Abstract #11093

Genomics, Transcriptomics, and Proteomics in the Clinical Setting: Integrating Whole Genome and RNA Sequencing With Quantitative Proteomics to Better Inform Clinical Treatment Selection

Shahrooz Rabizadeh,[1-4] Stephen C Benz,[2] Sheeno Thyparambil,[2,3] Todd Hembrough,[2,3] J Zachary Sanborn,[2] Charles J Vaske,[2] Patrick Soon-Shiong[1,4]

[1]NantWorks, LLC, Culver City, CA; [2]NantOmics, LLC, Santa Cruz, CA; [3]Oncoplex Diagnostics, Rockville, MD; [4]CSS Institute of Molecular Medicine, Culver City, CA

Background

- Next-generation sequencing (NGS) and quantitative proteomics enable the timely identification of a cancer patient's unique molecular signature, independent of anatomical tumor type, allowing the identification of clinically relevant targets for informed treatment selection
- Gene panels comprised of <500 genes are most often used to guide treatment selection; however, panels do not provide insights into altered protein expression
- To predict the downstream effects of gene alterations, orthogonal technologies such as RNAseq and proteomics are needed
  - RNAseq confirms the expression of mutated genes and enables the quantitation of gene expression and when integrated with DNA sequencing data using pathway-based modeling algorithms such as PARADIGM[1] can be used to infer protein expression within actionable signaling pathways
  - Mass spectrometry-based proteomics allows the quantitative measurement of expressed proteins that influence disease progression and sensitivity and resistance to therapeutics
- An important step in the evolution of precision cancer medicine is to utilize a comprehensive panomic approach to select therapies for patients
- We have developed a platform that integrates whole exome/whole genome sequencing data of patient matched tumor-normal samples with RNAseq, quantitative proteomics, and pathway analysis to identify clinically relevant targets

Methods

Study Population and Data Set

- A sequencing data set of patient matched tumor-normal samples was analyzed from The Cancer Genome Atlas (TCGA) CG-Hub database (https://cghub.ucsc.edu/)
- Whole exome sequencing data/RNAseq data were available for 3783 patients

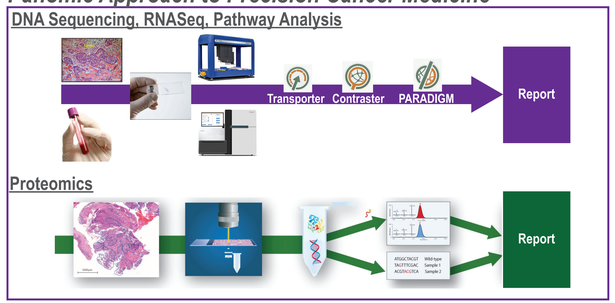
Data Analysis

- Transporter software platform encrypted and securely transfered unassembled data from sequencer to supercomputer used for analysis
- DNA sequencing data were processed using Contraster[2]
  - Gene panel analysis was limited to 328 genes
- RNAseq data confirmed the presence of gene mutations
  - Variants classified into high-expressed (>0.9 allele fraction) and low-expressed (<0.1 allele fraction)
  - RNAseq expression values calculated using a normal distribution across adjacent normal samples in TCGA; "high" expression was Z-score ≥3
- Associations between gene mutations and expression were determined:
  - Highly expressed gene mutations (whole exome versus gene panel)
  - Low/no expression of gene mutations (gene panel)
  - Highly expressed non-mutated genes (gene panel)
- PARADIGM used to reveal shared pathways among patients
  - Copy-number alterations derived from segmented data produced by Contraster
  - Transcriptomic data normalized to pool of normal samples across tissues, with transcript abundance identified as up- or down-regulated regulated when falling above the upper or below the lower 5th percentile for each gene
  - Clustering of PARADIGM activity values was performed using a custom version of Cluster 3.0.9

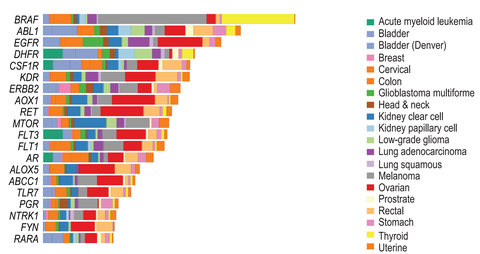
Proteomics

- Tissues were microdissected, solubilized, and enzymatically digested
- Peptides unique to proteins of interest were identified and labeled peptides were synthesized
- Absolute quantitation of protein targets was performed using selected reaction monitoring mass spectrometry

Panomic Approach to Precision Cancer Medicine

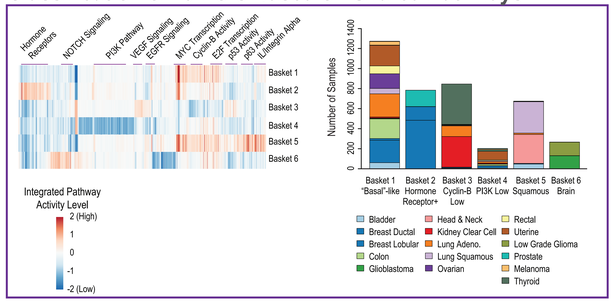


Mutated Targetable/Actionable Genes Across Cancer Types

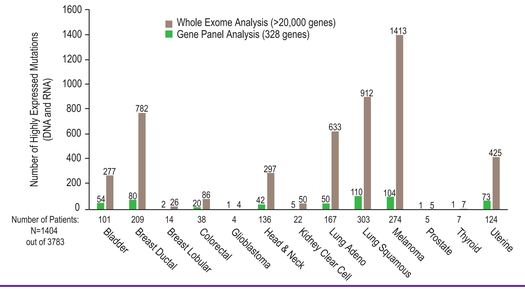


- A number of drugs targeting tumor mutations are approved in cancer indications

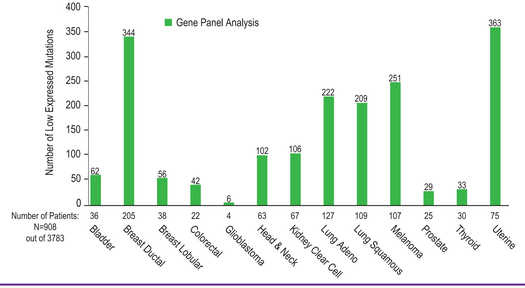
Classification of Tumors Based on Shared Pathways



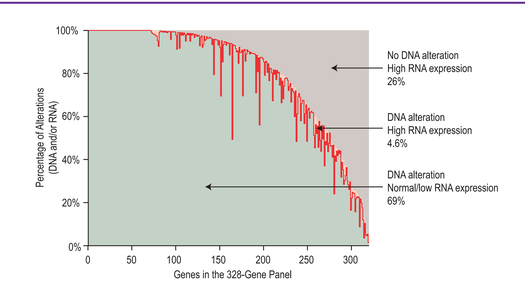
Highly Expressed Mutant Alleles: Whole Exome vs Panel



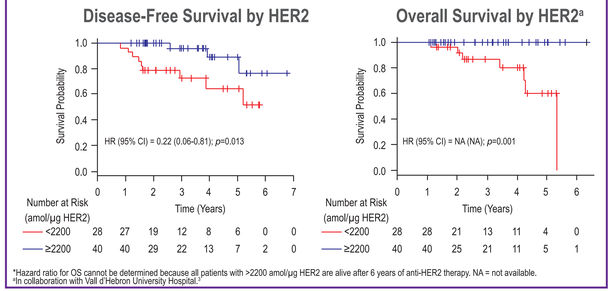
Low/No Expression of Mutant Alleles: Panel



Expression of Mutated and Non-Mutated Genes: Panel

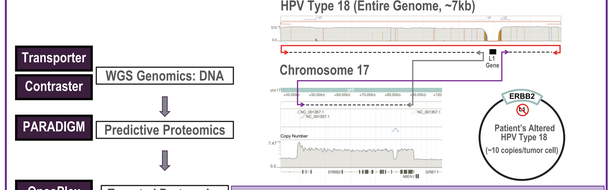


Predictive Value of Proteomics: HER2 as an Example



[a]Hazard ratio for OS cannot be determined because all patients with >2200 amol/µg HER2 are alive after 6 years of anti-HER2 therapy. NA = not available.
[b]In collaboration with Vall d'Hebron University Hospital.[3]

Panomics Case Study: End-Stage Cervical Cancer



Protein Target	amol/µg of Tissue Protein: Assay Limit of Detection (LOD)	Observed Range: Preclinical Expression Levels	Test Result HCS-12-17127 (C0608)
HER2	150	150-26,000	11,322

- *HER2* gene amplified 8-fold due to insertion of HPV DNA into patient's genome at chromosome 17
- Patient treated with anti-HER2 therapy; disease stabilization for 1.5 years

Conclusions

- Mutations in genes targeted by drugs approved based on anatomy are prevalent in other cancers independent of tissue type
- Expression matters
- Quantitative measurement of HER2 >2200 amol/µg is predictive of longer survival
- Panomic platform integrating genomic sequencing with quantitative protein expression analysis informed effective treatment for patient with end-stage cervical cancer with a drug not approved in that tissue type
- Precision cancer medicine will require reclassification of cancers based on their molecular profile and not on tissue type

References

1. Sanborn JZ, Salama SR, Grifford M, et al. *Cancer Res*. 2013;73:6036-6045.
2. Sedgewick AJ, Benz SC, Rabizadeh S, Soon-Shiong P, Vaske CJ. *Bioinformatics*. 2013;29:i62-70.
3. Nuciforo P, Thyparambil S, Aura C, et al. *Cancer Research*. 2014;75:4-11.

Acknowledgment

The results published here are in whole or part based upon data generated by The Cancer Genome Atlas project established by the NCI and NHGRI. Information about TCGA and the investigators and institutions who constitute the TCGA research network can be found at "http://cancergenome.nih.gov".

Kathryn Boorer, PhD of NantHealth, LLC provided writing assistance.