Clinical Cancer Research

Molecular Profiling of Patients with Pancreatic Cancer: Initial Results from the Know Your Tumor Initiative

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Abstract

Purpose: To broaden access to and implementation of precision medicine in the care of patients with pancreatic cancer, the Know Your Tumor (KYT) program was initiated using a turn-key precision medicine system. Patients undergo commercially available multiomic profiling to determine molecularly rationalized clinical trials and off-label therapies.

Experimental Design: Tumor samples were obtained for 640 patients from 287 academic and community practices covering 44 states. College of American Pathologists/Clinical Laboratory Improvement Amendments–accredited laboratories were used for genomic, proteomic, and phosphoprotein-based molecular profiling.

Results: Tumor samples were adequate for next-generation sequencing in 96% and IHC in 91% of patients. A tumor board reviewed the results for every patient and found actionable genomic alterations in 50% of patients (with 27% highly actionable) and actionable proteomic alterations (excluding chemopredictive markers) in 5%. Actionable alterations commonly found were in DNA repair genes (*BRCA1/2* or *ATM* mutations, 8.4%) and cell-cycle genes (*CCND1/2/3* or *CDK4/6* alterations, 8.1%). A subset of samples was assessed for actionable phosphoprotein markers. Among patients with highly actionable biomarkers, those who received matched therapy ($n = 17$) had a significantly longer median progression-free survival (PFS) than those who received unmatched therapy [$n = 18$; PFS = 4.1 vs. 1.9 months; HR, 0.47; 95% confidence interval (CI): 0.24–0.94; $P_{adj} = 0.03$].

Conclusions: A comprehensive precision medicine system can be implemented in community and academic settings, with highly actionable findings observed in over 25% of pancreatic cancers. Patients whose tumors have highly actionable alterations and receive matched therapy demonstrated significantly increased PFS. Our findings support further prospective evaluation of precision oncology in pancreatic cancer. *Clin Cancer Res; 24(20); 5018–27. ©2018 AACR.*

Introduction

Pancreatic cancer will likely become the second leading cause of cancer-related death in the United States by 2020 (1, 2). Standard-of-care (SOC) treatments for metastatic pancreatic ductal adenocarcinoma (PDAC) include FOLFIRINOX (3) and gemcitabine with nab-paclitaxel (4); however, most patients do not respond to these regimens, and the median overall survival (OS) remains less than 1 year. In an effort to optimize therapy, molecular profiling has been used to group patients with PDA into therapeutically "actionable" molecular subgroups (5–12). Targets have included homologous recombination repair (14%–17%; refs. 5, 9), HER2 amplification (2%; ref. 13), and mismatch repair gene deficiency (microsatellite instability; 2%–3%; refs. 14, 15).

Next-generation sequencing (NGS) and protein IHC panel–based examination of patients' tumors has previously been available at academic medical centers only, but is now more widely available with commercial lab testing (16). In this context, we launched a U.S. nationwide initiative, Know Your Tumor (KYT), a collaboration between Perthera Inc. and the Pancreatic Cancer Action Network (PanCAN), which utilizes Perthera's precision medicine system for multiomic molecular profiling of a nonselected patient population. This profiling is coupled with an analytics engine that incorporates previous treatment history and molecular–clinical evidence for biomarker therapy matching, and a cloud-based tumor board that provides a Health Insurance Portability and Accountability Act (HIPAA)-compliant portal to facilitate discussion among medical and scientific reviewers (17). The intent of the KYT program is to match patients with appropriate clinical trials and therapies based on actionable molecular anomalies, treatment history, and geographical locations. Here, we present our findings from the first 640 KYT patients.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Although targeted therapies have had limited success in unselected populations of patients with pancreatic cancer, molecular profiling could promote higher response rates by identifying individuals with actionable alterations. To survey the potential utility of molecular profiling, we performed multiomic molecular testing on 640 patients with pancreatic cancer using next-generation sequencing and IHC-based panels. The patients were from over 200 different high-volume and community practices, demonstrating that access to molecular testing need not be limited to large institutions. Profiling revealed that 50% of patients had an alteration predictive of potential response to targeted therapies, with 27% harboring highly actionable alterations. In a subset of patients with treatment-linked alterations who received matched treatment after profiling, progression-free survival (PFS) was significantly improved (after correcting for line of therapy) compared with patients with actionable findings who received standard of care, suggesting that precision medicine may lead to improved outcomes in patients with pancreatic cancer.

Materials and Methods

Patients and tumor samples

Patients with pancreatic cancer were identified through the PanCAN Patient Central call center, referred to Perthera, and enrolled through an Institutional review board–approved registry protocol. The study protocol, amendments, and the informed consent forms were approved by the New England institutional review board. Investigators obtained informed consent from each participant or participant's guardian prior to enrollment. The research was conducted in accordance with recognized ethical guidelines including the Declaration of Helsinki, CIOMS, Belmont Report, and U.S. Common Rule, as described during training in Good Clinical Practice guidelines (CITI Training). In general, a recent biopsy was required for molecular profiling, although under certain circumstances archived biopsies were used. Further details about the Perthera precision medicine process are provided in the Supplementary Methods.

Next-generation DNA sequencing

Tumor biopsy samples were sent to a Clinical Laboratory Improvement Amendments (CLIA) certified, College of American Pathologists (CAP)-accredited commercial laboratory [Foundation Medicine or PGDx (99% of the cases were sent to Foundation Medicine)] for next-generation DNA Sequencing (NGS) analysis of cancer-related mutations (Supplementary Table S1A and S1B).

IHC

Tumor biopsy samples were sent to Caris Life Sciences or NeoGenomics for IHC testing of a panel of 17 proteins with evidence that the markers are predictive of response to therapy (Supplementary Table S1C and S1D; ref. 18). Eighty-two percent of samples were sent to Caris Life Sciences, while 18% were sent to NeoGenomics.

Protein drug target activation analysis

Twenty tumor biopsy samples were sent on a research use only (RUO) basis to Theranostics Health, Inc. for quantitative measurement of phosphorylation of 21 well-described signaling proteins that are a direct read-out of deranged kinase drug target activity (Supplementary Table S1E). Full details are available in the Supplementary Methods.

Testing for microsatellite instability

Mismatch repair (MMR) proteins were assessed by IHC as above. However, due to recent data demonstrating clinical benefit with immune checkpoint inhibitors in tumors with microsatellite instability (14), we also tested 241 samples through either PCR fragment analysis (Caris Life Sciences) or a Foundation Medicine algorithm applied to NGS data for evidence of microsatellite instability in 114 intronic microsatellites as described previously (19).

Patient history and outcomes

Enrolled patients signed a HIPAA waiver, and patient coordinators received patient records from the treating oncologist. The medical review panel (MRP) reviewed the records prior to generating the multiomics report. For longitudinal outcomes assessment, patient records were obtained from the treating oncologist every month following delivery of the report. Progression-free survival (PFS) was calculated from the time of initiation of a certain therapy until disease progression or patient death. To reduce variability due to the timing of KYT enrollment and biopsy obtainment and to establish a more definitive starting point for the determination of OS, OS was calculated from the time of the biopsy used for molecular profiling until death.

Treatment options

The MRP was enabled through a cloud-based, online tumor board with at least two pancreatic cancer–focused medical oncologists and four cancer biologists and computational biologists. Upon receipt of the patient medical records and molecular profile, the MRP reviewed the data and presented several treatment options in the form of a report that is sent to the treating oncologist and the patient. Molecular alterations were defined as potentially actionable if there was: (i) literature supporting clinical evidence of a high response rate in patients with that molecular abnormality in any cancer type ("highly actionable" subgroup; see Supplementary Table S2C); or (ii) a possible implication of response to therapy, based on the underlying mechanism ("modifies options" subgroup).

Statistical analysis

All statistical analyses were performed in R, a statistical computing language. To compare biomarker frequencies between clinically defined subgroups, we used Fisher exact test (*fisher.test* function in the R *stats* package). Correction for multiple testing was performed using the Benjamini–Hochberg method for controlling false discovery rate (20).

Survival times for groups of patients were computed using the Kaplan–Meier estimator (the *Surv* and *survfit* functions in the R *survival* package). Differences between groups were evaluated for statistical significance using Cox proportional hazards models (the *coxph* function in the R *survival* package). To correct for the uneven distribution of covariates between groups, we utilized propensity score weighting. Propensity scores were calculated

from a logistic regression model of the treatment status based on three covariates (line of therapy, age, and gender). We then used the propensity scores to calculate weights for the Cox model as described in ref. 21.

Results

Enrollment details

As part of the KYT program, 1,245 patients with pancreatic cancer were referred to Perthera from June 16, 2014 to June 20, 2017 (Fig. 1). Of these, 1,020 patients consented to the program (82%), and 737 consented patients had tumor tissue collected, with testing completed and reports delivered for 640 patients or in process for 43 patients (as of June 20, 2017). These 640 patients were referred from 287 centers (175 community practices and 112 high volume tertiary cancer centers) across 44 states. After obtaining tissue from local pathologists, the median time to report delivery was 30 days; this time interval was 51 days or less in 90% of patients.

Patient characteristics

Of the 640 patients who had reports delivered, 591 had PDA and 49 had non-PDA histology. The majority entered the program with metastatic disease (458, 72%); however, some were enrolled with disease only in the pancreas: either localized, resectable (45, 7%), or locally advanced (137, 21%; Table 1).

Tissue sufficiency for molecular testing

The registry protocol called for sampling of the most accessible tumor deposit (see Supplementary Methods), and in 68% of patients, this was a metastatic deposit, while in 32%, the sample was from the primary lesion. There was sufficient tissue available for molecular profiling in 633 of 640 patients (99%; Fig. 2), with NGS results available for 616 (96%) and IHC results available for 580 (91%). Eighty of 640 (13%) of tumor samples were initially insufficient for NGS testing, but in 56 of those cases, archived tissue from a prior tissue sampling or surgery was available. Similarly, 72 of 640 (11%) of tumor samples were initially inadequate for IHC testing, but in 29 of those cases, archived tissue was available.

Genomic alterations detected by NGS

Targeted NGS testing detected at least one pathogenic mutation in 616 patients, with a median of four per patient (range, 1–27). MAPK pathway alterations were present in most patients, predominantly due to *KRAS* mutations (535 of 616, 87%; Table 1). In the 81 patients lacking *KRAS* mutations, other MAPK proteins were often mutated, including four *NRAS* and 14 *BRAF*

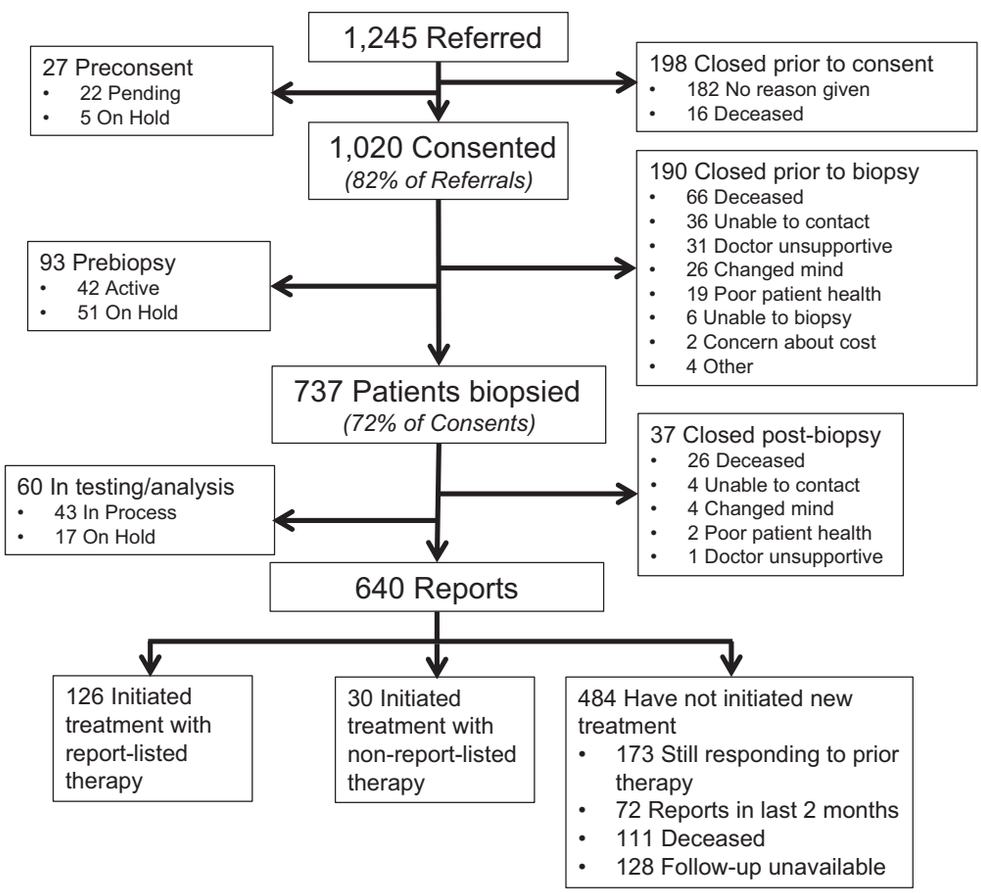

Figure 1.
Cumulative patient accrual in the KYT program. The number of patients referred, consented, biopsied, and completed as of June 20, 2017 by the Perthera process.

Table 1. Patient characteristics and KRAS mutation status. Gender, age, biopsy site, type of treatment institution, and KRAS mutation status are given across all patients (total columns) and in groups based on disease burden at the time of the biopsy used for the multiomic profiling described in this study (metastatic; locally advanced, unresectable [LAPC], or resected).

		Disease status		
	Total ($n = 640$)	**Metastatic ($n = 458$)**	**LAPC ($n = 137$)**	**Resected ($n = 45$)**
Gender				
Male	328 (51%)	245 (53%)	70 (51%)	13 (29%)
Female	312 (49%)	213 (47%)	67 (49%)	32 (71%)
Age, years				
<50	59 (9%)	41 (9%)	13 (9%)	5 (11%)
50–59	165 (26%)	124 (27%)	28 (20%)	13 (29%)
60–69	279 (44%)	196 (43%)	60 (44%)	23 (51%)
≥70	137 (21%)	97 (21%)	36 (26%)	4 (9%)
Tumor biopsy site				
Liver	259 (40%)	258 (56%)	1 (1%)	0 (0%)
Pancreas	207 (32%)	38 (8%)	125 (91%)	44 (98%)
Lung	41 (6%)	41 (9%)	0 (0%)	0 (0%)
Peritoneum	57 (9%)	57 (12%)	0 (0%)	0 (0%)
Other	76 (12%)	64 (14%)	11 (8%)	1 (2%)
Treatment setting				
High volume	414 (65%)	309 (67%)	77 (56%)	28 (62%)
Community practice	226 (35%)	149 (33%)	60 (44%)	17 (38%)
KRAS status[a]				
Mutated	535 (87%)	385 (88%)	115 (86%)	35 (80%)
WT, nonadenocarcinoma histology[b]	32 (5%)	21 (5%)	5 (4%)	6 (14%)
WT, other MAPK pathway alteration[c]	11 (2%)	9 (2%)	1 (1%)	1 (2%)
WT, other histology and other MAPK	5 (1%)	2 (0.5%)	3 (2%)	0 (0%)
True *KRAS* WT	35 (6%)	23 (5%)	10 (7%)	2 (4%)

[a]*KRAS* status by NGS was available in 618 of 640 patients.
[b]Nonductal adenocarcinoma histologies included neuroendocrine, acinar cell, and ampullary.
[c]Other MAPK pathway alterations included *NRAS* amplification/mutation and *BRAF* mutation.

alterations. Thirty-seven of 81 wild-type *KRAS* patients had non-adenocarcinoma histology, as determined by review of local pathology reports. A total of 535/579 (92%) PDA samples were *KRAS* mutated, while 33 of 616 samples (5.3%) were true *RAS* pathway wild-type lacking other MAPK pathway alterations. This was consistent with the frequency of *KRAS* mutations in published studies (5, 6, 9).

Mutations in DNA repair genes were the most common "highly actionable" alterations (92, 15%). The most frequently mutated DNA repair genes were *ATM* (28, 4.5%) and *BRCA2* (18, 2.9%), while 46 patients' samples (7.5%) harbored less common mutations in DNA repair genes, such as *PALB2*, *FANCA/C/G*, *RAD50*, and *CHEK1/2* (Fig. 3).

Additional highly actionable altered genes included receptor tyrosine kinases (RTK), including activating mutations in *ERBB2* (17, 2.8%) and several oncogenic fusions: *CCDC6-RET*, *NCOA4-RET*, *STRN-ALK*, *LMNA-NTRK1*, and *ETV6-NTRK3*. Three activating mutations in codon 132 of *IDH1* were also observed (22).

Abnormalities that lack a clear alteration-to-drug response connection were frequently detected including *TP53* mutations. Cell-cycle genes were also frequently mutated, with 45% tumors harboring alterations of *CDKN2A*, as well as 8.1% with alterations in "actionable" cell-cycle genes (amplifications in *CDK4*, *CDK6*, *CCND1*, *CCND2*, or *CCND3*). In addition, 19% had some molecular defect in the PI3K/AKT/mTOR pathway including *STK11* mutations (4.7%), PI3K mutations (3.7%), and AKT amplifications (2.8%). Frequencies were comparable with those from other published datasets (5, 6, 23). Notable exceptions were increased mutation frequencies in *TP53* and *CDKN2A* relative to public datasets (Supplementary Table S3; Supplementary Fig. S3).

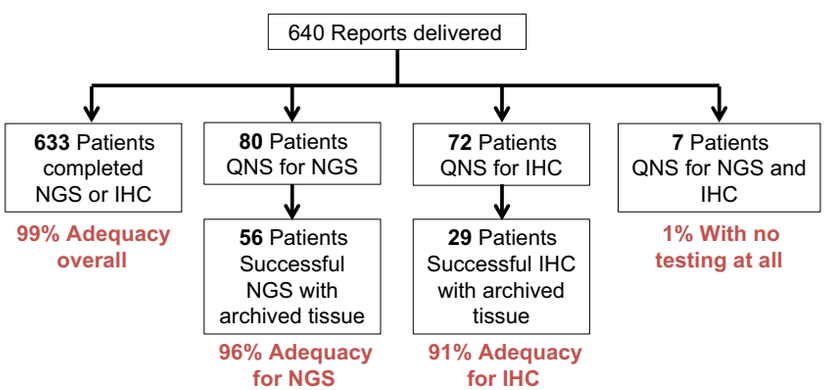

Figure 2.
Tissue sufficiency for molecular testing. Biopsy samples for which NGS or IHC testing could not be completed were deemed "quantity not sufficient" (QNS), indicating that the tumor cell content of the sample was too low. Additional tissue was obtained from a different portion of the biopsy that was initially QNS, from a previous (archived) biopsy, or, in several cases, from a new biopsy.

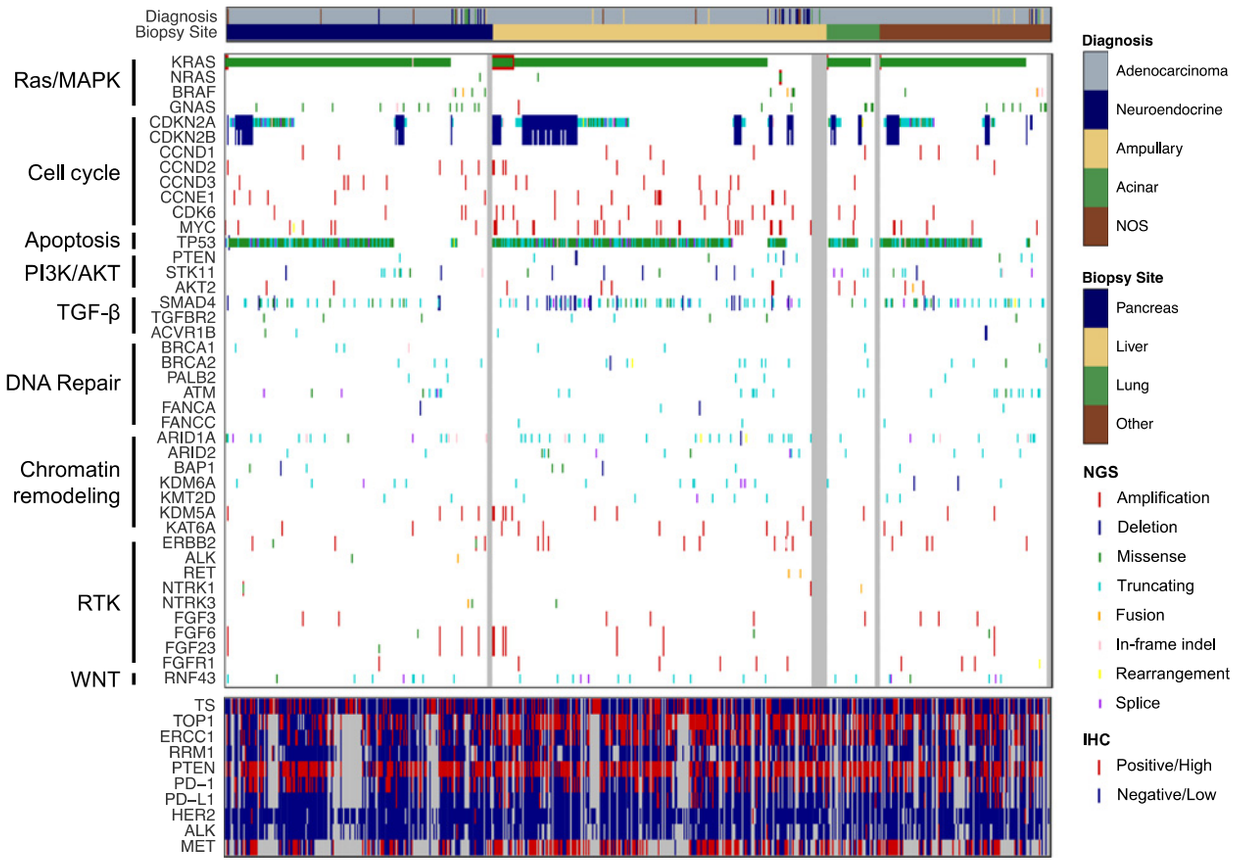

Figure 3.

Genomic and proteomic profiling of 640 pancreatic cancer biopsies. Patients were grouped according to site of biopsy. Within each site of biopsy, patients were ordered according to *KRAS* and *TP53* mutation. Gray indicates that data were unavailable, which generally occurred when samples were found to have low tumor cell content. For NGS data, pathways are indicated to the left of the gene names. NOS, pathologic diagnosis not otherwise specified.

Protein expression by IHC

IHC profiling of up to 17 proteins was performed on 580 of 640 (91%) patients. Seven were potential markers of sensitivity to SOC chemotherapies in PDA, with negative expression of thymidylate synthase in 73% of patients (sensitivity to 5-fluorouracil), negative expression of the DNA excision repair protein ERCC1 in 63% of patients (sensitivity to platinum agents), and positive expression of topoisomerase 1 in 57% of patients (sensitivity to irinotecan). Negative expression of ribonucleoside diphosphate reductase (a potential marker of gemcitabine sensitivity) occurred in 93% of patients.

The other 10 proteins measured expression of RTKs such as HER2, ALK, c-MET, as well as MMR proteins. Evidence of MMR deficiency was identified in only three of 386 patients (0.8%). Loss of expression of MLH1 and PMS2 occurred simultaneously in two patients, both of whom had insufficient tumor content for microsatellite instability (MSI) testing. One patient had loss of MSH6 expression. HER2 was overexpressed in four of 531 (0.8%) patients, and ALK was overexpressed in four of 454 (0.9%) patients (one of whom had an *ALK* fusion). PD-L1 was positive in 26 of 440 (6%) patients.

Biomarker frequencies in clinical subgroups

Several genes were mutated at different frequencies in patient demographic subgroups (Supplementary Table S4). For example, *KRAS* mutations were observed less frequently in patients under the age of 62, the median patient age (OR, 0.34, $Q = 0.002$ in all pancreatic cancers).

Phosphoprotein analysis

The levels of activated/phosphorylated proteins were measured in a subset of 20 consecutively consented patients (Supplementary Fig. S5). The most frequently activated (score of two or three) proteins were pERK T202/Y204 (9 of 20, 45%) and p4EBP1 T37/T46 (9 of 20, 45%). In keeping with recent findings (24), no significant correlations between protein activation and the associated genomic alterations, including KRAS and phosphorylated ERK, were found (Supplementary Fig. S6).

Therapeutic implications

Molecular profiles were used to match patients to both molecularly targeted and SOC therapies. NGS alterations were actionable in 50% of patients ("highly actionable" and "modifies options" in Fig. 4; *KRAS* and *CDKN2A/B* were excluded from the 50%) and IHC alterations were actionable in 5% of patients

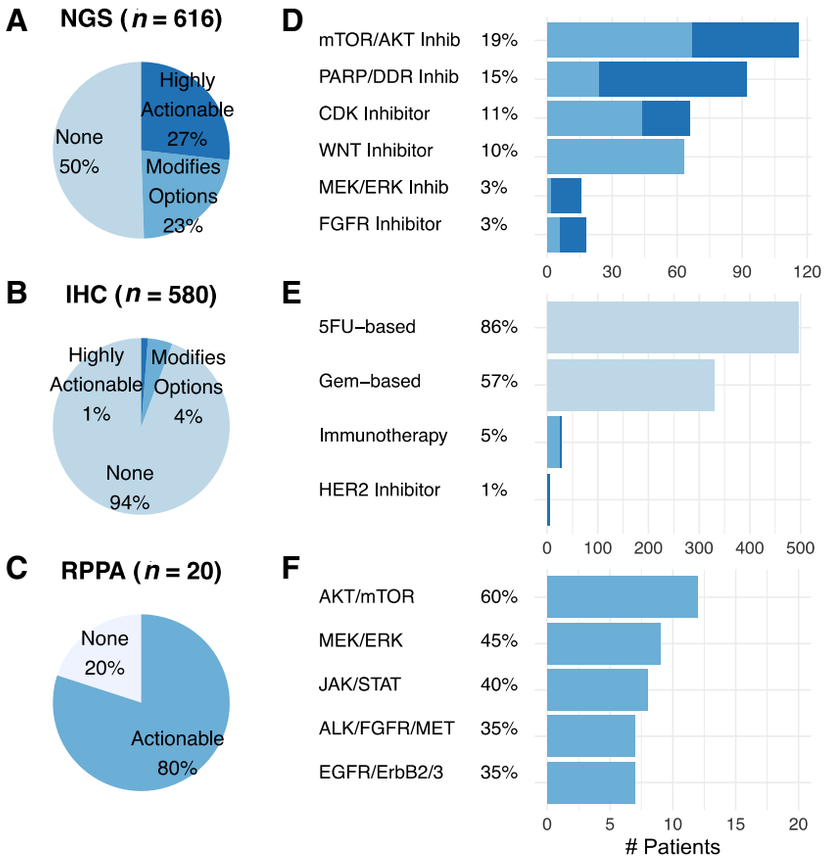

Figure 4.
Actionable biomarkers from patient samples. The percentage of patients with actionable biomarkers found on NGS (**A**), IHC (**B**), and RPPA (**C**) platforms. NGS alterations are divided on the basis of level of actionability: "highly actionable" genes are *BRCA1, BRCA2, PALB2, ATM, CHEK1/2, FANCA/C/G, STK11, AKT1/2/3, TSC1/2, CDK4/6, FGFR1/2/4, ERBB2, RET, NTRK1, NTRK3, BRAF, ALK*, and *ROS1*; genes that modify treatment options are those that affect a drug target but (to date) have no demonstrated clinical predictive value; genes such as *KRAS, CDKN2A*, and *CDKN2B* are not included as they have been proven to not be predictive of response in pancreatic cancer (see Supplementary Table S2 for the complete list). Specific therapy options are listed for each platform (**D, E,** and **F** for NGS, IHC, and RPPA, respectively), with percentages indicating the frequency of each option in patients that completed profiling on the particular platform. Actual numbers of patients are the labels of the horizontal axes in **D** to **F**.

(excluding chemopredictive markers; Fig. 4B and E). Eighty percent of patients had activated/phosphorylated protein targets identified (Fig. 4C and F).

Common classes of drugs presented as treatment options based on NGS alterations included inhibitors of MEK, CDK4/6, mTOR, and PARP, alone or in combination (Fig. 4D). Of the 165 patients with highly actionable NGS alterations, nearly all (162, 98%) had associated FDA-approved therapies. Only *IDH1* mutations lacked an FDA-approved therapy. *KRAS* mutations are considered to have low-direct actionability (25), but emerging data suggest that inhibition of two signaling pathways downstream of KRAS may control pancreatic cancer growth (26). While *CDKN2A/B* mutations are not directly predictive of response to cell-cycle inhibitors, loss of cell-cycle control is common in pancreatic cancers. Thus, CDK inhibitor-based combination trials were commonly listed as options.

Outcomes summary

As of June 20, 2017, the KYT program had delivered Perthera reports for 640 patients. Of these, 156 have begun a new treatment regimen (Fig. 5A), 173 remain on a treatment initiated prior to receipt of the report, 111 passed away prior to beginning a new treatment, and 200 have no follow-up information available (Fig. 1).

Of the 156 patients who have begun new treatments after receiving a report, 126 (81%) have chosen treatment options presented in the report, while 30 (19%) did not. The types of treatments chosen by the 126 patients utilizing the report to select a therapy were: SOC (80 of 126, 63%), off-label molecular targeted (20 of 126, 16%), and clinical trials (26 of 126, 21%). Pancreatic cancer clinical trial enrollment historically has been less than 5% of patients (27). The 21% enrollment observed here (for the first 126 patients with follow-up data) suggests that availability of an integrated physician report with well-annotated actionability evidence from multiomic profiling results may promote trial enrollment. Outcomes data from the 173 patients still on prior therapy and the 200 with no available follow-up could affect this number. Moreover, patients that gave consent to the current study may introduce some bias in this estimate of trial enrollment, due to potentially higher likelihood of these patients consenting to additional studies.

We focused further analysis on patients with highly actionable molecular alterations who received either matched therapy or who received SOC. The subgroup of patients who utilized report-listed therapies, had highly actionable biomarkers, and did not stop treatment early due to toxicity was analyzed. In this subgroup, 17 patients received molecular targeted therapies that matched biomarkers (5 in the second line of therapy and 12 in the third line or later), and 18 patients received only SOC agents or molecularly targeted therapies that did not match biomarkers (12 in the second line of therapy and six in the third line or later). The matched therapies included five patients on PARP inhibitors, four on HER2 inhibitors, and three on TKIs that target oncogenic fusions (Fig. 5B).

Kaplan–Meier estimates of median PFS (Fig. 5C; Supplementary Fig. S8) in the highly actionable patients that received

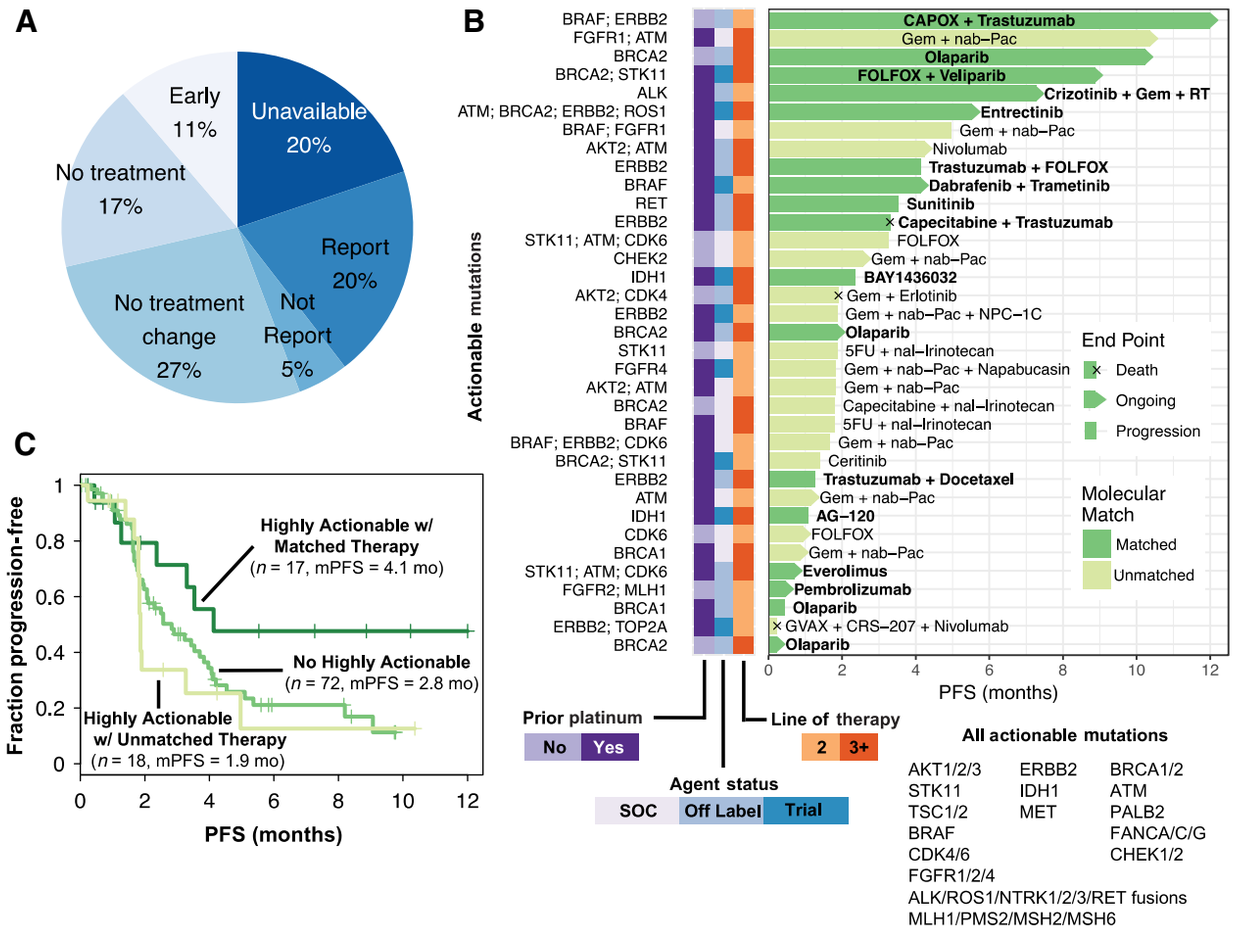

Figure 5.

Treatment outcomes in patients with identified highly actionable biomarkers. **A,** Across 640 patients that completed the KYT program, 126 (20%) have subsequently utilized a report-listed therapy. In the subset of these patients with highly actionable biomarkers ($n = 35$), 17 have utilized molecularly targeted therapies (PFS indicated by dark green bars in **B**), either off label or in clinical trials. **C,** Kaplan–Meier survival curves of patients with highly actionable alterations that received matched therapy had higher median PFS than patients with no highly actionable biomarker after correcting for line of therapy; $P = 0.03$.

matched therapies, highly actionable patients that did not receive matched therapies, and patients with no highly actionable biomarkers did not differ significantly in a univariate model (4.1, 1.9, and 2.8 months, respectively; log-rank $P = 0.13$). After correction for line of therapy, age, and gender using a Cox proportional hazards model with inverse propensity score weighting (Supplementary Table S7), the median PFS in highly actionable patients who received matched therapy was significantly longer than that of the highly actionable patients who received unmatched therapy (HR, 0.47; 95% CI, 0.24–0.94; $P = 0.03$). Patients with highly actionable alterations (regardless of whether treatment was matched) also had longer PFS than those who lacked highly actionable biomarkers (HR for highly actionable vs. no actionable markers was 0.65; 95% CI, 0.45–0.93; $P = 0.02$). The median PFS of patients that were placed on second-line chemotherapy (for all patients with and without highly actionable markers) was 3.4 months, which is comparable with historical controls derived from a trial of nanoliposomal irinotecan plus 5-fluorouracil [NAPOLI-1 trial (28): median PFS in experimental arm = 3.1 months; median PFS in control arm = 1.5 months].

The median OS in patients with highly actionable biomarkers who received molecularly matched therapies in any line of therapy was 1.5 years (Supplementary Fig. S9), compared with 0.9 years in the highly actionable/unmatched subgroup and 1.4 years in the subgroup with no highly actionable markers. Unlike PFS, the comparisons of OS in patients with highly actionable biomarkers versus patients with no highly actionable biomarkers, as well as the comparison of patients on matched therapy versus patients with highly actionable biomarkers on unmatched therapy, did not reach statistical significance (Supplementary Table S7).

Discussion

We demonstrate herein that a nationwide program using an integrated precision medicine operating system for precision oncology is quite feasible in a highly aggressive cancer type with considerable challenges in obtaining tumor tissue, regardless of the clinical setting and paucity of tumor sample. The process was successfully deployed for 640 patients from 112

high-volume academic hospitals and 175 community practices, demonstrating that the process is available for every patient with cancer.

In almost every case, multiomic information was successfully obtained. Commonly altered pathways included DNA repair (15%), cell cycle (11%), and AKT/mTOR (19%). These frequencies are largely consistent with multiple prior studies (5–12), as we have recently reviewed (29), although some genes were mutated at a higher frequency in the patients reported here compared with other published datasets. This likely reflects the lack of germline filtering in the FoundationOne assay, but is confounded by the possibility that the deeper sequencing of a targeted panel relative to whole-genome sequencing could uncover more variants. We did not observe major differences in mutation frequency between biopsies from primary and metastatic sites, consistent with recent reports (30).

One strength of the Perthera system is a multiomic profiling approach for assessing alterations to the same gene/protein/phosphoprotein that provides a potentially more robust pathway-centered view of the underpinning biology. For example, measuring the phosphorylation of HER2 may add value to NGS and IHC measurements as has been seen in breast cancer (31–35). The addition of protein phosphorylation measurements to genomic profiling may yield increased frequency of identification of actionable targets because activation of important drug targets was found in nearly all of the patients measured. Given the debate that exists in using IHC-based predictive markers, we were judicious in our selection of which markers to include, and to that end, we recently performed a comprehensive review of the literature for several of the chemopredictive biomarkers to support our choices (18). Utilizing a standardized operating process that incorporates multiomic data, including those from protein-based IHC, moves beyond a genomics-only view of cancer and provides a consistent process that can incorporate new protein-based predictive biomarkers in the future, such as hyaluronan (HA), which is a promising protein marker that could be important in selection of promising pancreatic cancer therapies (36).

Despite the high frequency of actionable biomarkers found, in this study 63% of patients were still placed on SOC regimens. While physicians and patients were not surveyed for the reasons behind therapeutic decision making, this lack of deviation from standard chemotherapy could be due to the difficulty of accessing clinical trials, lack of "level 1" evidence supporting the actionability of certain biomarkers, and challenges with payer reimbursement (37). In fact, that is one of the limitations observed in virtually all of the molecular profiling efforts for patients with pancreatic cancer, that there is no prospective evidence of benefit to molecularly targeted therapy. However, this most likely reflects the reality that such trials have not yet been completed (at least to a level that would qualify as "level 1 evidence"). Alternatively stated, there is no evidence that biomarker selected and molecularly targeted therapy has failed to demonstrate benefit for patients in pancreatic cancer, and in fact, there is a growing body of anecdotal and early phase clinical trials that have demonstrated benefit. For example, O'Reilly and colleagues demonstrated a highly promising 78% response rate for patients with BRCA-mutated pancreatic cancer treated with gemcitabine, cisplatin, and veliparib (38). We have recently submitted a case series of patients with pancreatic cancer that harbor NTRK or ROS1 fusions who benefitted from entrectinib (Pishvaian, et al., manuscript submitted); Dhir and colleagues discussed the benefit of crizotinib in a patient with pancreatic cancer with an ALK translocation (11); and Le and colleagues included four patients with MSI-high pancreatic cancer, two of whom benefitted from single-agent pembrolizumab (14).

With regards to broad profiling efforts, recent evidence suggests that survival is higher when patients with actionable findings are treated with biomarker-matched targeted agents compared with chemotherapy (39). A randomized clinical trial failed to show improvement in PFS (40), but several flaws were noted including inappropriate use of agents as monotherapy in settings where they would be expected to lack efficacy (41). When our analysis focused on patients with highly actionable biomarker findings and evaluated clinical outcomes between patients who were matched to the suggested therapy to those who were not, it was found that therapy-matched patients had significantly improved PFS (Supplementary Table S7). Patients with highly actionable biomarkers had improved PFS relative to patients lacking highly actionable biomarkers, independent of matched status of treatment, suggestive of a favorable prognostic effect. However, the comparison of matched versus unmatched patients within the highly actionable subgroup suggests that there is predictive significance as well. This was admittedly a small sample size of 17 patients who received matched therapy, and the results could be confounded by a potential influence of certain biomarkers (such as mutations in the homologous recombination, DNA damage repair genes) on prognosis, but the results are consistent with similar profiling efforts.

This finding supports the growing body of evidence that patients whose molecular profiling produces highly actionable findings should be considered for matched therapy whenever possible. This is even more germane to the care of patients with pancreatic cancer wherein ASCO (42) and NCCN guidelines prescribe the consideration of clinical trial-based experimental therapies in all lines of treatment. Given that over 25% of the KYT patients had highly actionable biomarker findings identified by our overall process and principally ranked treatment options based on those findings, it is evident that this is a critical outcome of the KYT program to date. Expansion of multiomic profiling efforts will include incorporation of germline testing as well as potentially incorporating CLIA/CAP RNA-Seq panels and expanded use of phosphoproteomic-based assays to identify even higher numbers of patients with highly actionable findings.

Finally, we report in this first cohort of patients from the KYT program that we have successfully encouraged 21% of patients with pancreatic cancer to enroll in clinical trials. These data suggest that patient-engaging programs like KYT coupled to effectively annotated treatment recommending systems for the oncologist may enhance accrual into clinical trials (43). Our study is nonrandomized with heterogeneously treated patients and clinical history, which may introduce bias, particularly due to selection of patients with an interest in clinical research and the potential for patients with poor performance status being ineligible for trials of targeted therapies. As a real-world evidence type study, we believe that programs such as ours play a critical and important role in precision medicine as a "signal finding" effort that can highlight important therapeutic target–drug combinations that warrant further exploration in more rigorous prospective studies.

Disclosure of Potential Conflicts of Interest

S. Mikhail is a consultant/advisory board member for Eisai. V.J. Picozzi reports receiving commercial research grants from Aduro, Immunomedics, Celgene, Oncomed, Lilly, Fibrogen, Halozyme, Tahio, Takeda, and Phoenix Bioscience, and holds ownership interest (including patents) in Amgen and Gilead. D. Sohal is a consultant/advisory board member for Foundation Medicine. E.M. Blais holds ownership interest (including patents) in Perthera. J.R. Brody is a consultant/advisory board member for Perthera. E.F. Petricoin is an employee of and holds ownership interest (including patents) in Perthera and Ceres Nanosciences, and is a consultant/advisory board member for Perthera, Ceres Nanosciences, ADVX Investors Group, and AzGen Scientific. No potential conflicts of interest were disclosed by the other authors.

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