6.25mm^2	0.9581	0.9525	0.9575	0.9583	0.9618	0.9576
3.125mm^2	0.9059	0.8878	0.7356	0.8705	0.7297	0.7236

Figure 1. HTG EdgeSeq Performance Evaluation

(A) . FFPE sample input levels between 25 and 3.125 mm2 for two NSCLC samples were tested using the HTGEdge Seq OBP in four HTGEdge Seq runs (2 HTGEdge processors on 2 days). Normalized CPM counts generated from the HTG EdgeSeq data analysis were plotted to calculate R2 values to evaluate concordance between sample inputs. Good concordance indicated by R2 values close to 1 (highlighted in green) was observed for all runs for sample input levels of 25, 12.5 and 6.25 mm2.

(B) Two (2) FFPE NSCLC tissue samples were tested using the HTG Edge Seq OBP in four different HTGEdge Seq runs performed on two (processors on two days. Sample input levels were between 25 and 3.125 mm2. R2 values above 0.9000 (highlighted in green) indicate good concordance and Day to Day and Processor to Processor reproducibility for sample inputs at or above 6.25 mm2.

RESULTS

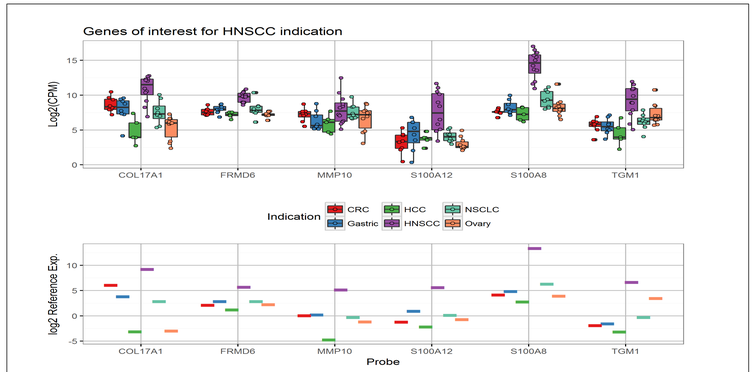

Figure 2. Comparison of custom panel gene expression to TCGA expression data- HNSCC indication .

Twelve (12) HNSCC FFPE tissue samples (12 mm2 sample input) were analyzed in triplicate with the HTG custom panel. Log2(CPM) expression values for HNSCC are shown here. All probes performed as expected and according to specification; gene expression signatures were consistent with the expected signatures for the different cancer indication tested. Expected differential expression of the selected genes was based on RNA-Seq data as reported in TCGA.

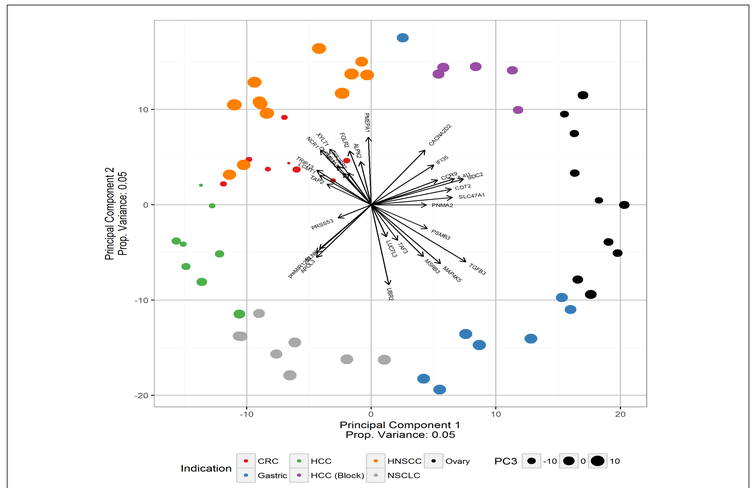

Figure 3. Principal Component Analysis (PCA) showing clustering of samples by indication

A PCA and sparse PCA were performed using all HTG EdgeSeq data. The colored spots in the covariance plot represent the first 3 principle components from the PCA. The arrows were derived from the sPCA and 30 non-zero probes

RESULTS

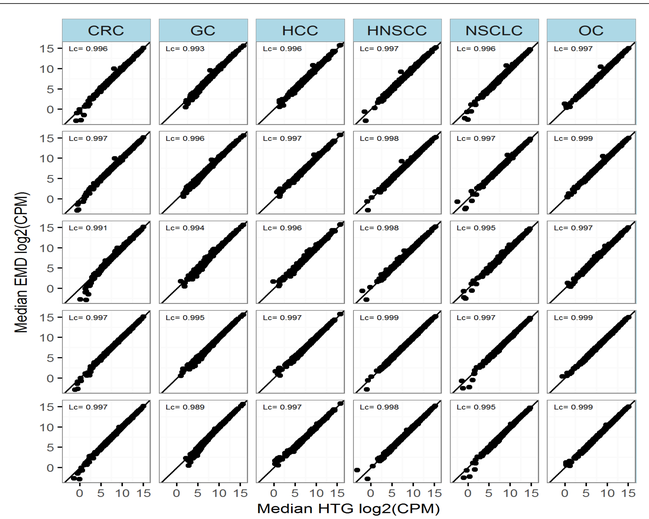

Figure 4. Inter-site Repeatability – Summary of Lin's CCC values for all inter-site correlation

The same sixteen (16) replicate FFPE lysate samples from each of six (6) cancer indications were processed under identical conditions at HTG Molecular Diagnostics and EMD Serono. LCCC was calculated comparing the median log2(CPM) values per indication obtained at EMD Serono to the median values from all replicates per indication from all 5 plates processed at HTG Molecular Diagnostics.

CONCLUSIONS

- **The designed panel met all the predefined quality and analytical acceptance criteria.**
- **The here reported results correlated with TCGA RNA-Seq data, and the validation and precision studies confirmed the excellent performance of the pane.**
- **This customized gene panel may be a powerful tool for predictive and prognostic biomarker identification to inform the development of new cancer therapies.**

REFERENCES

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ACKNOWLEDGEMENTS AND DISCLOSURES

This research was supported financially by Merck KGaA, Darmstadt, Germany. VD, DF and JS are employees of Merck KGaA, Darmstadt, Germany. DO, DW, JFS-G, HG, TC, JW, AM, TC and ZF are employees of EMD Serono Research and Development Institute, Inc., Billerica, MA, USA, A business of Merck KGaA, Darmstadt, Germany.