Actionable Targets in Pancreatic Cancer Detected by Immunohistochemistry (IHC), Microarray (MA) Fluorescent *in situ* Hybridization (FISH) and Mutational Analysis

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I. Abstract

Background: A great need exists for new therapeutic approaches for patients with pancreatic cancer.

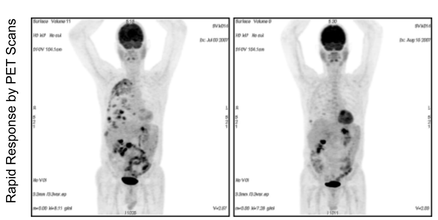
Methods: The study cohort included 1029 patients analyzed for a.) up to 29 different immunohistochemical biomarkers – (e.g. COX-2, MGMT, PGP, RRM1, TOPOI, TOP2A, SPARC etc.); b.) in up to 450 patients' specimens a whole genome expression analysis was performed using HumanHT-12 v4 beadChips (Illumina Inc., San Diego, CA); c.) in up to 695 patients FISH for c-Myc, EGFR, HER2 and TOP2A gene copy amplifications; and d.) in up to 783 patients sequencing for KRAS, EGFR, PIK3CA and BRAF, was performed.

Results: IHC identified actionable targets included; 74% high COX-2; 57% negative ERCC1; 8% negative MGMT; 22% negative MRP1; 47% negative PGP; 77% low RRM1, 44%high SPARC; 30% high TOP2A; 61% high TOPOI and 73% negative TS. Other biologically important findings by IHC for possible new therapeutics included 27% negative PTEN; and 20% high PDGFR. Microarray results presented multiple overexpressed targets for consideration including 36% of specimens with overexpressed adenosine deaminase; 28% asparagine synthase; 17% BCL2; 20% survivin; 23% carboxylesterase; 67% DNMT1; 40% thymidine phosphorylase; 49% EPHA2 (and others in the src family of kinases); 57% FOLR2; 41% HDAC1; 62% HIF1α; 23% IL2RA (CD25); 46% NFkB1; 48% OGFR; 32% RARA; 26% VEGFR; and 43% vitamin D receptor. FISH yielded 2% amplified EGFR and 10% amplified Her2/neu. Sequencing noted 73% mutated KRAS and 3% mutated PIK3CA.

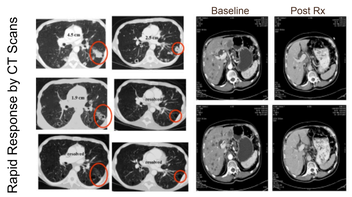
Conclusions: Examining actionable targets in patients' pancreatic cancers (a)reiterates the commonality and importance of KRAS mutations in this disease (needs renewed targeting effort); (b)suggests that TOPO2 inhibitors (particularly if transport into tumor can be improved) should be examined in this disease; (c)suggests other pathways to target including DNA repair, epigenetic, Src and inflammation; (d) suggests protein turnover, amino acid targets and folate receptor 2 as fresh areas to explore against the disease. Supported in part by a Stand Up To Cancer Dream Team Award and by Caris Life Sciences.

II. Background

- New therapeutic approaches are needed
- Prior experience profiling pancreatic cancer taken directly from 16 patients yielded clinically significant target – SPARC (secreted protein acid rich in cysteine)[1,2]
- There was improved tumor accumulation of nab-paclitaxel (albumin-bound 30 nanometer particle form of paclitaxel) through the albumin-binding SPARC[3]
- When nab-paclitaxel was combined with gemcitabine there was substantial clinical activity[4]

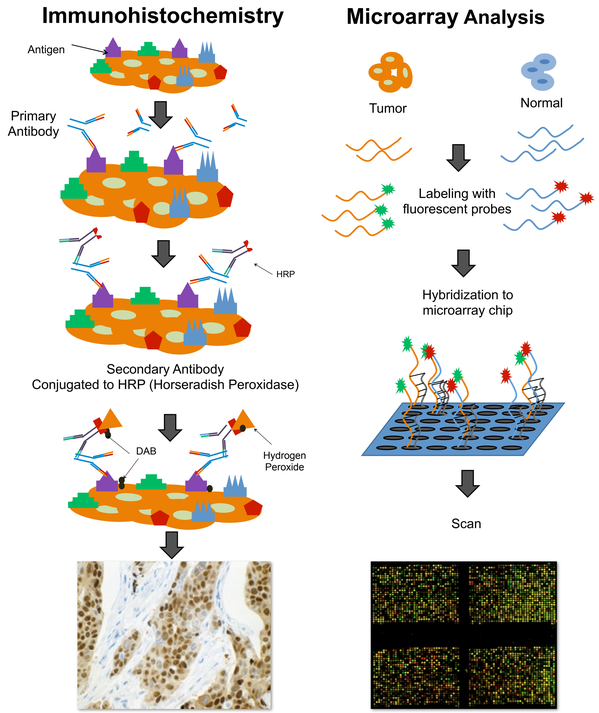


II. Background (continued)



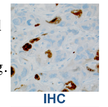
- Because of continued profiling with the Caris Target Now™ we have data on 1029 patients' pancreatic cancer specimens
- High likelihood of other actionable targets (with more certainty because of larger sample size)

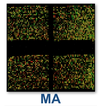
III. Methods

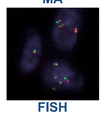


III. Methods (continued)

- Tumors received from multiple sites around the world were examined
- Immunohistochemistry (IHC) – up to 29 different IHC biomarkers e.g. COX2, MGMT, etc)
- Whole genome expression analysis human HT-12 V4 bead chips (Illumina Inc), San Diego, CA
- Florescent *in situ* hybridization (FISH) for gene copy number
- Mutational analysis (sequencing) of specific genes
  -Sanger sequencing method







IV. Results

A. Potential targets by Immunohistochemistry (up to n=1029 patients)

Marker	Percent Actionable
RRM1 (↓)	77%
COX2 (↑)	74%
TS (neg)	73%
TOPO 1 (↑)	61%
ERCC1 (neg)	57%
PGP (neg)	47%
SPARC (↑)	44%
TOP2A (↑)	30%
PTEN (neg)	27%
MRP1 (neg)	22%
PDGFR (↑)	20%
MGMT (neg)	8%

B. Potential targets by Microarray (up to n =450)

Marker	% overexpressed	Marker	% overexpressed
LCK*	72%	HDAC1	41%
DNMT1	67%	Thymidine phosphorylase	40%
HIF1α	62%	Adenosine deaminase	36%
HCK*	62%	RARA	32%
LYN*	60%	Asparagine synthase	28%
FOLR2	57%	VEGFR	26%
EPHA2*	49%	IL2RA (CD25)	23%
OGFR (opioid growth factor)	48%	Carboxylesterase	23%
NFkB	46%	Survivin	20%
FYN*	45%	BCL2	17%
Vitamin D receptor	43%	YES1*	9%

* = Src family member

IV. Results (continued)

C. Fluorescent in-situ Hybridization (FISH) (up to n= 695)

Target	% Amplified
C-Myc	33%
Her2neu	10%
EGFR	2%
TOP2A	0%

D. Mutational Analysis (sequencing) (up to n= 783)

Gene Target Sequenced	% Mutated
KRAS	73%
PIK3CA	3%
BRAF	0%
cKit	0%

V. Conclusions

A. need renewed effort to target mutated KRAS

B. Confidence in data is based on finding of known actionable targets (e.g. ERCC1 (neg)-platinum; RRM1(↓) – gemcitabine; SPARC(↑) – nab-paclitaxel; TOPO1 (↑) - irinotecan; TS (neg) – 5FU [Although clinical response rates to those single agents are lower than the incidence of the targets]

C. most generalizable targets (>30%) (not already addressed clinically)

• KRAS	73%
• COX2	74%
• DNMT1	67%
• SRC family	9-72%
• HIF1α	62%
• Folate receptor 2	57%
• OGFR (opioid growth factor)	45%
• Vitamin D receptor	43%
• HDAC1	41%
• Adenosine deaminase	36%
• CMYC	33%

D. Need follow up clinical trials to address several of the generalizable targets (epigenomic, repair, inflammation, purine, pyrimidine metabolic pathways, SRC, FOLR2)

VI. References

1. Von Hoff DD and colleagues, J. Clin Oncol. 2006; 18s, 138s
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3. Trieu V and colleagues, New Targets and Delivery Systems for Cancer Diagnosis and Treatment (SVCC) 2007 San Diego Abstract #53
4. Von Hoff DD and colleagues, J Clin Oncol. 2011, 34: 4548-4554
5. Moertel CG. NEJM. 286:813-815, 1972.