Preliminary Observations of Blood-based Molecular Testing in a Subset of Patients with Pancreatic Cancer Participating in the Know Your Tumor (KYT) Initiative

Michael J. Pishvaian[1,2], Lynn M. Matrisian[3], Andrew Hendifar[4], Anitra W. Engebretson[3], Lola Rahib[3], William A. Hoos[3], Sam Mikhail[5], Vincent Chung[6], Vincent Picozzi[7], Craig Heartwell[1], Kimberly Mason[1], Katelyn Varieur[1], Metasebia Aberra[1], Joseph Bender[1], Subha Madhavan[1,2], Emanuel Petricoin III[1],, Jonathan Brody[8]

[1]Perthera, Inc, McLean, VA; [2]Lombardi Comprehensive Cancer Center, Georgetown University Medical Center Washington, D.C.; [3]The Pancreatic Cancer Action Network, Manhattan Beach, CA; [4]Cedars-Sinai Medical Center, Los Angeles, CA; [5]Ohio State University, Columbus, OH; [6]City of Hope Cancer Center, Duarte, CA; [7]Virginia Mason Medical Center, Seattle, WA; [8]Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA

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Corresponding email: pishvaim@georgetown.edu

Summary

Background:
While molecular profiling in solid tumors remains routine practice in cancer diagnostics, modern technologies have enabled detection of biomarkers in stray cells, exosomes, and traces of DNA in blood and other body fluids. Obtaining an adequate tissue sample for molecular profiling to guide therapy selection for patients with advanced cancers can be clinically difficult, and/or patients may not want to undergo a biopsy. Recent research has shown that Blood-based (BB) tests, including cell free DNA (cfDNA) and exosome/circulating tumor cell based-analyses can act as surrogates for tumor tissue (TT) molecular testing (1). This concept must be rigorously validated as there are biological and technical limitations of BB tests. Validation of BB tests in pancreatic ductal adenocarcinoma (PDA) is possible because >90% of PDAs harbor *KRAS* mutations (2) – thus providing a reliable "internal control."

Methods:
The Pancreatic Cancer Action Network (PanCAN) and Perthera initiated an IRB-approved registry trial for patients with PDA wherein we facilitated commercially available, CLIA certified multi-Omic profiling including next generation DNA sequencing (NGS), immunohistochemistry, and phosphoproteomics on patient tumor samples. In a subset of these patients, we incorporated BB cfDNA testing.

Results:
From 06/2014 to 12/31/2015, molecular profiling was available for 175 pts. Actionable findings, defined based on a high response rate in pts with an identified molecular abnormality (in any cancer type), or based on a mechanism/pathway-defined implication of response to treatment were identified in 40% of pts, primarily based on NGS. A *KRAS* mutation has been identified 90% (146/162) of KYT patients with confirmed PDA based on TT NGS in general. 30 BB test results (cfDNA NGS) were available. In 22 patients we were able to compare the cfDNA NGS directly with TT NGS. BB testing identified a *KRAS* mutation in 5/22 (23%) compared to 19/22 (86%) from the tumor tissue. Of the 8 patients who did not have corresponding TT NGS, a *KRAS* mutation was found on BB testing in 3 samples (38%). The *KRAS* mutation rate in the 30 BB patients overall was 8/30 (27%). When the 30 BB cases were filtered for patients with extensive metastatic disease and progressive disease at the time of blood sampling, the *KRAS* mutation rate increased to 4/13 (31%). Actionable findings (i.e. linked to a specific therapeutic option) were identified in 8/30 (27%) cases, of which 4/30 (13%) had both an actionable mutation and a *KRAS* mutation.

Conclusions:
Although we are aware of the limitations of this study, we recommend that for patients who have biopsiable disease, a tumor tissue test should still be the gold standard for molecular profiling. For those without biopsiable disease, the KYT program presents an opportunity to determine the parameters that influence the probability of obtaining reliable molecular profiling information from a BB sample.

Citations and Acknowledgments

(1) Zill OA. et al. Cancer Discov. 2015 Oct;5(10):1040-8.
(2) Knudsen ES et al. Gastroenterology. 2016 Jan;150(1):48-63.
(3) Waddell N et al. Nature. 2015 Feb 26;518(7540):495-501.
(4) Leichman, et al. ASCO 2012 Meeting. J Clin Oncol 30, 2012 (suppl; abstr 4052)

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Background

- To date, hundreds of PDA genomes have been sequenced identifying multiple actionable pathways that maybe "druggable" including: 1) DNA repair; 2) WNT signaling ; 3) NOTCH signaling; 4) cell cycle genes; 5) chromatin remodeling (2).
- The best example of a personalized approach to PDA are tumors that harbor a *BRCA1/2*, Fanconi Anemia, or *PALB2* mutations (i.e., BRCAness) for PARP inhibitor/Platinum based therapy (3).
- BB-cfDNA testing is a novel, noninvasive "liquid biopsy" with the potential utility of identifying actionable mutations in patients where a conventional biopsy is not an option. Theoretically, mutations identified in cfDNA represent tumor specific genetic alterations that could be used in guiding precision therapy.
- >90% of PDAs harbor *KRAS* mutations and thus can serve as a validation marker of a "liquid biopsy" taken from a PDA patient's blood sample.

Methodologic Details

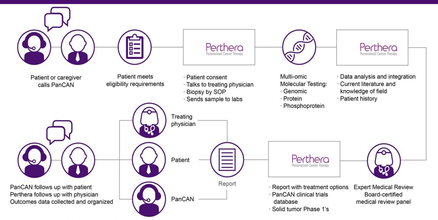

Figure 1: The PanCAN Perthera KYT Workflow Algorithm: Identified patients are referred for tumor collection. Perthera facilitates the process of tumor biopsy, coordinates molecular testing, and reviews the results with the disease-specific medical review panel. A report is then delivered to the patient and treating oncologist, and patients are followed longitudinally for outcomes.

Patient Consent and Testing
- New England IRB-approved registry protocol & HIPAA waiver
- Testing order forms completed by Perthera and patient insurance billed directly
- Perthera oversees tissue process regarding timing and adequacy of samples

Biopsies and Molecular Profiling
- Primary oncologist schedules biopsy, and Perthera contacts IR/surgeon
- Perthera SOP delivered to IR/surgeon AND path – requesting 4-6 18-20 gauge core needle biopsies, each individually embedded longitudinally, then shipped to Perthera
- FFPE samples sectioned and sent for CAP/CLIA accredited testing.
- Blood based cfDNA testing on whole blood sent for CAP//CLIA accredited NGS

Definition of Actionability
Literature supports a high response rate in patients with that molecular abnormality (any cancer type)
OR
Possible implication of response to therapy, based on mechanism or pathway

Molecular Biomarker Weighting and the Perthera Drug Scoring Algorithm:
Molecular Vector:
- Chemotherapy markers alone (low rank) → Pathway-level (high score)

Disease-Specific Vector:
- Untested in that disease (low score) → Clinically proven benefit (high score)

Patient History Vector:
- Patient has received the agents (low score) → Treatment naïve (high score)

Patients

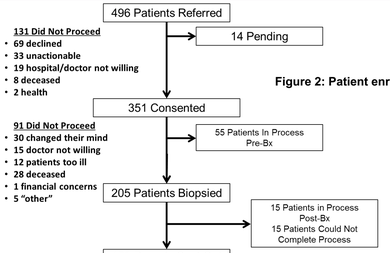

Figure 2: Patient enrollment.

	N	%
Gender		
Male	95	55%
Female	80	45%
Age		
< 50	16	9%
50 to 59	49	28%
60 to 69	77	44%
≥ 70	33	19%
Biopsy Site		
Liver	88	50%
Pancreas	35	20%
Abdomen	14	8%
Lung	12	7%
Other	26	15%

	N	%
# Prior Therapies		
1	41	23%
2	41	23%
≥ 3	52	30%
Unknown	41	24%
Prior Therapy		
5-FU-based	26	15%
Gemcitabine-based	38	22%
Both	68	39%
Neither	6	3%
Unknown	37	22%

Tables 1 and 2: Patient Demographics

Results

NGS Testing:
30 patients underwent BB cfDNA testing:
- 24 patient samples were tested by Guardant Health
- 6 patient samples were tested by Cynvenio

22 out of 30 patients underwent tumor tissue NGS:
- NGS on tumor tissue was performed by Foundation Medicine

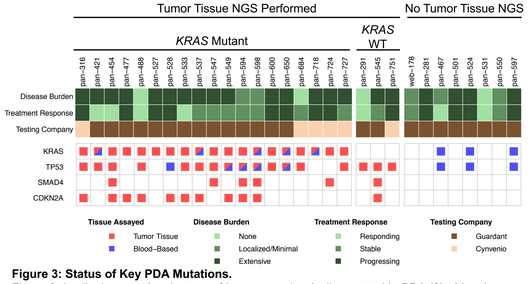

Figure 3: Status of Key PDA Mutations.
Figure 3 details the mutational status of key genes classically mutated in PDA (2). Mutations are shown for TT NGS analysis (**Red**) and BB cfDNA analysis (**Blue**). Of the 22 patient samples for which TT was available, *KRAS* was mutated in 19 (86%). Of note, in the larger KYT group, the *KRAS* mutation rate is 90% for confirmed PDA. However, the *KRAS* mutation rate in the BB cfDNA samples is only 8/30 (27%) overall.
• Importantly, this percentage was only slightly increased when the degree of tumor burden and treatment response are considered. For patients with extensive disease burden, and for whom the disease was progressing at the time of cfDNA sampling, the KRAS mutation rate was 4/13 (31%).
• A similar gap is true for other key mutations. The % mutation for other genes in TT vs. BB cfDNA is: *TP53* 18/20 (80%) vs. 8/30 (27%); *SMAD4* 6/20 (30%) vs. 0/30 (0%); and *CDKN2A* mutation OR *CDKN2A/2B* loss 11/20 (55%) vs. 0/30 (0%).

Results

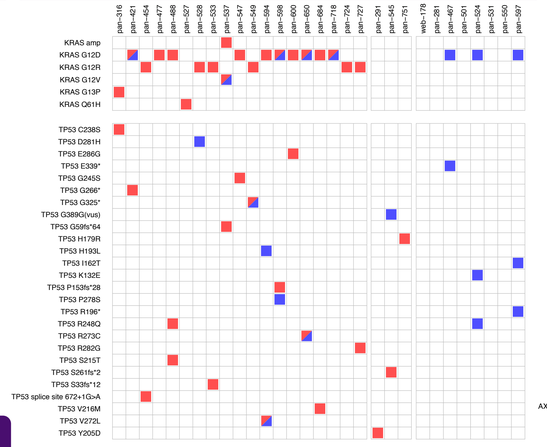

Figure 4: Examination of specific PDA Mutations. Highlighted herein are the specific *KRAS* and *TP53* mutations identified through TT NGS analysis (**Red**) and BB cfDNA analysis (**Blue**). These results demonstrate the breadth of mutations in key genes that are identified by any NGS analysis. Specific mutations in one patient are often, though not always identified by both TT NGS and BB cfDNA testing.

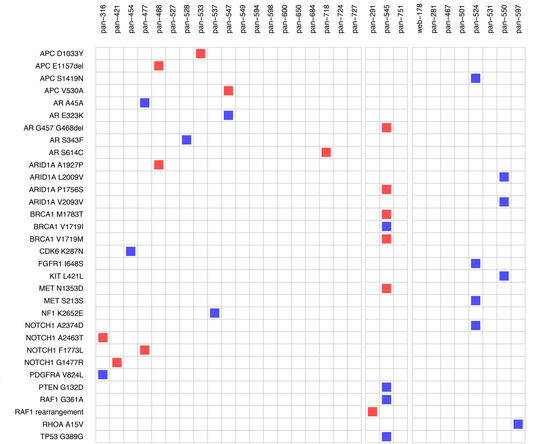

Figure 5: Comparison of variants of undetermined significance identified by TT and BB cfDNA NGS testing. Both TT-based (**Red**) and BB cfDNA-based (**Blue**) NGS testing reveal a number of VUSs. Surprisingly, there is relatively little overlap between the results.

Results

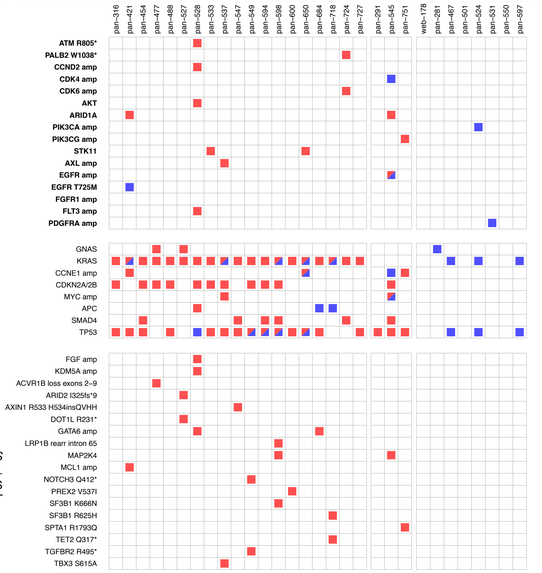

Figure 6: Examination of "Actionable" Mutations. A number of gene mutations are identified by both TT and cfDNA NGS testing. Highlighted in bold are the "actionable" mutations, as defined in the methods. There are many more "actionable" mutations identified by TT NGS testing. Surprisingly, there was little overlap between the TT NGS identified "actionable" genes, and those identified by BB cfDNA testing. Additionally, in 3 cases, an "actionable" mutation was identified by BB cfDNA testing in the absence of a corresponding KRAS mutation, raising concern over the reliability of the "actionability" of the finding. The lowest section highlights mutations only tested by TT NGS, but not BB cfDNA NGS.

Conclusions and Future Directions

- Based on this pilot study, given that BB-cfDNA testing could not identify known *KRAS* mutations in a small percentage of patients with disseminated and metastatic disease, we conclude that BB-cfDNA testing is not ready for clinical decision making, especially in the arena of precision therapy for PDA.
- Tumor tissue testing should still remain the gold standard.
- Future studies evaluating BB-cfDNA platforms in PDA cohorts should consider: 1) side by side testing with TT and 2) the use of *KRAS* mutations as a gold standard biomarker for this disease.
- Sequencing germline (constitutional gDNA *not* from the tumor) should be considered in an effort to define true somatic events.