Multiplatform molecular profiling of 2400 pancreatic adenocarcinomas to identify targets for therapeutic intent

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Abstract #4136

Background: Pancreas adenocarcinoma (PC) is a challenging disease with overall single digit 5-year survivorship. Few validated biomarkers exist for directing treatment in PC. Only one targeted agent (erlotinib) is FDA-approved and was developed in an unselected population (Moore, et al, J Clin Oncol, 2007). Identification of individual PC genomic and phenotypic profiles may yield targets with novel therapeutic application.

Methods: 2400 cases referred internationally to Caris Life Sciences were evaluated using a combination of sequencing (Sanger or next generation sequencing (NGS)), protein expression (immunohistochemistry), and/or gene amplification (CISH or FISH).

Results: The published PC molecular profile (24 cases, Jones, et al, Science, 2008) is consistent with the 2400 cases evaluated; KRAS was the most common mutation N=1190/1460 (82%) followed by TP53, N=175/310 (59%), and SMAD4, N=39/324 (12%).
Mutations in BRAF, EGFR, HER2, FLT3, HRAS, PDGFRA and PTEN were identified exclusively in KRAS WT cases.

	IHC			ISH	Sequencing										
	PR	RRM1	SPARC	HER2	BRAF	cKit	cMET	EGFR	HER2	FLT3	HRAS	PDGFRA	PTEN	SMAD4	TP53
KRAS MT (82%)															
Total N	1099	1092	1092	603	451	346	254	278	252	250	216	248	247	250	242
% Positive	2	21	35	4.5	-	1	4	-	-	-	-	-	-	14	65
KRAS WT (18%)															
Total N	248	248	248	111	87	71	50	54	49	50	43	49	49	49	47
% Positive	8	19	38	7.3	8	3	8	2	2	2	5	4	2	6	21

Conclusions: 18% of PC cases were KRAS WT, representing a significant minority of patients with PC. Aberrant signaling through the RAS/MAPK pathway through oncogenic mutations in HRAS, BRAF, EGFR, HER2 and PDGFR was found in a very small subset of KRAS WT cases (8%), and the likely benefit of anti-EGFR-based therapies is limited to those patients with KRAS wild-type tumors lacking downstream oncogenic activation of this pathway. IHC evaluation of certain markers, e.g., RRM1, SPARC, etc. may help select drugs and refine treatment decision-making for certain patients. Evaluating these profiles with clinical outcomes will provide valuable insight into the clinical behavior in genomically defined subsets and will assist in developing rational combinations of targeted agents in PC.

Methods

All 2400 pancreatic cancer cases underwent molecular profiling at Caris Life Sciences between 2008 and 2013. The original diagnosis of pancreatic cancer was obtained from the ordering physician and verified by a pathology team at Caris Life Sciences. Testing on formalin-fixed, paraffin-embedded tumor samples included a combination of immunohistochemistry (IHC; thresholds shown below), in situ hybridization (ISH) performed by either fluorescent or chromogenic methods, and Sanger or next-generation sequencing (NGS). All IHC results were read by a board-certified pathologist by measuring the intensity of the stain and percent staining. The KRAS testing included both Sanger and NGS. FISH was interpreted by a molecular cytogeneticist, while CISH was read by a board-certified pathologist. Clinical molecular geneticists provided the NGS interpretation. Statistical analysis was performed using JMP.

Biomarker	THRESHOLDS		
AR	=0+ or <10% or ≥1+ and ≥10%	PTEN	=0+ or ≤50% or ≥1+ and >50%
cKIT	=0+ and=100% or ≥2+ and ≥30%	RRM1	<2+ or <50% or ≥2+ and ≥50%
cMET	<50% or <2+ or ≥2+ and ≥50%	SPARC	<30% or <2+ or ≥2+ and ≥30%
ER	=0+ or <10% or ≥1+ and ≥10%	TLE3	<30% or <2+ or ≥2+ and ≥30%
HER2	≤1+ or =2+ and ≤10% or ≥3+ and >10%	TOP2A	=0+ or <10% or ≥1+ and ≥10%
MGMT	=0+ or ≤35% or ≥1+ and >35%	TOPO1	=0+ or <30% or <2+ or ≥2+ and ≥30%
PGP	=0+ or <10% or ≥1+ and ≥10%	TS	=0+ or ≤3+ and <10% or ≥1+ and ≥10%
PR	=0+ or <10% or ≥1+ and ≥10%	TUBB3	<30% or <2+ or ≥2+ and ≥30%
ERCC1	<2+ or ≤3+ and <10% or =2+ and <50% or ≥3+ and ≥10% or ≥2+ and ≥50%		

Results: Biomarker Status in PC

Figure 1. Distribution of immunohistochemistry (IHC), in situ hybridization (ISH), and DNA sequencing (NGS or Sanger) in 2400 pancreatic adenocarcinomas. The tables below show the overall distribution. and total cases tested. The numbers vary as technologies and test menu options changed over time. Percentages in red indicate cases where mutations were identified. Percentages in purple indicate cases where no mutations were identified. KRAS and TP53 mutation rates are consistent with findings in the literature.

Protein Expression, IHC

	AR	BCRP	c-kit	cMET	EGFR	ER	ERCC1	Her2	MGMT[$]	PDGFR
Total Positives	3	608	72	374	20	9	493	17	1581	140
Total Cases Tested	2054	664	1349	757	46	2075	1679	2142	2142	668
% Positive	0.1	91.6	5.3	49.4	43.5	0.4	29.4	0.8	73.8	21.0
	PGP[$]	PR	PTEN[$]	RRM1[$]	SPARC	TLE3	TOP2A	TOPO1	TS[$]	TUBB3
Total Positives	970	59	913	404	859	166	803	942	460	246
Total Cases Tested	1812	2063	2187	2100	2359	770	1850	2091	2106	501
% Positive	53.5	2.9	41.7	19.2	36.4	21.6	43.4	45.1	21.8	49.1

[$]Expression of the biomarker below the threshold is considered predictive of a positive response to therapy

Amplification, ISH

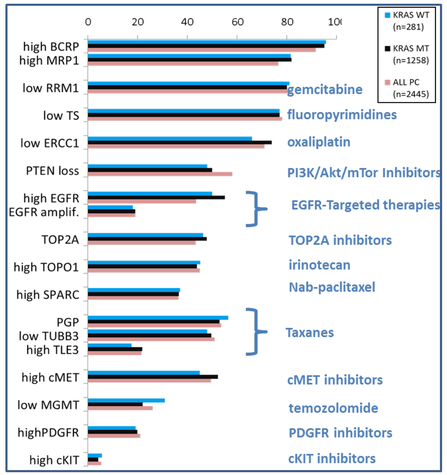
	cMET	EGFR	Her2	TOP2A
Total Positives	7	167	45	8
Total Cases	516	895	933	218
% Positive	1.4	18.7	4.8	3.7

DNA Sequencing

	ABL1	AKT1	ALK	APC	ATM	BRAF	CDH1	c-KIT	cMET	CSF1R	CTNNB1	EGFR	ERBB2	ERBB4	FBXW7
Total Positives	2	0	0	22	12	7	0	4	19	1	2	3	1	2	1
Total Cases Tested	384	397	398	395	386	647	399	516	398	396	398	434	393	395	392
% Positive	0.5	0.0	0.0	5.6	3.1	1.1	0.0	0.8	4.8	0.3	0.5	0.7	0.3	0.5	0.3
	FGFR1	FGFR2	FLT3	GNA11	GNAQ	GNAS	HNF1A	HRAS	IDH1	JAK2	JAK3	KDR	KRAS	MLH1	MPL
Total Positives	0	0	1	2	0	8	2	2	1	0	7	0	1260	3	0
Total Cases Tested	398	395	393	294	126	398	336	335	398	399	399	394	1539	399	394
% Positive	0.0	0.0	0.3	0.7	0.0	2.0	0.6	0.6	0.3	0.0	1.8	0.0	81.9	0.8	0.0
	NOTCH1	NPM1	NRAS	PDGFRA	PIK3CA	PTEN	PTPN11	RB1	RET	SMAD4	SMARCB1	SMO	STK11	TP53	VHL
Total Positives	0	0	0	2	14	2	0	2	0	45	0	0	6	224	3
Total Cases Tested	383	397	488	391	585	388	399	392	399	392	394	363	380	378	376
% Positive	0.0	0.0	0.0	0.5	2.4	0.5	0.0	0.5	0.0	11.5	0.0	0.0	1.6	59.3	0.8

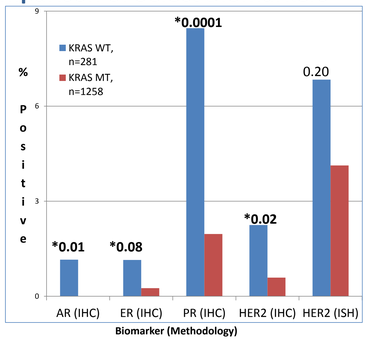
Differences between KRAS Wild Type and KRAS Mutated Cases

Figure 2. Differences in KRAS MT versus KRAS WT tumors based on IHC or EGFR amplification. Biomarkers were tested, regardless of KRAS status. The highest rates of protein overexpression are in BCRP, MRP1, MGMT, and PGP, which are all associated with drug resistance. The overexpression of drug pumps may help explain why pancreatic cancer responds poorly to traditional chemotherapy. The lowest protein expression rates were found in RRM1, TS, ERCC1, and cKIT. Associations to potential drug therapies are shown.



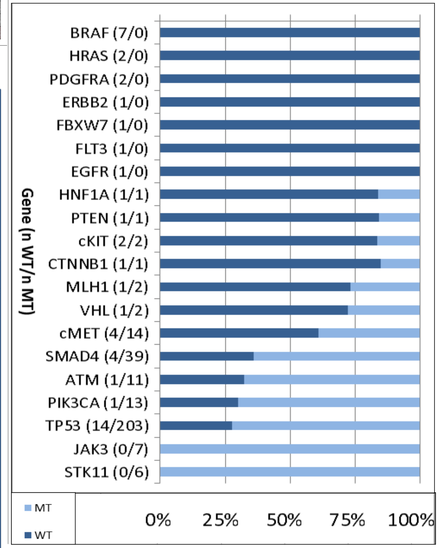
KRAS WT versus MT Comparison of Select Biomarkers

Figure 3. Distribution of AR, ER, PR, and HER2 in KRAS wild type versus KRAS mutated pancreatic cancer. The figure on the right shows hormonal biomarkers and HER2. As shown, KRAS WT tumors show *statistically significant* higher rates of protein expression for AR, HER2, and PR. For ER IHC the protein expression difference did not quite reach statistical significance, while HER2 amplification rates were not significantly different. The value of hormonal biomarkers in PC is unknown. However, pancreatic cancers with HER2 overexpression and/or amplification are potential candidates for newer HER2-targeted therapy.



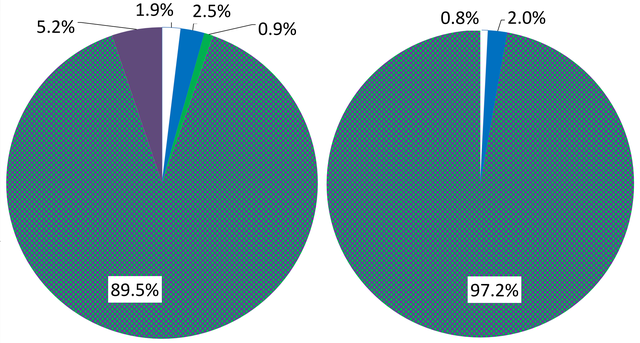
KRAS WT versus MT Comparison of Gene Alterations: mutation ratio

Figure 4. Differences in Sequence Analysis Between KRAS mutated and wild type pancreatic cancer. Testing was performed using Sanger and/or NGS. KRAS WT cases with results=58; KRAS MT=398. Not all cases were tested for KRAS (insufficient tissue or other reason). Only genes where the difference in mutation rate was greater than 0.5% between KRAS WT and KRAS MT are shown (excludes ABL1, APC, CSF1R, ERBB2, ERBB4, GNA11, GNAS, IDH1, and RB1). Genes not mutated in any of the tested pancreatic adenocarcinomas are also omitted (AKT1, ALK, CDH1, FGFR1, FGFR2, GNAQ, JAK2, KDR, MPL, NOTCH1, NPM1, NRAS, PTPN11, RET, SMARCB1, SMO). Most of the NGS biomarkers shown are potentially actionable, especially in the setting of clinical trials recruitment. Clinical trials are currently investigating PARP inhibitors in pancreatic cancer, which tend to have defective homologous recombination in biomarkers like ATM and MLH1.



Multiplatform identification of actionable targets

Figure 5. Potentially actionable targets identified using a multiplatform approach. Drug associations are determined using Caris Molecular Intelligence™ recommendations based on biomarker status and published evidence, which includes peer-reviewed literature and/or NCCN Guidelines, but independent of cancer type. KRAS WT cases (B) with associated protein alterations and gene mutations have increased potential for targeted therapies (97.5%) compared to PC cases overall (A: 89.5%). On average, 8 therapies were associated with benefit, per case, based on biomarker status.



Conclusions

- Using a multiplatform approach, the distinct molecular profiles exhibited by KRAS WT and KRAS-mutated patients highlight the need for a more personalized approach to this aggressive disease. The differences shown even, within pancreatic cancer subpopulations (e.g. KRAS WT versus mutated populations), illustrate tumor heterogeneity.
- Erlotinib, in combination with gemcitabine, may be more beneficial in KRAS wild type PC, as EGFR mutations are seen only in KRAS WT. The addition of gemcitabine to agents like erlotinib or nab-paclitaxel is bolstered by the absence of RRM1 protein expression.
- TP53 mutations are seen in 75% fewer pancreatic cancer KRAS WT patients and suggest improved survival in this subpopulation.
- Higher protein expression rates , increased gene copy number, and gene mutations in HER2 (ERBB2) were more common in the KRAS WT cohort, making newer HER2-targeted therapy a consideration to improve outcomes in this population.
- Profiling may assist in the clinical trial recruitment. Mutations in ATM, for instance, can be used to enroll PC patients into PARP inhibitor trials. SMAD4 mutations could lead to targeting the TGF-B pathway. The need to find newer, better agents is especially acute in KRAS-mutated patients.
- Hormone and/or HER2 targeted therapies may be of benefit in KRAS WT PC.

References

1. Moore, MJ, W Parulekar, et al. (2007). « Erlotinib Plus Gemcitabine Compared With Gemcitabine Alone in Patients With Advanced Pancreatic Cancer: A Phase III Trial of the National Cancer Institute of Canada Clinical Trials Group ». J Clin Oncol. 25:1960-1966.
2. Jones, S, KW Kinzler, et al. (2008). «Core Signaling Pathways in Human Pancreatic Cancers Revealed by Global Genomic Analyses ». Science. 321:1801-1806.