3983. A Cross Comparison of Technologies for the Detection of Immune System Related Gene Expression Signatures in Clinical FFPE Samples of Metastatic Prostate Cancer Patients

Kimberly Kruse[1], Fang Yin Lo[1], Ryan Fleming[1], Douglas Chung[1], Vickie Satele[1], Lindsey Maassel[1], Tuuli Saloranta[1], Inah Golez[1], Saman Tahir[1], Sally Dow[1], Evan Anderson[1], Spencer Chee[1], Raghavee Venkatramanan[1], Steven Anderson[2], Peter S. Nelson[3], Colm Morrissey[4], Anup Madan[1], Sharon Austin[1] and Kellie Howard[1]

[1]Covance, Redmond, WA; [2]Covance, Durham, NC; [3]Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA; [4]Department of Urology, University of Washington, Seattle, WA

Introduction

The success of immunotherapy for the treatment of metastatic cancers relies on the prediction and identification of potential neo-antigens. In recent years expression levels of these neo-antigens along with other immune system related genes have been evaluated in an effort to better understand response rates for immunotherapy in various cancers. Gene expression levels can be assessed by numerous techniques including hybridization-based or direct sequencing technologies. Two platforms-HTG Molecular and NanoString nCounter have been utilized to profile changes in gene expression and offer unique advantages for analyzing challenging specimens such as formalin-fixed paraffin embedded (FFPE) tissues. The NanoString nCounter platform utilizes hybridized fluorescent probes targeted against genes of interest for a non-amplified measurement of gene expression. Several studies have been shown that the NanoString platform has good sensitivity, specificity and reproducibility for the assessment of gene expression levels from FFPE samples. The HTG platform is relatively new and also uses a hybridization based method to enrich genes of interest without first isolating RNA. To determine the robustness of the HTG platform, we profiled a set of 30 metastatic prostate cancer samples using the HTG Molecular EdgeSeq Immuno-Oncology Assay. In these experiments, we found that expression data obtained by using both extracted RNA and lysate from FFPE slides was highly reproducible (Spearman coefficient > 0.85). In addition, the expression profile of targeted genes obtained by using different slides from the same blocks was also highly correlated (Spearman coefficient > 0.90). Our experiments also showed a high correlation between gene expressions profiles obtained by HTG, the NanoString PanCancer Immune Profiling panel and RNA-Seq from the same set of 30 metastatic prostate cancer samples. Further analysis to evaluate and compare the sensitivity of different platforms is being performed and results of these will be presented.

Experimental Methods

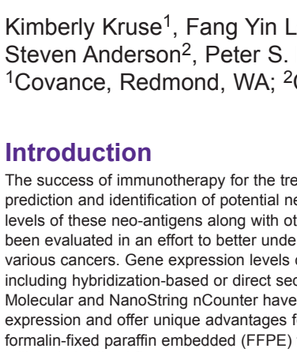


Figure 1. Alternate technologies for immune profiling: Nanostring PanCancer Immune Profiling Panel.

The Immuno-Oncology assay is comprised of 549 genes implicated in the host immune response to tumors. Applications include:

- Characterizing immune response in tumors
- Measuring total T-cell load
- Evaluating drug targets
- Monitoring patient response to drug treatment
- Profiling tumor infiltrating cells (TILs)
- Assessing tumor microenvironment
- Evaluating prognostic drug signatures

Figure 2. Alternate technologies for immune profiling: HTG EdgeSeq Immuno-Oncology Assay.

Metastatic Site	Number
Abdominal LN	1
Adrenal	2
Hilar LN	3
Independent met L Lung	1
L retroperitoneal LN	1
Liver	10
Lung	4
Mediastinal LN	1
Nodule Met	1
Periaortic LN	1
Periaortic Mass	2
R Iliac Pelvic	1
Spleen	2

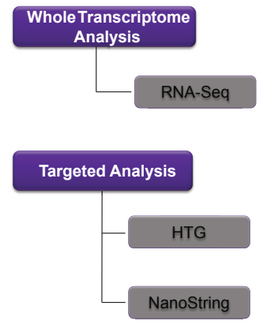


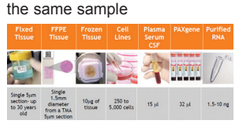
Figure 3. Investigating the transcriptome of metastatic prostate cancer samples workflow.

Sample	Slides			RNA		
	Run1	Run 2	Run 3	Run1	Run 2	Run 3
13-042_L1_Hilar LN			SC-848819		US-1613801	
13-042_H2_Liver			SC-848825		US-1613836	
12-021_H3_Liver			SC-848827		US-1613918	
13-084_H6_Liver			SC-848831		US-1613799	
14-039_E2_Lung			SC-848833		US-1613806	
13-042_M1_R Iliac Pelvic			SC-848834	US-1613849		
14-053_H7_Liver			SC-848836	US-1613833		
14-105_H4_Liver			SC-848838		US-1613905	
12-021_I 2_Lung			SC-848840		US-1613809	
15-010_H4_Liver			SC-848841		US-1613822	
14-077_K3_LN			SC-848842		US-1613816	
13-104_I4_Abdominal LN			SC-848845	US-1613856		
13-099_H8_Liver			SC-848847	US-1613832		
14-053_O6_L retroperitoneal LN			SC-848849	US-1613841		
13-117_H7_Liver			SC-848853	US-1613828		
14-039_G1_Adrenal			SC-848855		US-1613848	
15-023_I4_Spleen			SC-848856		US-1613825	
14-039_K3_Liver			SC-848860		US-1613922	
15-010_K3_Periaortic LN			SC-848862	US-1613823		
13-099_K4_Independent met L Lung			SC-848863	US-1613829		
13-104_P8_Periaortic Mass			SC-848865	US-1613803		
15-023_L7_R Hilar LN			SC-848866		US-1613821	
14-031_N3_Hilar LN			SC-848867		US-1613924	
14-105_I4_Spleen			SC-848870	US-1613835		
14-077_N5_Liver	SC-848911			US-1616844		
14-053_K4_Mediastinal LN	SC-848916			US-1613820		
14-105_N1_Adrenal	SC-848929			US-1613850		
13-104_Q3_Nodule Met	SC-848932			US-1613907		
14-031_M3_Lung Met	SC-848934			US-1613923		
14-031_K3_Periaortic Mass	SC-848955	SC-848909		US-1613804	US-1613804	
				UHR	UHR	

Figure 4. HTG EdgeSeq experimental plan: samples shown in darker colors were repeated at different input amounts.

Experimental Design to Evaluate the Robustness of the HTG EdgeSeq Platform

- Determine the correlation between different FFPE sections/slides from the same sample
- Determine the correlation between FFPE sections/slides and extracted RNA from the same sample



- Examine inter and intra-day reproducibility
- Effect of input amount of sample
- Determine the correlation with RNA-Seq data
- Determine the correlation with the NanoString PanCancer Immune Profiling Panel

Results

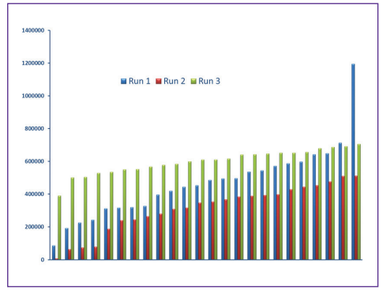


Figure 5A. Number of reads obtained on the HTG EdgeSeq with both RNA and FFPE samples. The samples are ordered based on reads. There is a large variability in read numbers when both RNA and FFPE are analyzed.

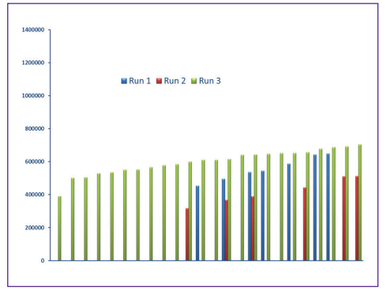


Figure 5B. Number of reads obtained on the HTG EdgeSeq with only FFPE samples. The samples are ordered based on reads. There is much more uniform coverage when the RNA samples are removed.

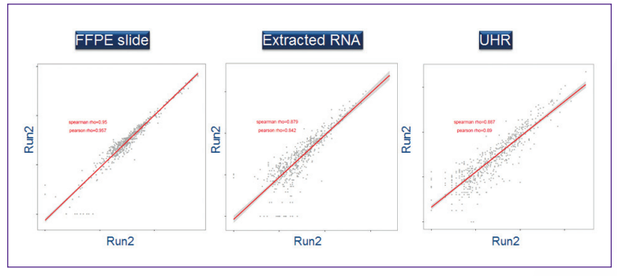


Figure 6. Intra-assay precision: correlation between samples run on the same day on the HTG EdgeSeq platform. FFPE performed higher than both extracted RNA and control RNA.

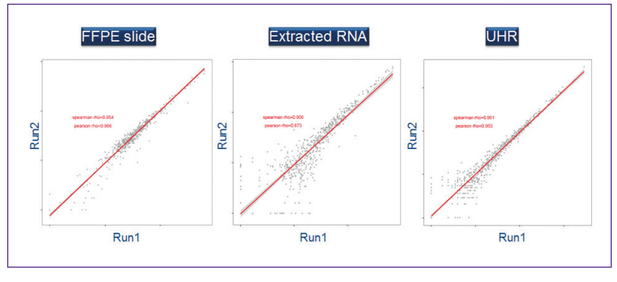


Figure 7. Inter-assay precision: correlation between samples run on the same day on the HTG EdgeSeq platform. FFPE performed much better than extracted RNA and was comparable to the RNA control.

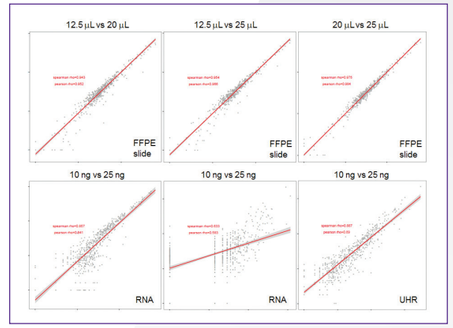


Figure 8. Comparison of different RNA mass input amounts of the same sample on the HTG EdgeSeq platform. FFPE performed much better than extracted RNA.

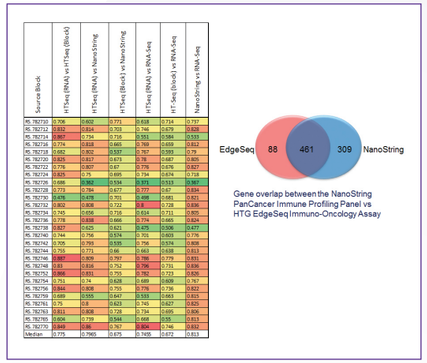


Figure 9. Overall platform comparison. Individual samples are listed on the left-hand side of the figure. Each platform comparison is listed at the top. Darker red means a higher correlation of gene expression from genes in common across the platforms shown on the right (n=461).

Conclusions

- Current results show that the NanoString and HTG platforms performed equally well
- Platform selection would be determined by sample type, TAT and availability
- HTG EdgeSeq: high correlation between different FFPE sections/slides from the same sample (pearson rho>0.95) and at different concentrations (pearson rho>0.952-0.984)
- HTG EdgeSeq: lower correlation between extracted RNA processed in different runs (pearson rho>0.84) and at different concentrations (pearson rho>0.583-0.890)
- 96% of samples showed correlation values >0.6 for FFPE vs RNA on the HTG EdgeSeq (median 0.775)
- 83% of samples showed correlation values >0.6 between RNA processed on HTG EdgeSeq vs RNA-Seq data (median 0.7455) and 80% for FFPE (median 0.672)
- 90% of samples showed correlation values >0.6 between RNA processed on HTG EdgeSeq vs NanoString data (median 0.795) (based on common genes) 83% for FFPE (median 0.675)

Future Directions

- Determine the gene expression profile on FFPE lysates utilizing the NanoString platform
- Determine the LLOQ for each platform to look at low expressing genes
- Determine whether any probe-specific bias exists in either platform

Presented at AACR 2017

COVANCE
SOLUTIONS MADE REAL®