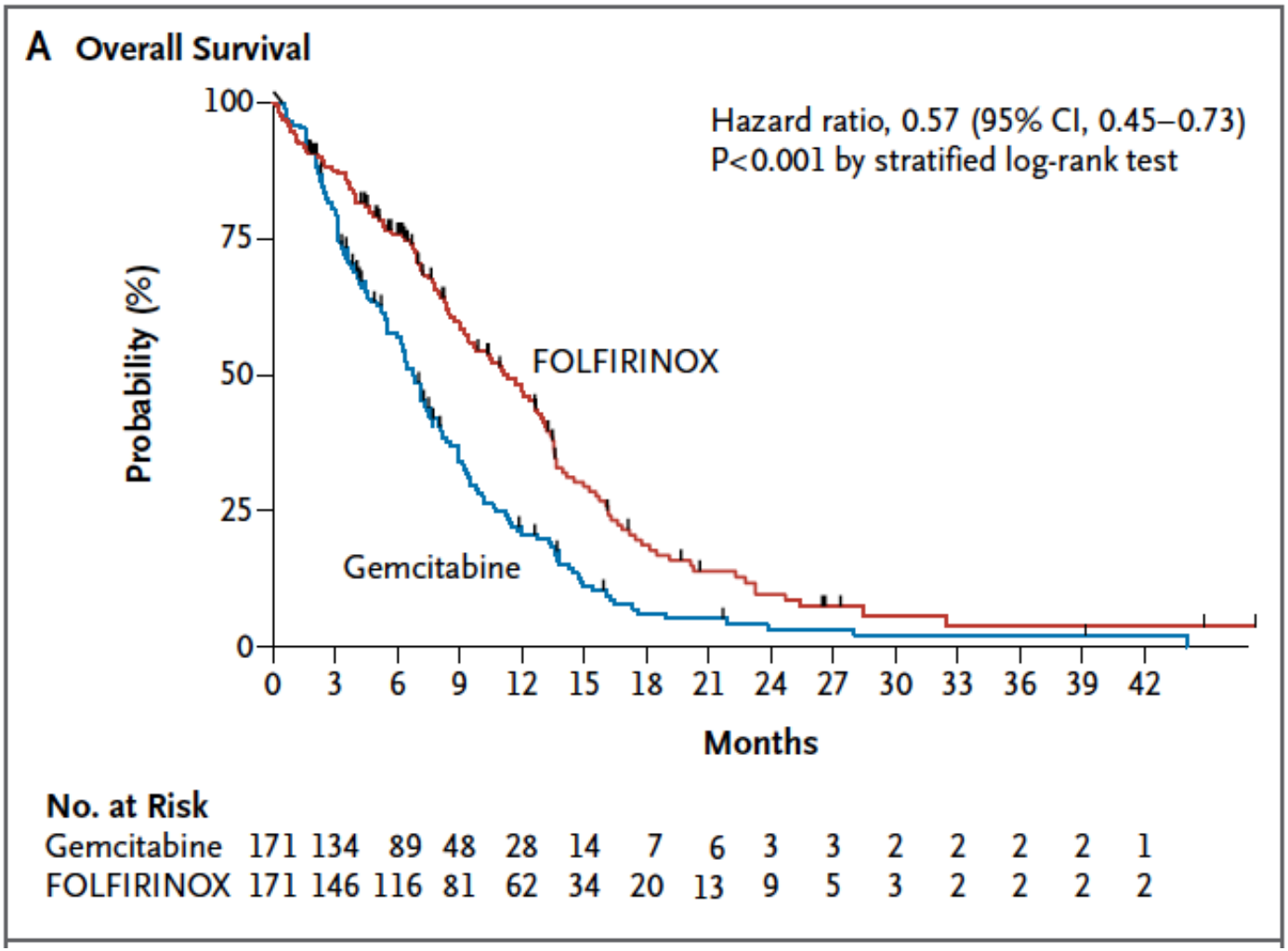
Pancreatic Cancer: Prospect for Personalize Treatment

Manuel Hidalgo, M.D., Ph.D.

Disclosure

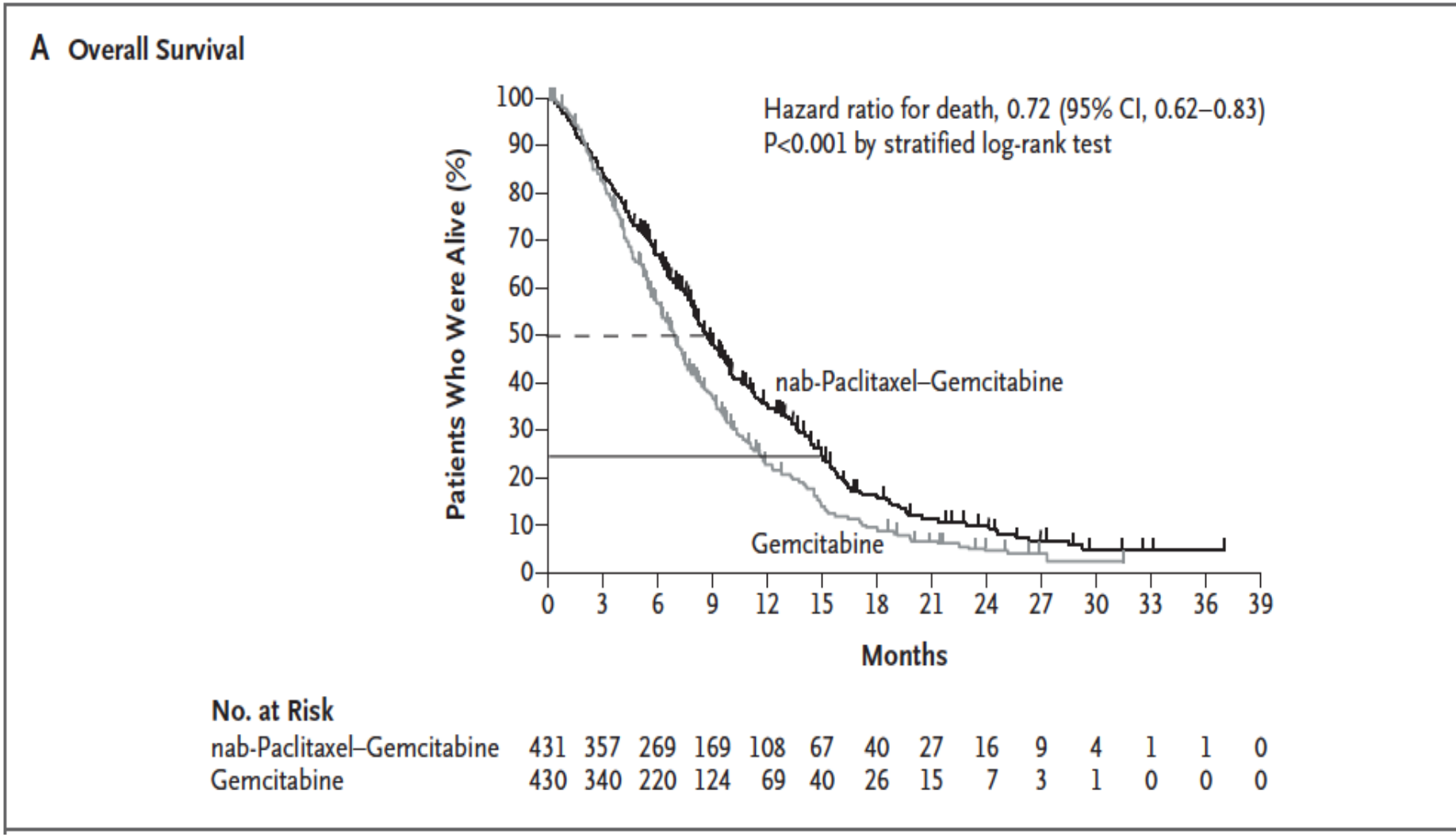
- Champions Oncology
  - Founder and Stock Holder

Overall Survival



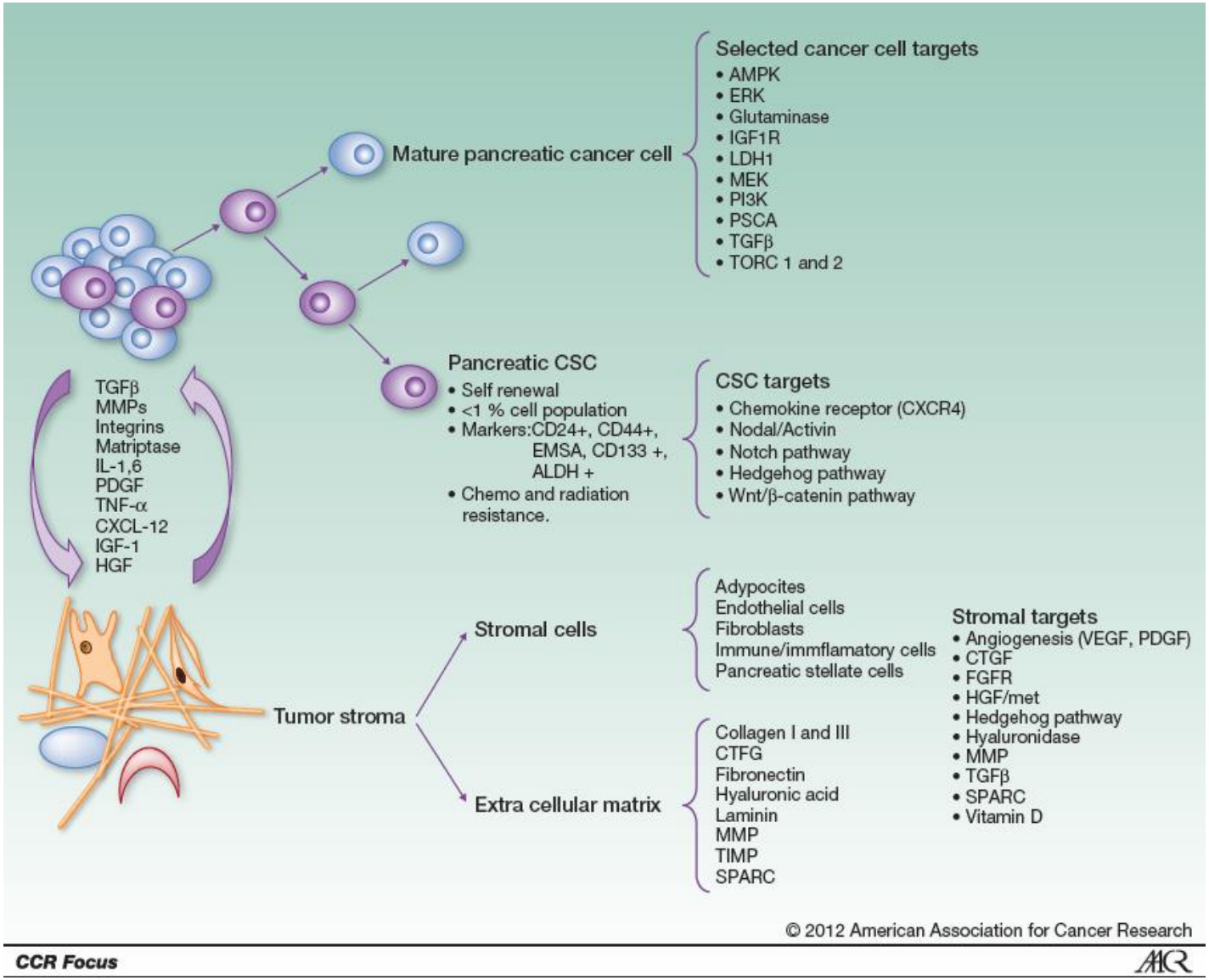
Conroy et al, NEJM 2011

Overall Survival



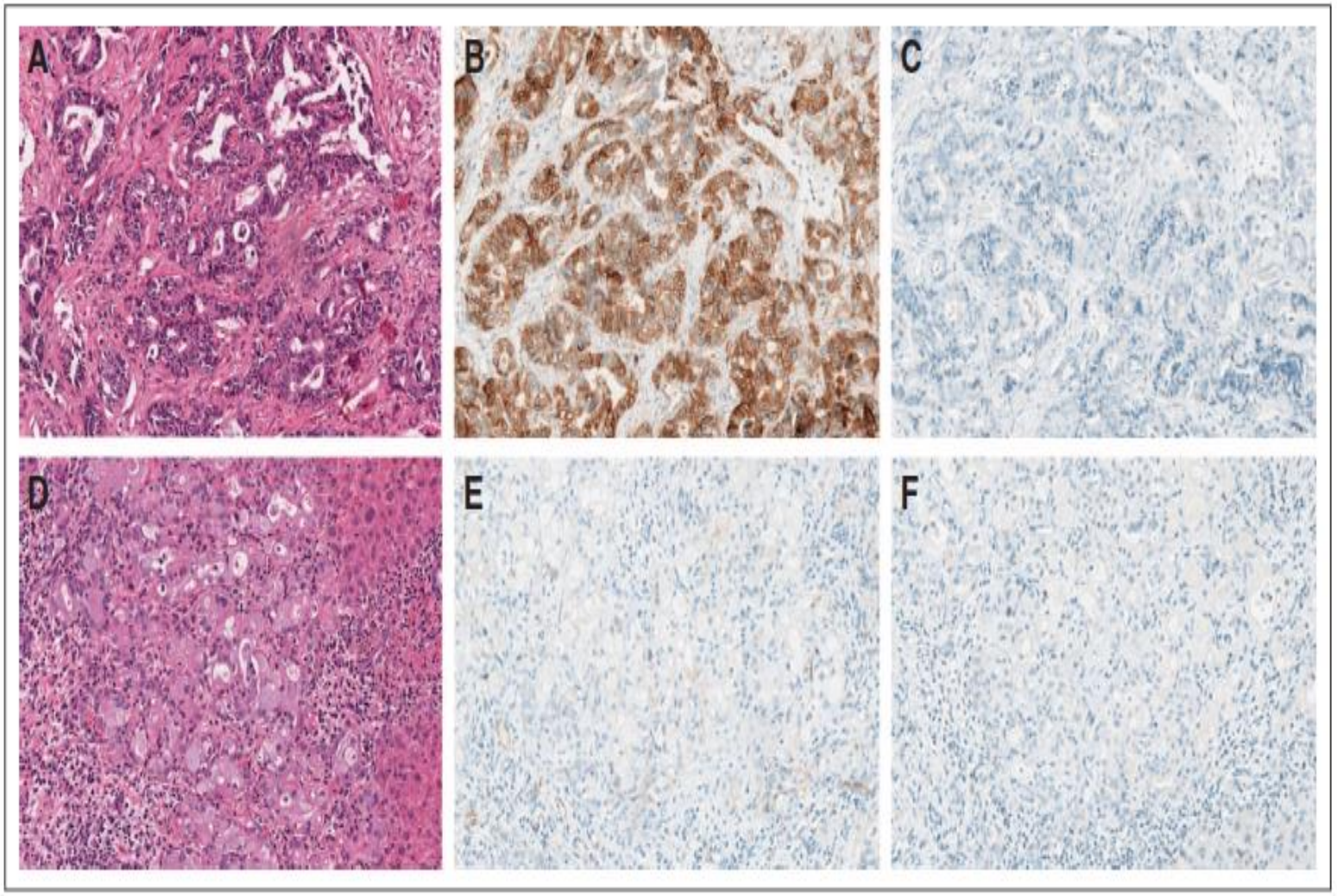
Von Hoff et al., NEJM 2013

Multifaceted Biology of PDA



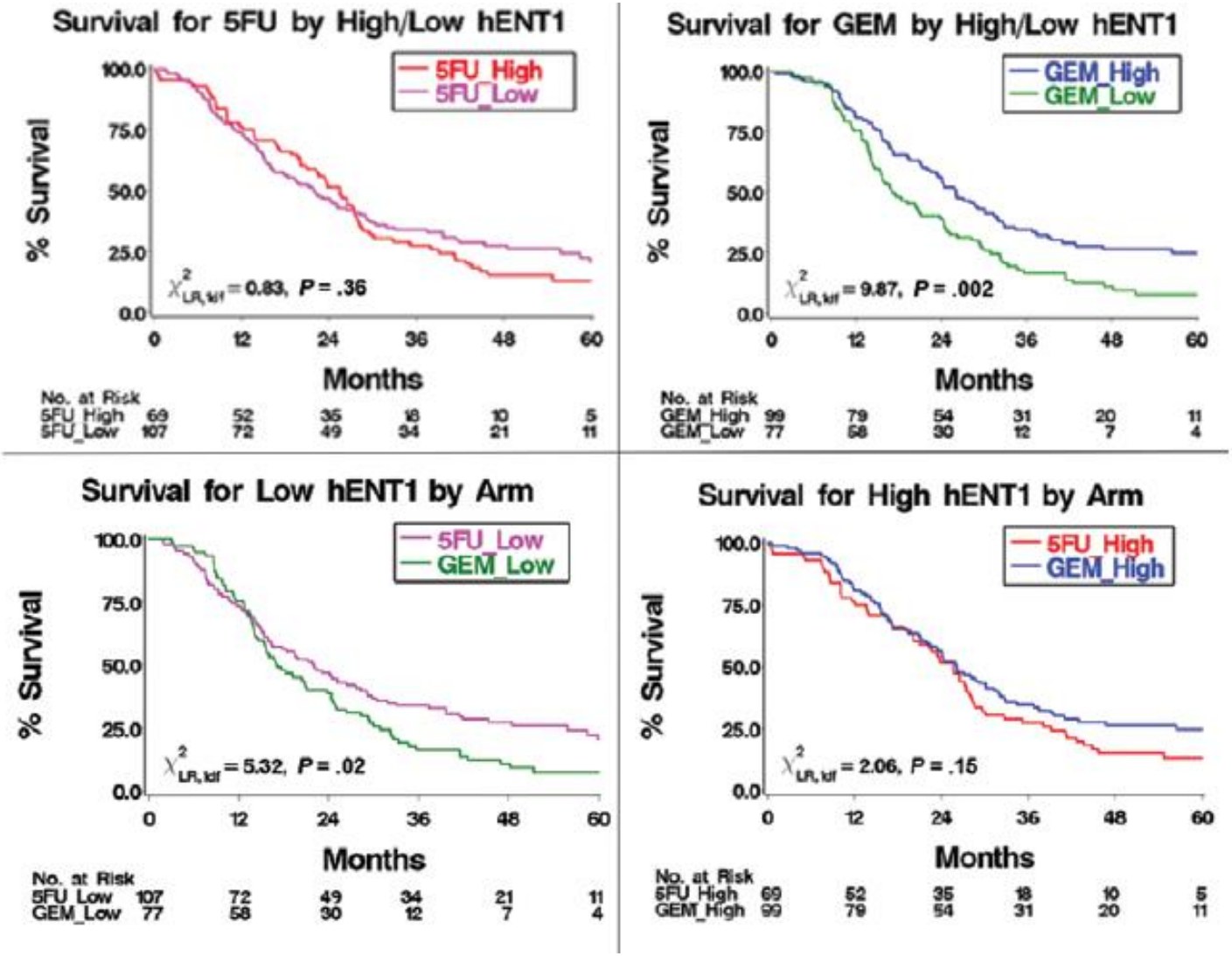
M Hidalgo, CCR 2012

hENT1 Expression in PDAC



Poplin et al, JCO 2013

hENT1 Expression in ESPAC-3



Greenhalf et al, JNCI 2013

SPARC Expression in Pancreatic Adenocarcinoma: Development of a Robust, Predictive Immunohistochemical Assay and Scoring Method.

Illei PB[1], Conde E[2], Dominguez N[2], Plaza C[2], Redondo P[2], Suárez-Gauthier A[2], Heise C[3],Hidalgo M[2],, López-Rios F[2]
[1]The Johns Hopkins Hospital, Baltimore, [2]Laboratorio de Dianas Terapeuticas, Madrid and [3]Celgene Corporation, San Francisco.

ABSTRACT

Background: SPARC is a glycoprotein involved in the regulation of extracellular matrix deposition and remodeling. Our aim was to develop a robust SPARC IHC assay and scoring method that would be adaptable and reproducible in pathology laboratories worldwide. Overexpression of SPARC assessed by IHC has been associated with poorer prognosis and improved survival in patients with advanced pancreatic carcinoma (PC) treated with gemcitabine plus nab-paclitaxel, an albumin-containing formulation of paclitaxel (Celgene, Summit, NJ), in a single arm Phase I/II trial (VonHoff et al., 2012). Another objective was to assess value for predicting response to this therapy using tissue of the Phase I/II clinical trial.
Design: Three SPARC antibodies were evaluated on formalin-fixed paraffin-embedded tissues of 50 resected PC. (Invitrogen clone ON-1; Novocastra clone 15G12; and Sigma-Aldrich polyclonal). Staining was assessed in tumor and stromal cells. Based on these results, a novel scoring method was developed and 20 samples from the nab-paclitaxel plus gemcitabine Phase I/II trial stained using the Invitrogen antibody and a Ventana Benchmark Ultra autostainer by two laboratories (Baltimore and Madrid). Slides were scored using a published method (Infante et al., 2007) and a novel method similar to HER2 assessment in gastric carcinomas (Ruschoff J et al., 2010) All readers were blinded to each other and to the clinical data.
Results: The Invitrogen antibody had the most intense and specific staining in the carcinoma and stromal cells. Concordance was high (85-95%) for all scoring criteria between the different pathologists. Kaplan-Meier survival analysis of overall survival (OS) using the Infante method showed no significant difference between positive and negative tumors. In contrast, Kaplan-Meier survival analysis of OS according to stromal SPARC expression using the novel scoring method showed significant survival benefit in tumors with SPARC positive stroma.
Conclusion: The Invitrogen antibody produced the most specific stain and was the easiest to interpret. These preliminary results also suggest that SPARC expression analysis using IHC and a novel scoring method is reproducible. This IHC methodology will be used to assess the correlation of clinical outcome with SPARC expression from a randomized Phase 3 trial of nab-paclitaxel followed by gemcitabine versus gemcitabine alone in metastatic PC.

INTRODUCTION

SPARC (secreted protein acidic and rich in cysteine) is a glycoprotein involved in the regulation of extracellular matrix deposition and remodeling. Nab-paclitaxel, an albumin-bound formulation of paclitaxel particles (Celgene, Summit, NJ), has shown clinical activity in different tumor types, including pancreatic adenocarcinoma (PC). Specifically, in patients with PC the overexpression of SPARC assessed by immunohistochemistry (IHC) has been associated with (a) poorer prognosis in resectable PC and (b) improved survival in patients with advanced PC treated with gemcitabine plus nab-paclitaxel (VonHoff et al, JC), 2012). The goals of this study were to develop a robust SPARC IHC assay and scoring method, and to assess its value for predicting response to nab-paclitaxel based therapy using archived tissue of the Phase I/II clinical trial.

METHODS

Three SPARC antibodies were evaluated on 50 formalin-fixed paraffin-embedded resected PC. (Invitrogen clone ON-1; Novocastra clone 15G12; and Sigma-Aldrich polyclonal). Staining was assessed for intensity and extent (percent) in both tumor and stromal cells. Based on these results, a novel scoring method was developed and 20 samples from the gemcitabine plus nab-paclitaxel Phase I/II trial were stained using the Invitrogen antibody and a Ventana Benchmark Ultra autostainer by two laboratories (Baltimore and Madrid).

Table 1

Scoring criteria

Infante scoring criteria	Novel Method
Positive:	Positive:
• Intensity moderate (++), to strong (+++)	• Intensity moderate (++), to strong (+++)
• Extent ≥10%	• Extent ≥25%
Negative:	Negative:
• Intensity absent to weak (+)	• Intensity absent to weak (+)
• Extent of stain < 10%.	• Extent of stain < 25%.

Scoring guide for novel method

Positive (+++): Staining seen at 2-4X magnification in >50% of stroma
Positive (++): Staining seen at 10-20X magnification in ≥25% of stroma
Negative (+): No staining or only seen at 40X magnification in <25% of stroma

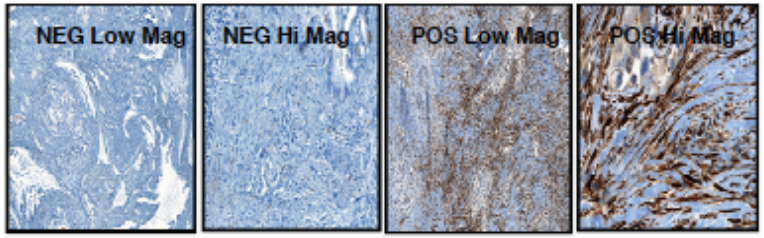


Figure 1. Examples of SPARC IHC stains with the Invitrogen ON-1 antibody

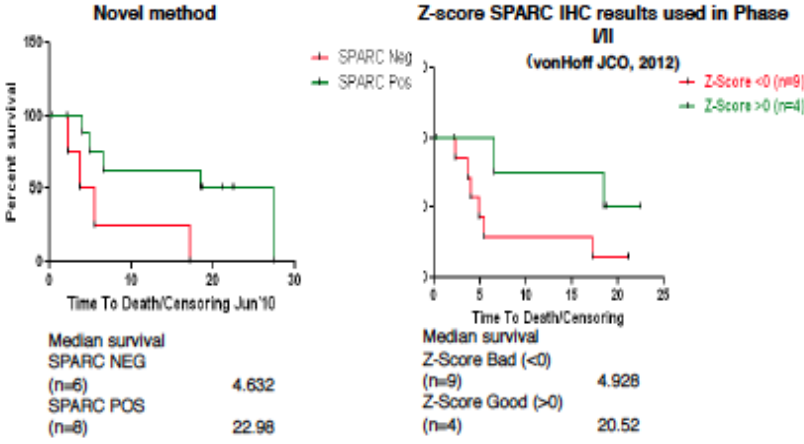


Figure 2. SPARC expression with ON-1 IHC and novel scoring confirms association with overall survival in a subset of samples from the Phase I/II study of nab-paclitaxel + Gemcitabine

METHODS Cont-d

Staining was scored using a previously published method (Infante *et al.*) and a novel method based on the proposed scoring system for assessing HER2 in gastric carcinomas (Ruschoff J *et al.*) that are summarized in Table 1. All three readers were blinded to each other and to the clinical data.

RESULTS (cont)

- The polyclonal antibody had the least staining in cells. The Invitrogen antibody had the most intense and specific staining in the carcinoma and stromal cells. (Figure 1)

- For the 50 cases of PC, concordance was high for all scoring criteria between the three different pathologists.
  92% (46 of 50) Infante method
  90% (45 of 50) Novel method

- Of the 20 cases from the Phase I/II trial, only 14 had evaluable tissue. Kaplan-Meier analysis of overall survival using the novel scoring method confirmed the survival advantage observed in the Phase I/II trial (Figure 2).
  High concordance was observed for these cases with both scoring methods

CONCLUSIONS & FUTURE DIRECTIONS

- The Invitrogen antibody produced the most specific stain and was the easiest to interpret.
- These preliminary results suggest that SPARC expression analysis using IHC with Invitrogen ON-1 antibody, and a novel scoring method is technically reproducible.
- This IHC methodology will be used to assess the correlation of clinical outcome with SPARC expression from a randomized Phase 3 trial of nab-paclitaxel followed by gemcitabine versus gemcitabine alone in metastatic PC.

Abstract Confirmation #: 2033
Abstract Publication #: 1771
Poster # 166

Stromal SPARC - Multivariate Analysis in SPARC *Evaluable Population*

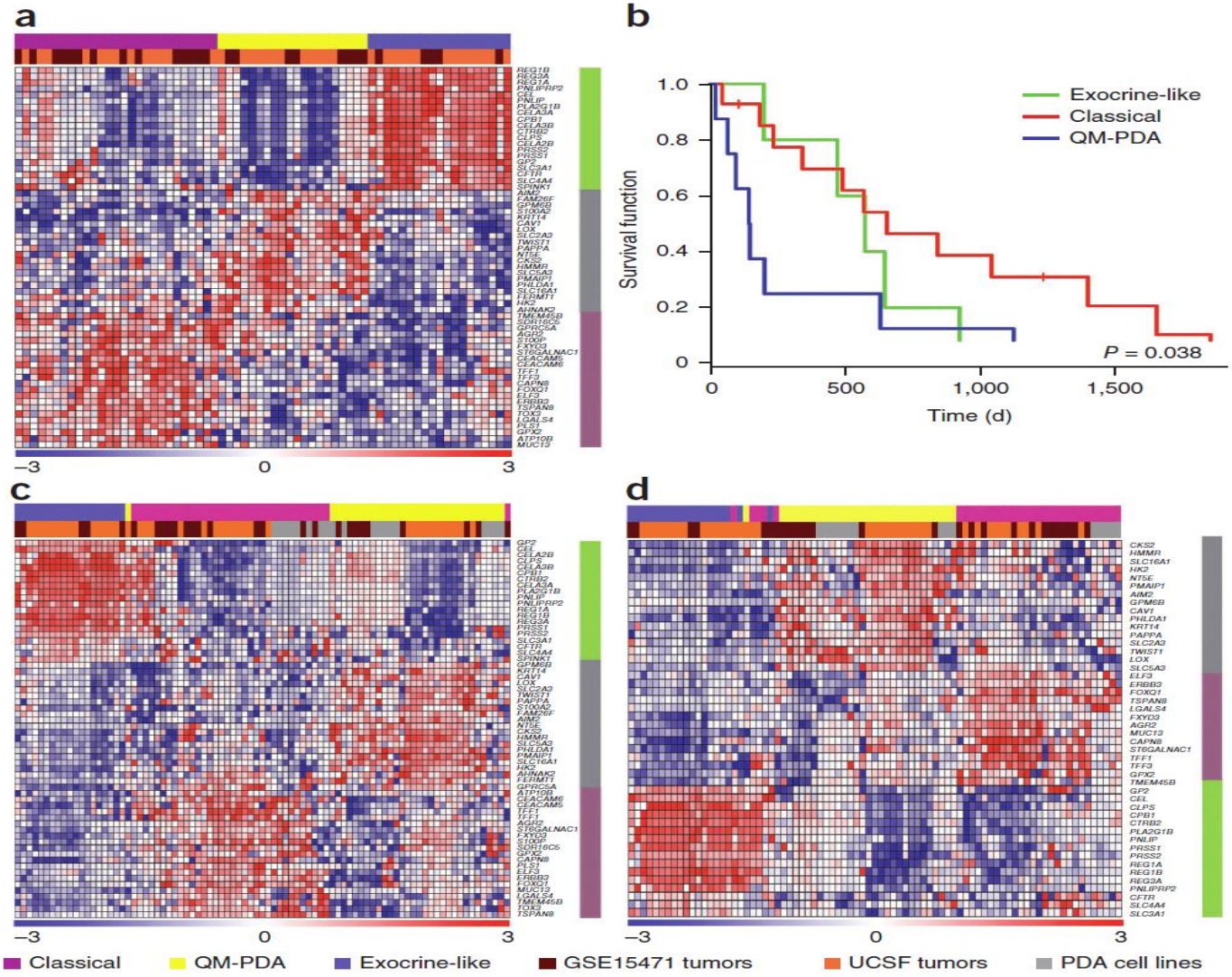
Covariate[a]	HR	95% CI	*P* Value
Treatment Group (*nab*-P + Gem vs Gem)	0.65	0.47 - 0.89	0.007
KPS (70-80 vs 90-100)	1.50	1.09 - 2.06	0.012
Presence of Liver Metastases (Yes vs No)	2.12	1.31 - 3.41	0.002

- In a multivariate analysis, stromal SPARC was not a significant, independent predictor of overall survival[1]
- Treatment, Karnofsky performance status, and presence of liver metastases were significant predictors of OS, consistent with the ITT analysis[2]

[a] A stepwise procedure was carried out using the following factors: treatment group, age, sex, KPS, Geographic region, pancreatic cancer primary location, presence of biliary stent, previous whipple procedure, presence of liver metastasis, presence of pulmonary metastasis, peritoneal carcinomatosis, stage at diagnosis, number of metastatic sites, baseline level of CA 19-9, and stromal SPARC.

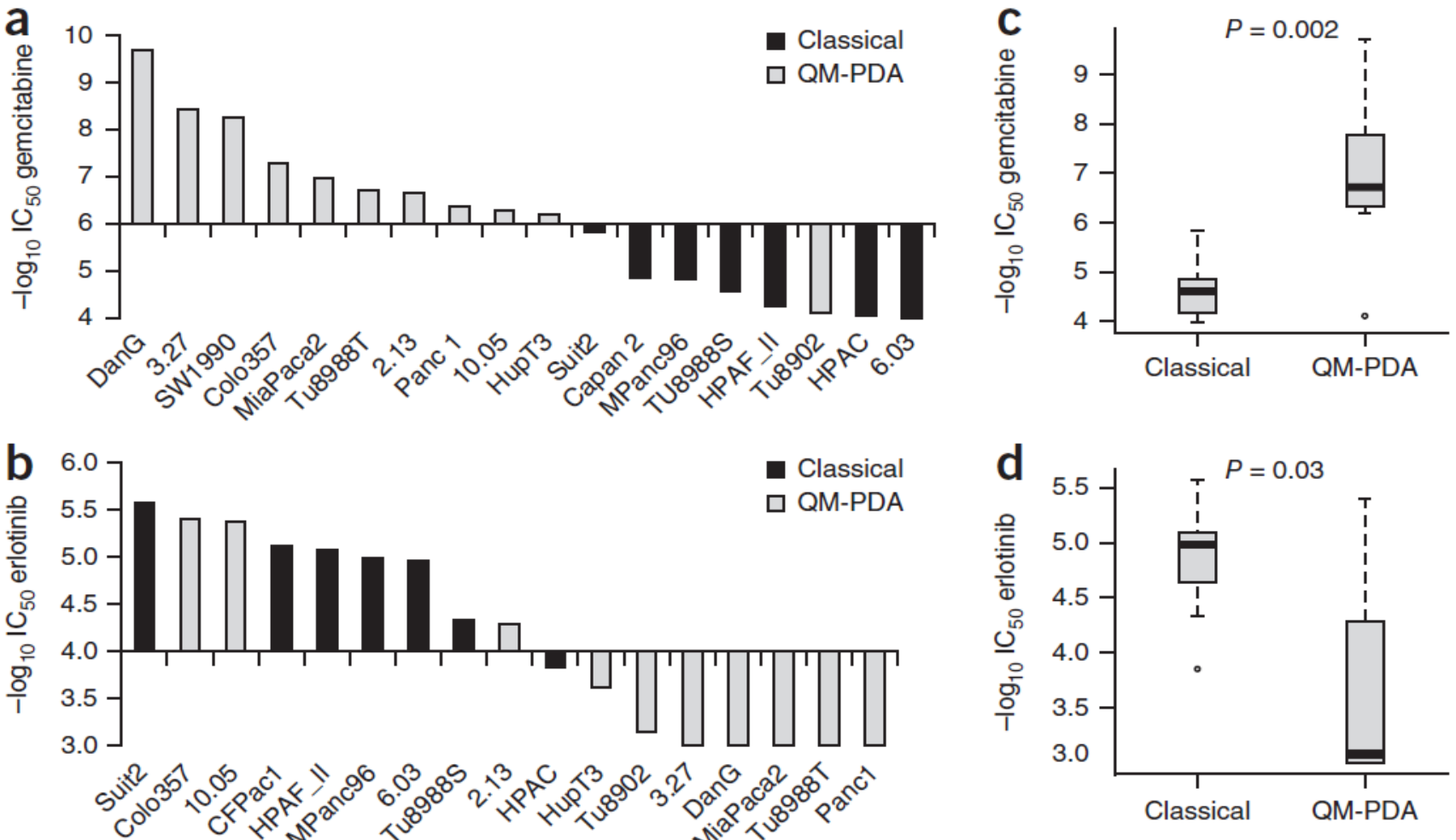
Hidalgo M, et al. Oral presentation at: World GI 2014 [abstract O-0004]. 2. Von Hoff DD, et al. *N Engl J Med*. 2013;369:1691-1703.

Molecular Subtypes of PDA



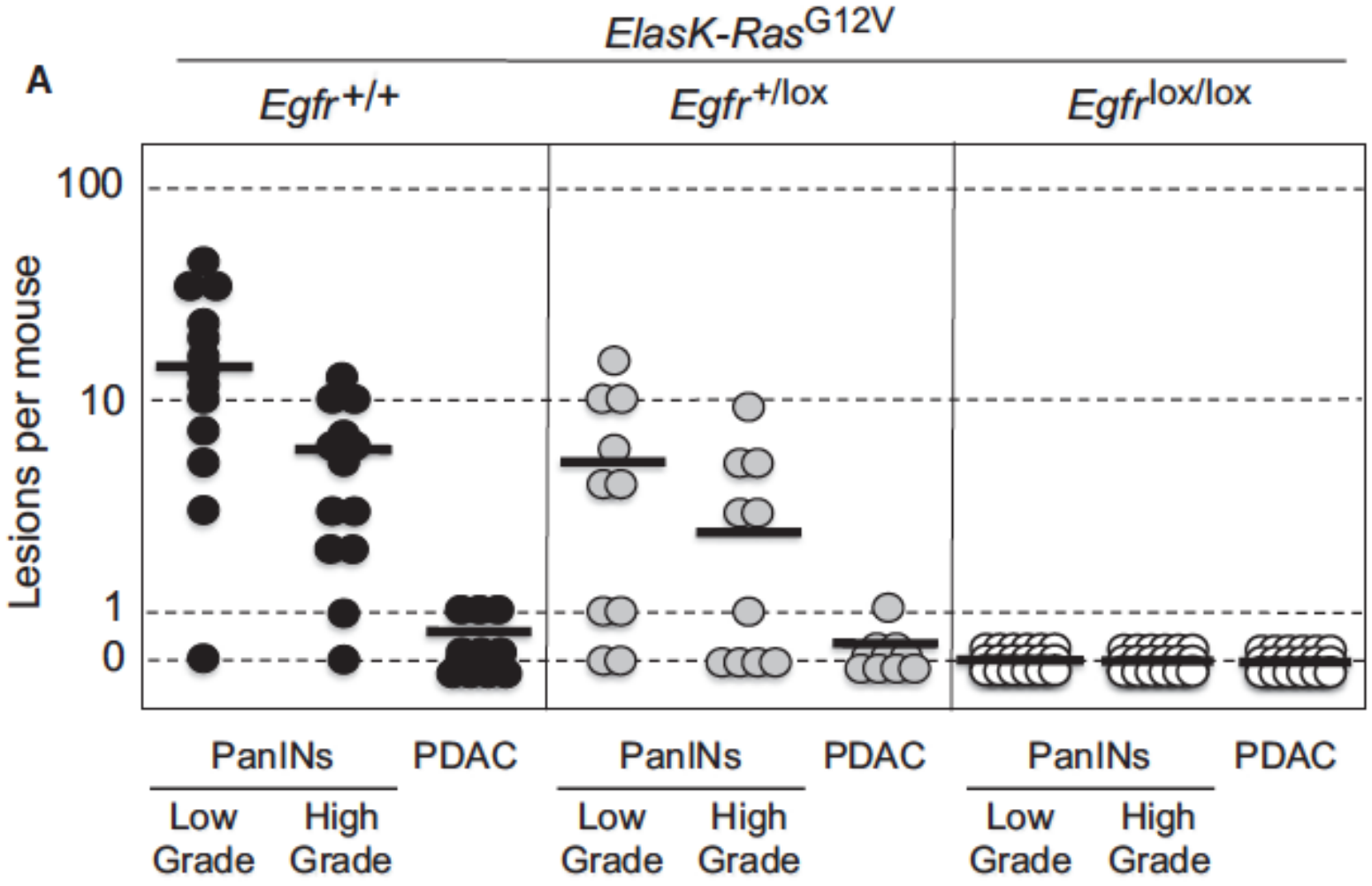
Collison et al, Nat Med 2011.

Drug Response in Molecular Subtypes of PDA



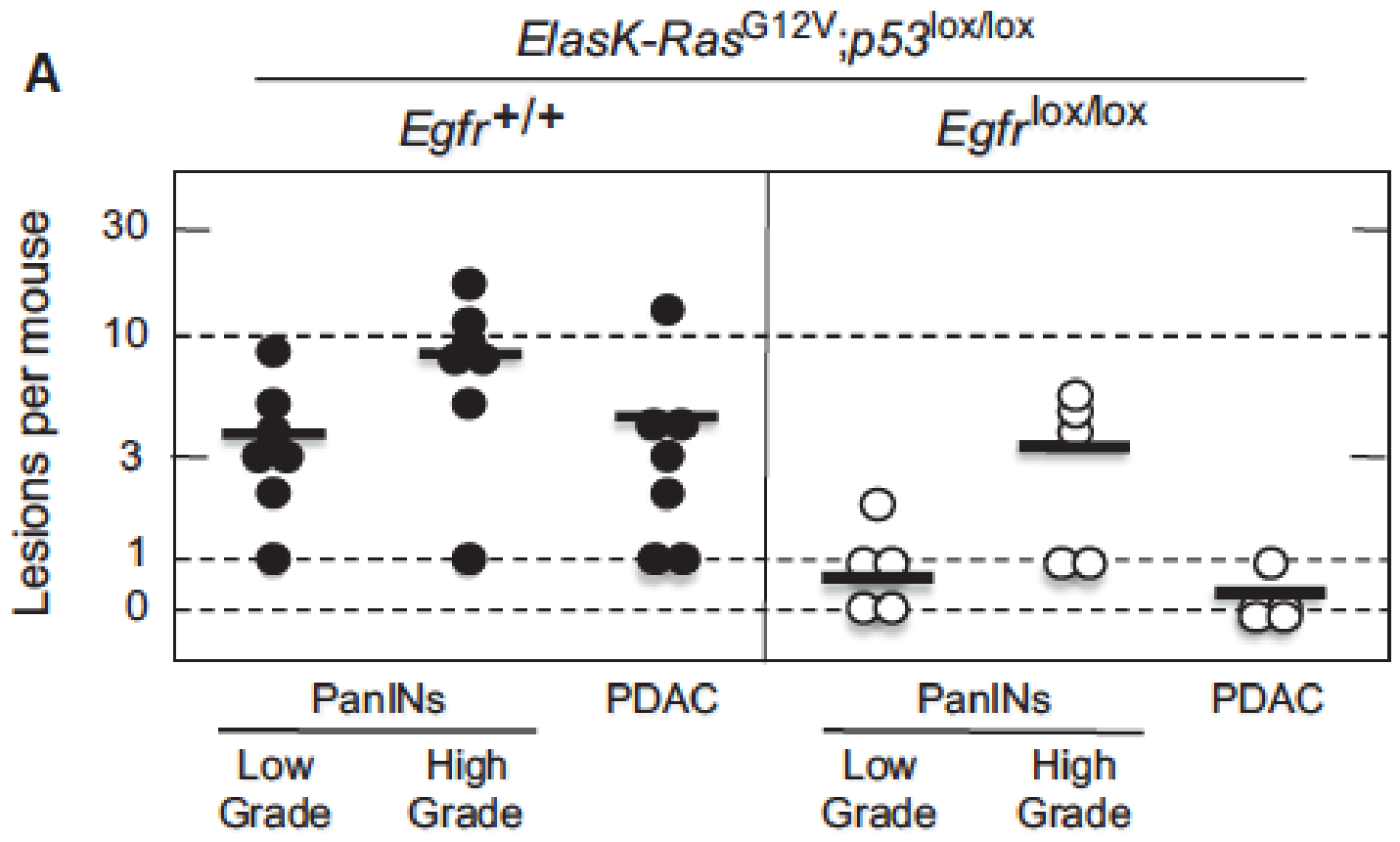
Collison et al, Nat Med 2011.

EGFR Expression is Needed for KRAS Driven PDA Tumorigenesis



Navas et al, Can Cell 2012.

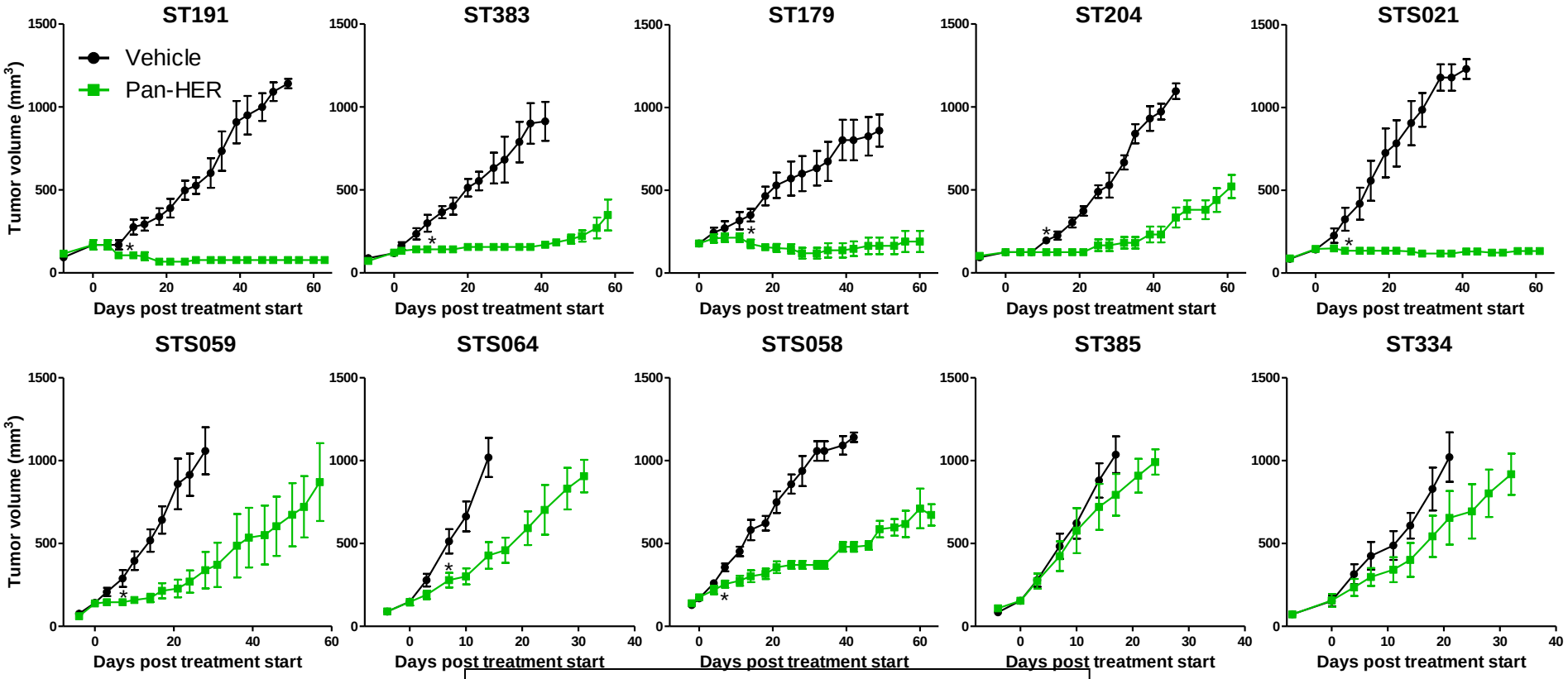
Lost of EGFR Delays but not Prevents PDA Tumorigenesis in p53 -/-



Navas et al, Can Cell 2012.

Striking Pan-HER Response in Pancreatic PDX Models

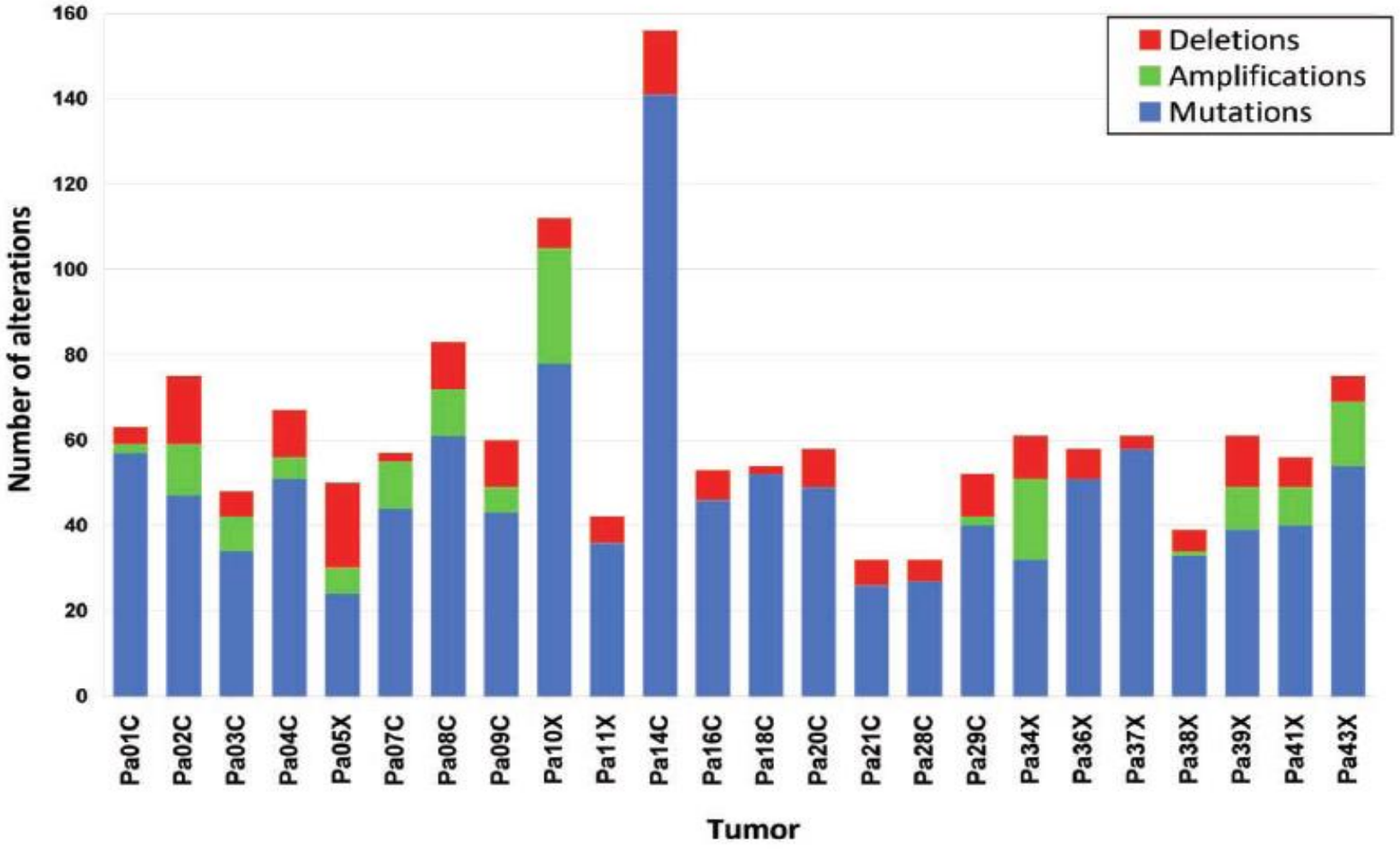
- Patient-derived xenograft models with mutated *KRAS* and known resistance to HER family targeted therapeutics selected



Pan-HER dosed at 50 mg/kg, 3xqw, 10 doses in total

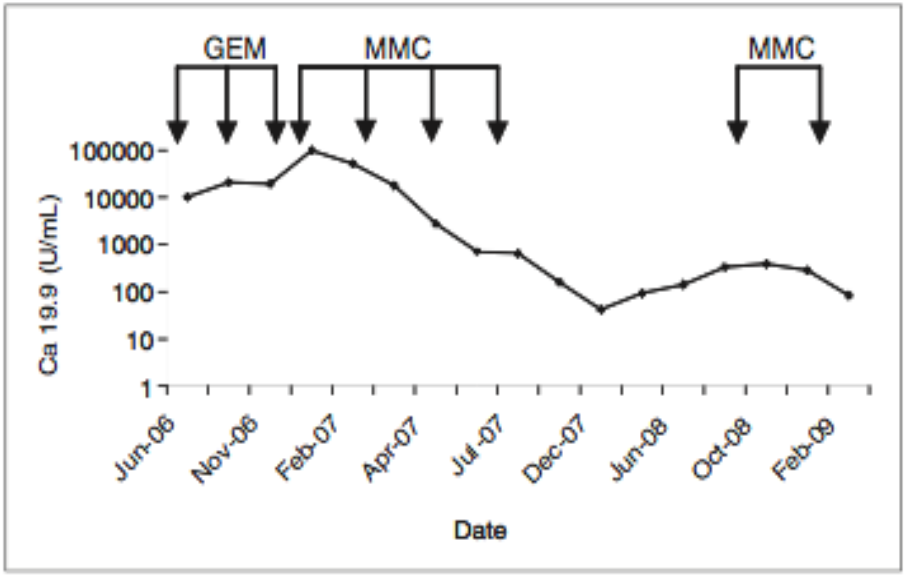
- Target expression and/or modulation not indicative of responsiveness

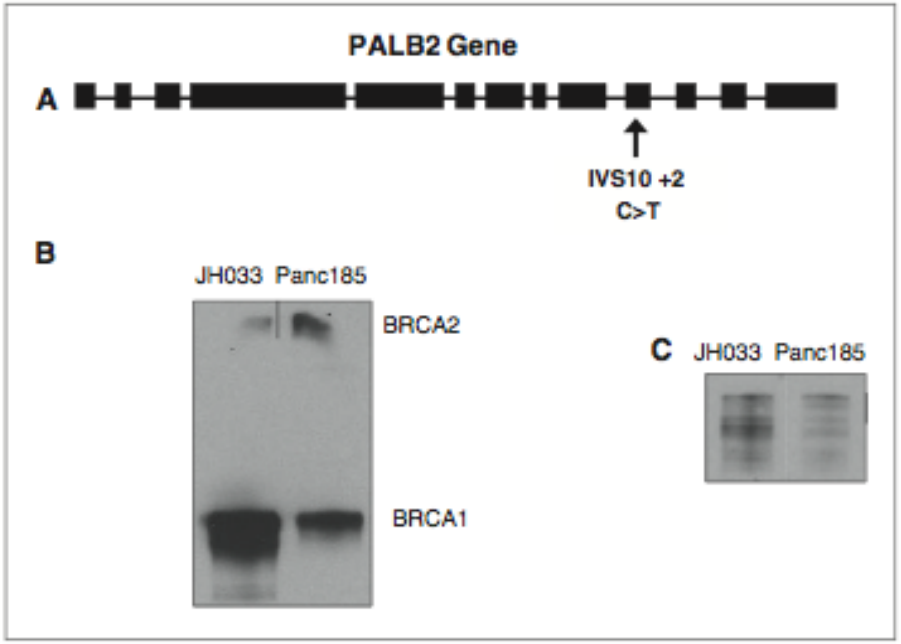
Genomic Diversity of Pancreas Cancer

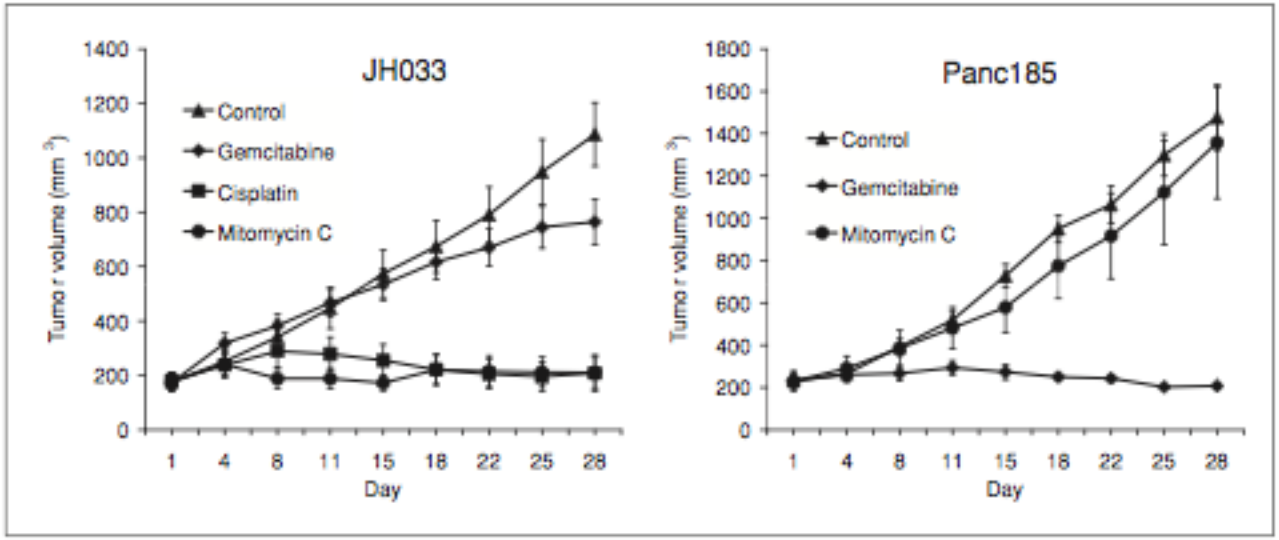


Jones et al, Science 2008.

Targeting PALB2 Mutations

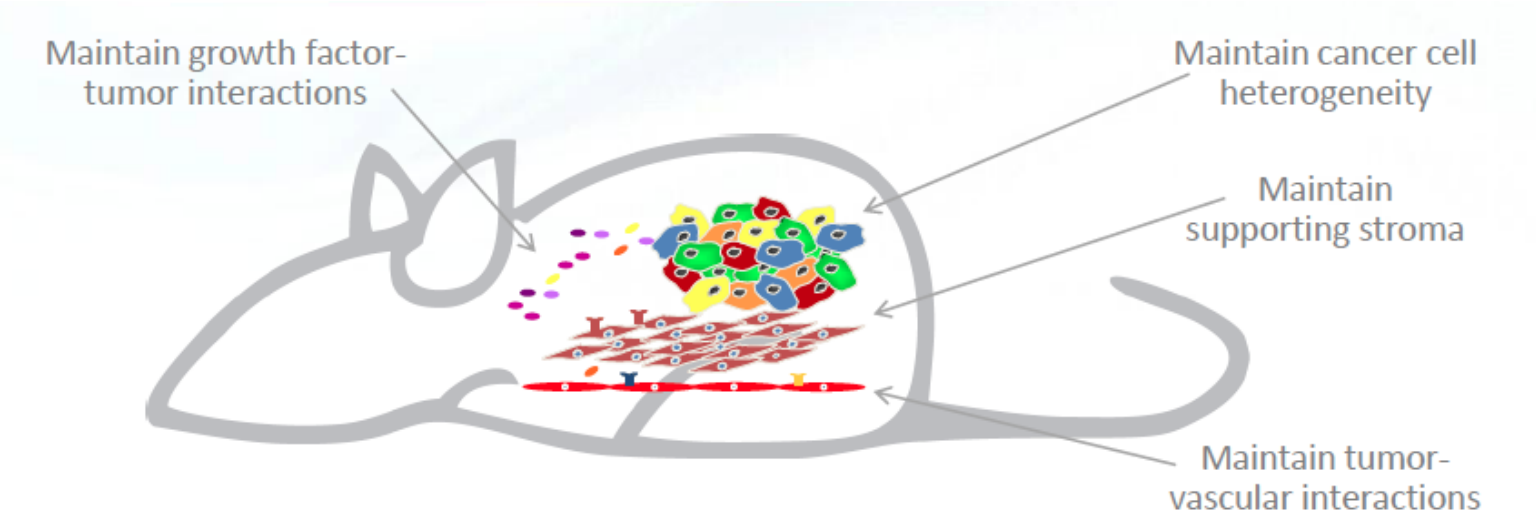






Villaroel, MCT 2011

Salient Features of Avatar Models



- Maintains fundamental genotype
- Maintains heterogeneity of original cancer
- Exploring means to shorten cycle times

Xenograft Predict Treatment Outcome

Spotlight on Clinical Response

Molecular Cancer Therapeutics

A Pilot Clinical Study of Treatment Guided by Personalized Tumorgrafts in Patients with Advanced Cancer

Manuel Hidalgo[1,4,5,6], Elizabeth Bruckheimer[3], N.V. Rajeshkumar[1], Ignacio Garrido-Laguna[1], Elizabeth De Oliveir
Belen Rubio-Viqueira[4,5], Steven Strawn[3], Michael J. Wick[7], James Martell[3], and David Sidransky[1,2]

Spotlight on Clinical Response

Molecular Cancer Therapeutics

Personalizing Cancer Treatment in the Age of Global Genomic Analyses: *PALB2* Gene Mutations and the Response to DNA Damaging Agents in Pancreatic Cancer

Maria C. Villarroel[1,2], N.V. Rajeshkumar[1,2], Ignacio Garrido-Laguna[1,2], Ana De Jesus-Acosta[1,2], Sián Jones[1,2], Anirban Maitra[1,2], Ralph H. Hruban[1,2], James R. Eshleman[1,2], Alison Klein[1,2], Daniel Laheru[1,2], Ross Donehower[1,2], and Manuel Hidalgo[1,2]

JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Gemcitabine Plus *nab*-Paclitaxel Is an Active Regimen in Patients With Advanced Pancreatic Cancer: A Phase I/II Trial

Daniel D. Von Hoff, Ramesh K. Ramanathan, Mitesh J. Borad, Daniel A. Laheru, Lon S. Smith, Tina E. Wood, Ronald L. Korn, Neil Desai, Vuong Trieu, Jose L. Iglesias, Hui Zhang, Patrick Soon-Shiong, Tao Shi, N.V. Rajeshkumar, Anirban Maitra, and Manuel Hidalgo

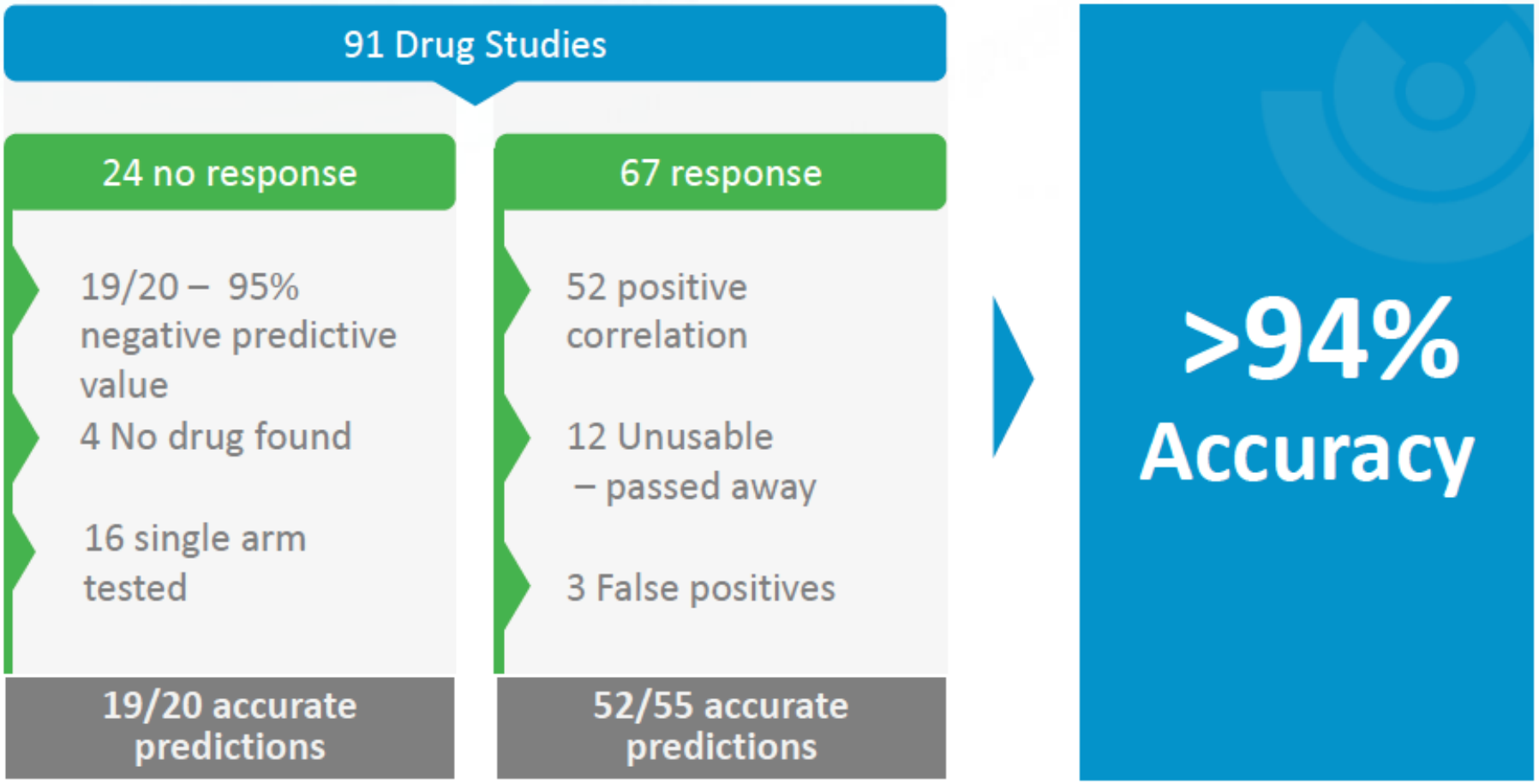
and Personalized Medicine

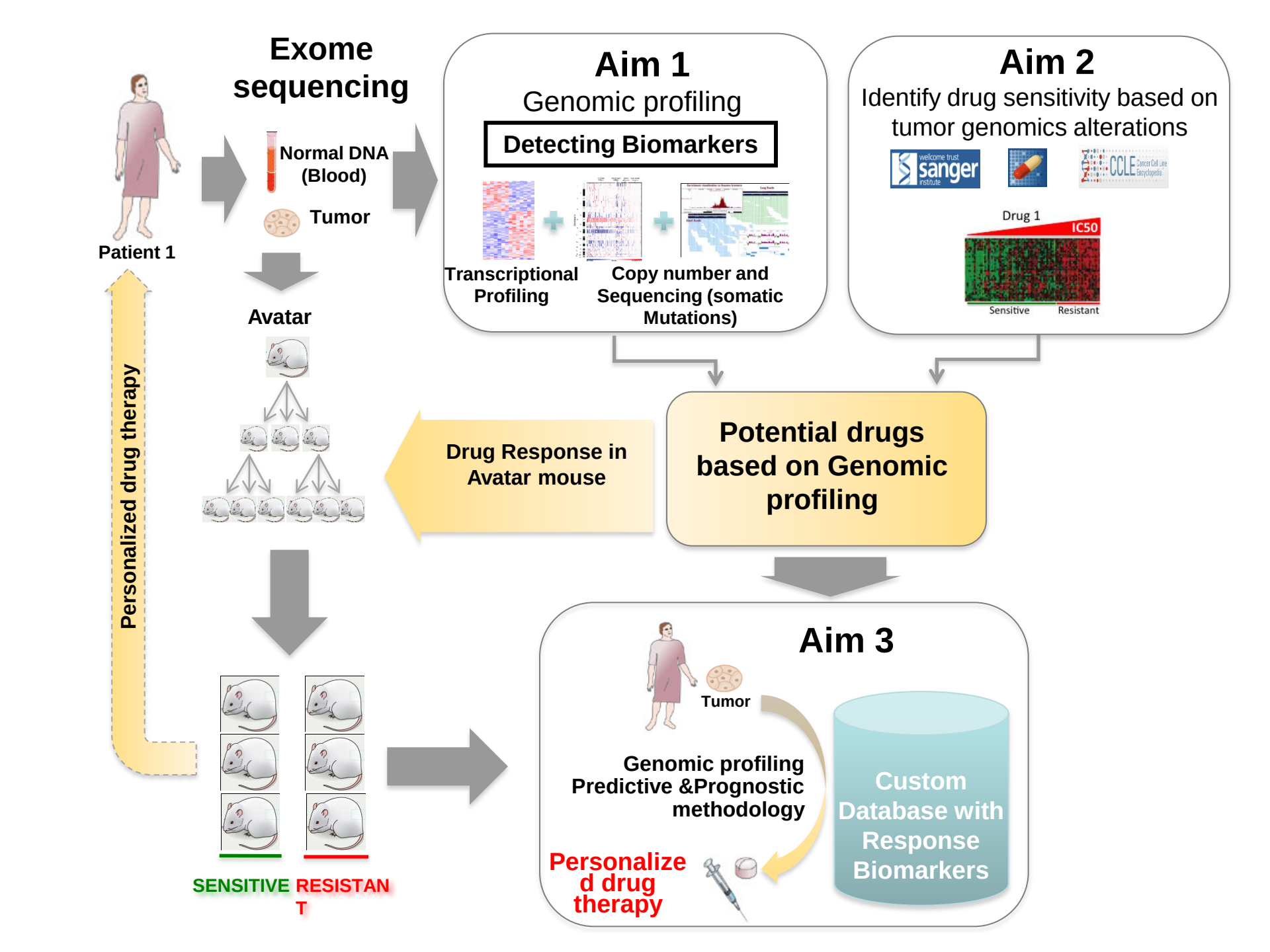
Clinical Cancer Research

Tumor Engraftment in Nude Mice and Enrichment in Stroma-Related Gene Pathways Predict Poor Survival and Resistance to Gemcitabine in Patients with Pancreatic Cancer

Ignacio Garrido-Laguna[1], Maria Uson[1], N.V. Rajeshkumar[1], Aik Choon Tan[1], Elizabeth de Oliveira[1], Collins Karikari[1], Maria C. Villaroel[1], Ana Salomon[1], Gretchen Taylor[1], Rajni Sharma[1], Ralph H. Hruban[1], Anirban Maitra[1], Daniel Laheru[1], Belén Rubio-Viqueira[2,3,4], Antonio Jimeno[1], and Manuel Hidalgo[1,2,3,4]

Overall Results

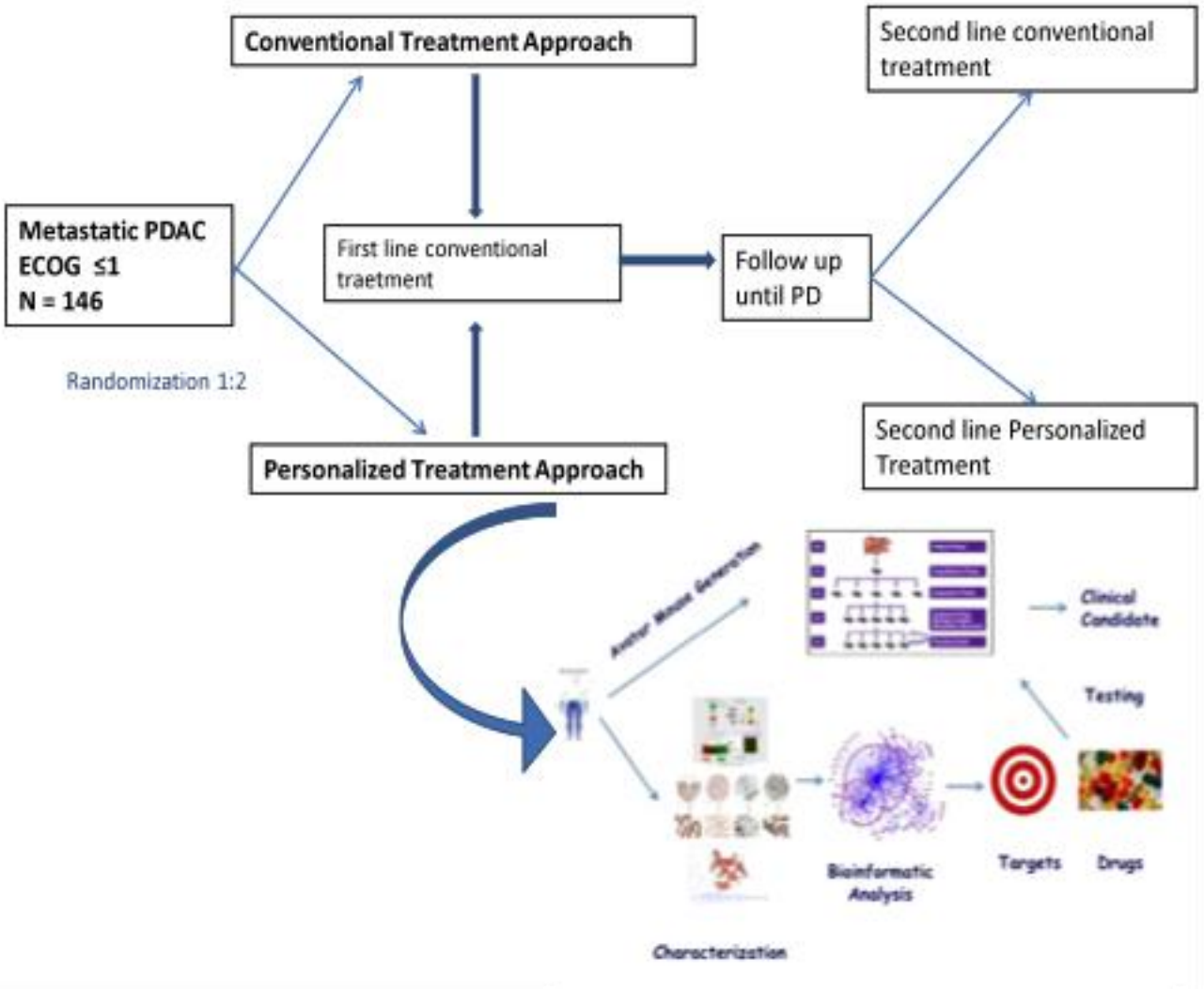



Exome sequencing
Patient 1
Normal DNA (Blood)
Tumor
Avatar
Personalized drug therapy
SENSITIVE
RESISTANT
Aim 1
Genomic profiling
Detecting Biomarkers
Transcriptional Profiling
Copy number and Sequencing (somatic Mutations)
Aim 2
Identify drug sensitivity based on tumor genomics alterations
Drug 1
IC50
Sensitive
Resistant
Potential drugs based on Genomic profiling
Drug Response in Avatar mouse
Aim 3
Tumor
Genomic profiling Predictive &Prognostic methodology
Personalized drug therapy
Custom Database with Response Biomarkers

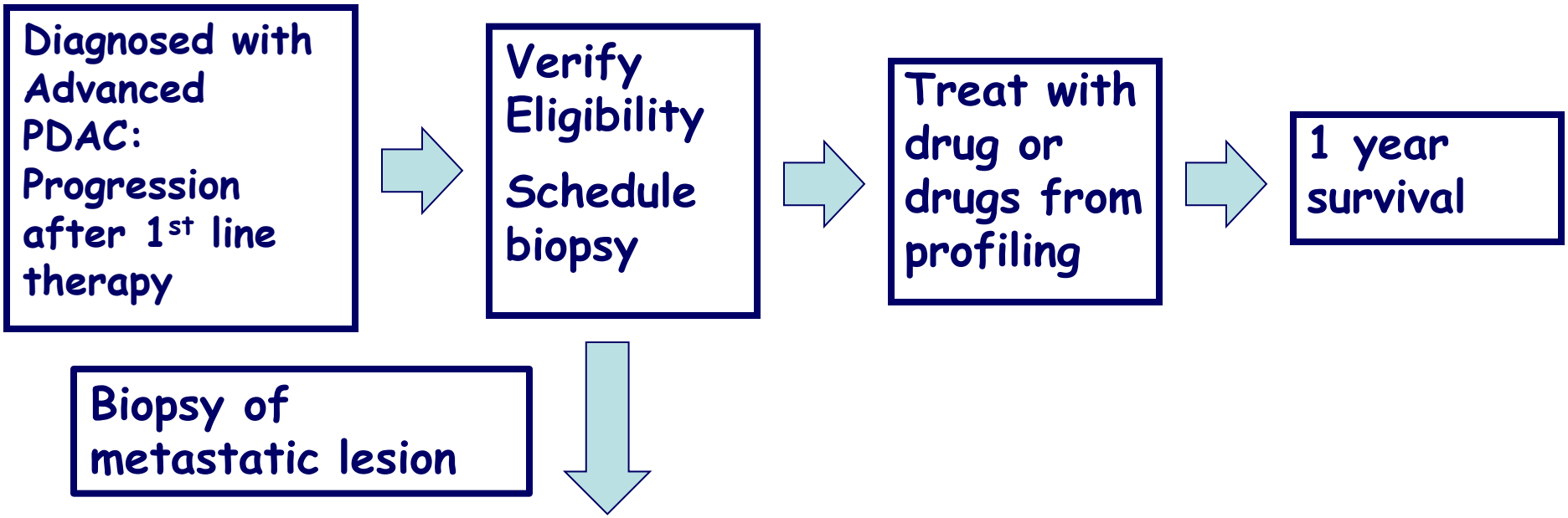
N#	TUMOR TYPE	N# MUTS	RELEVANT SOMATIC MUTS	N# CNV	RELEVANT CNV	PUTATIVE TARGETS.
1	Neuroendocrine tumor	5	CREB3L3, ITPR2, MYO5B	0	0	CREB3L3
2	Glioblastoma	63	EPHA3, NF1, PTPN11 ,FAS , CDKN2A	0	0	NF1
3	High grade pancreatic neuroendocrine	62	ARID1A, ARID1B, JAKMIP2, JARID2 PIK3C2A, PIK3CA, SSTR2, DDR2, TP53	6	GNG11	PI3KCA, DDR2
4	PDAC	38	KRAS, UBA1, FAM83H, SMAD4, SLC15A2, PIWIL3, SLC3A2, SLC22A17, TP53.	10	0	-
5	Melanoma Uveal	5	GNA11, TAOK3	0	0	GNA11
6	Colon cancer	71	APC, DICER 1, TP53, CHEK1, SOS1	63	0	CHEK1
7	PDAC	952	BRCA1, EZH2, FGFR2, FN1, IGF1R, KDR, KRAS, MET, MPL, PRKCB, PIK3C2G, PTK2B.	0	0	FGFR2, IGF1R, PIK3C2G, MET, BRCA1
8	Melanoma	29	BAI3, DNAH5, MDN1, NRAS	2	SKT19	NRAS
9	PDAC	18	SMAD4, KRAS, ERBB2IP	0	0	ERBB2IP
10	PDAC	21	KRAS, XPC, P53	0	0	XPC
11	PDAC	29	KRAS. P53,SMAD4	3	0	-
12	Renal Carcinoma	25	BAP1	965	ZAP70, FGFR3, NOTCH1, TERT, STK11, GNA11, ZNF668, SOCS1, IRS2	BAP1, FGFR3, NOTCH1, STK11, GNA11.
13	Glioblastoma	64	MLLT10, PBRM1	27	EGFR, CDKN2A, ERRFI1	EGFR
14	Cervix Cancer	73	ALK, NOTCH, FANCB, MAOA	186	ERBB2, JAK3, AKT	ALK, AKT, ERRB2
15	PDAC	573	BRCA2,GAK	0	0	BRCA2
16	PDAC	48	KRAS, NOTCH1, NF2, MMP21	35	0	NF2, NOTCH1

Primary tumor	Gene/Pathway Targeted	Matched treatment	Best Response (RECIST)	Time on treatment (months)	Present status
Neuroendocrine tumor	CREB3L3 mutation	Sandostatin + Metformine	EE. CR by PET	18	On treatment
Glioblastoma	NF1 mutation	Everolimus + Erlotinib + Bevacizumab	PD	3	Exitus
High grade pancreatic neuroendocrine tumor	PI3KCA, DDR2 mutations	Dasatinib	PD	3	Exitus
Uveal melanoma	GNA11 mutation	1st: Protein Kinase C inhibitor. 2nd: Carboplatin + Paclitaxel + Pi3k inhibitor	EE	4	Exitus
PDAC	XPC mutation	Mytomicin C	EE (clinical benefit)	3	On treatment
Renal	BAP1 mutation	Mytomicin C + Irinotecan	EE	4	On treatment
Glioblastoma	EGFR amplification	Erlotinib	EE	3	On treatment
PDAC	BRCA2	Mytomicin C	EE	5	On treatment

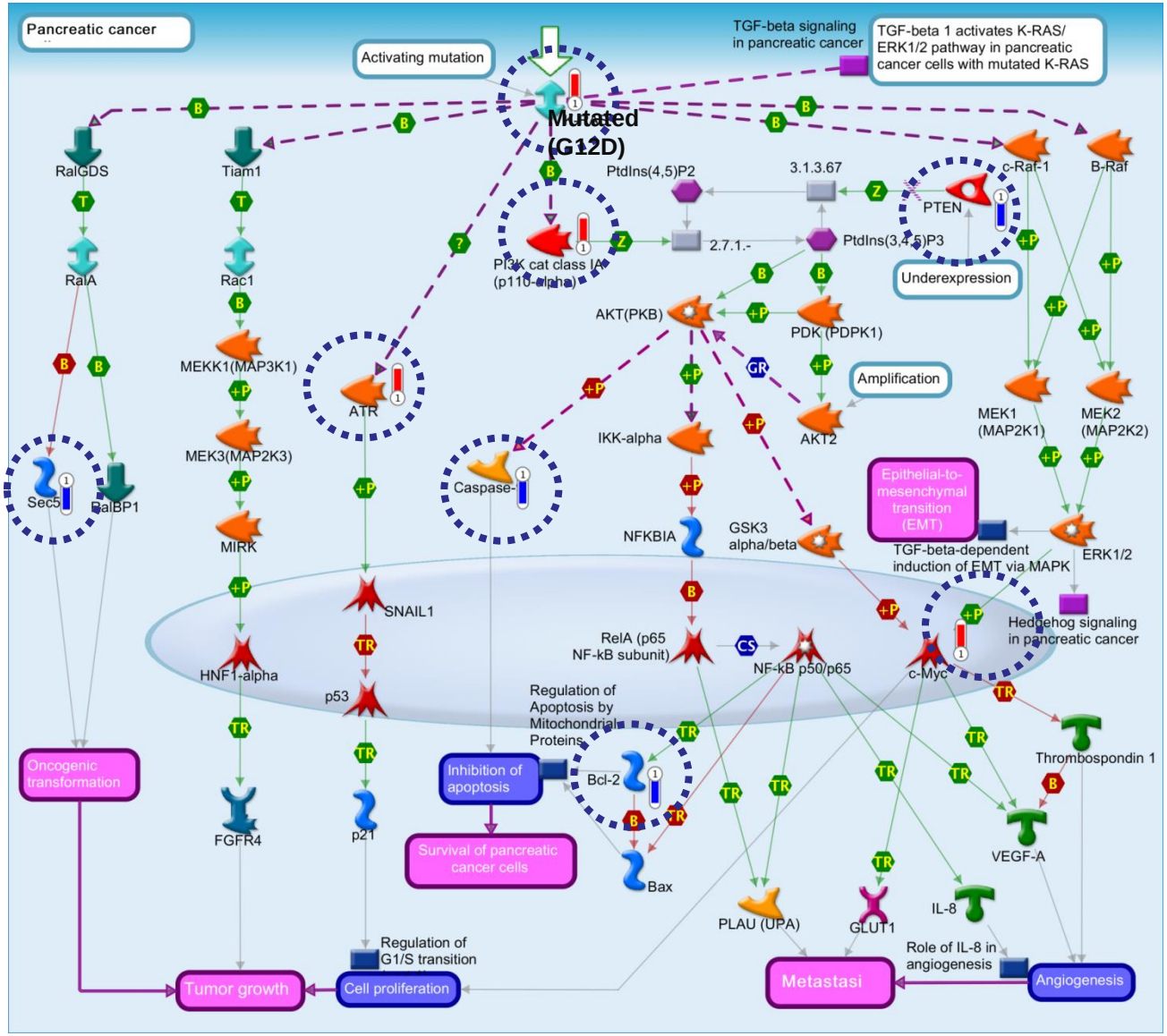
The Avatar Clinical Trial



Study Schema



Aberrations in K-RAS Signaling Pathways

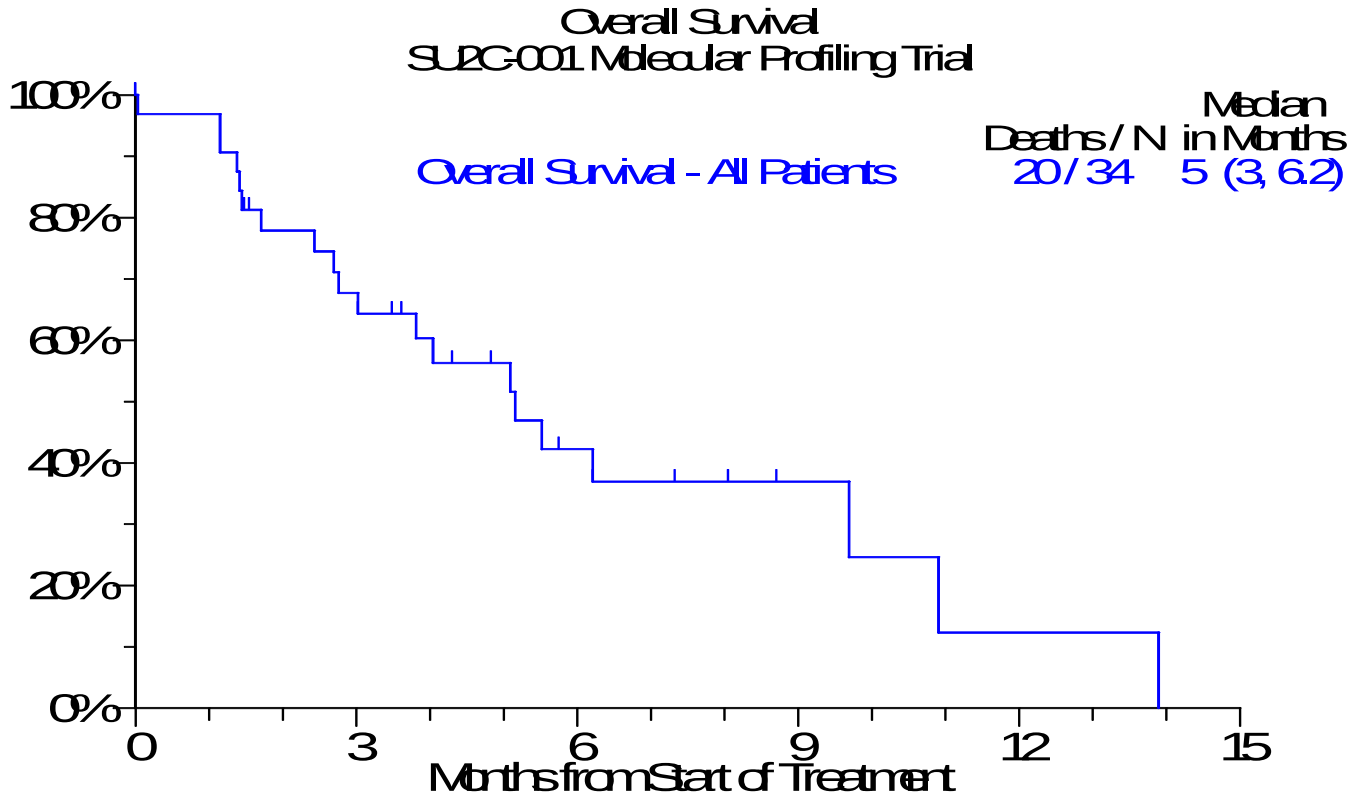


Patient Specific Maps (Patient #36 shown)

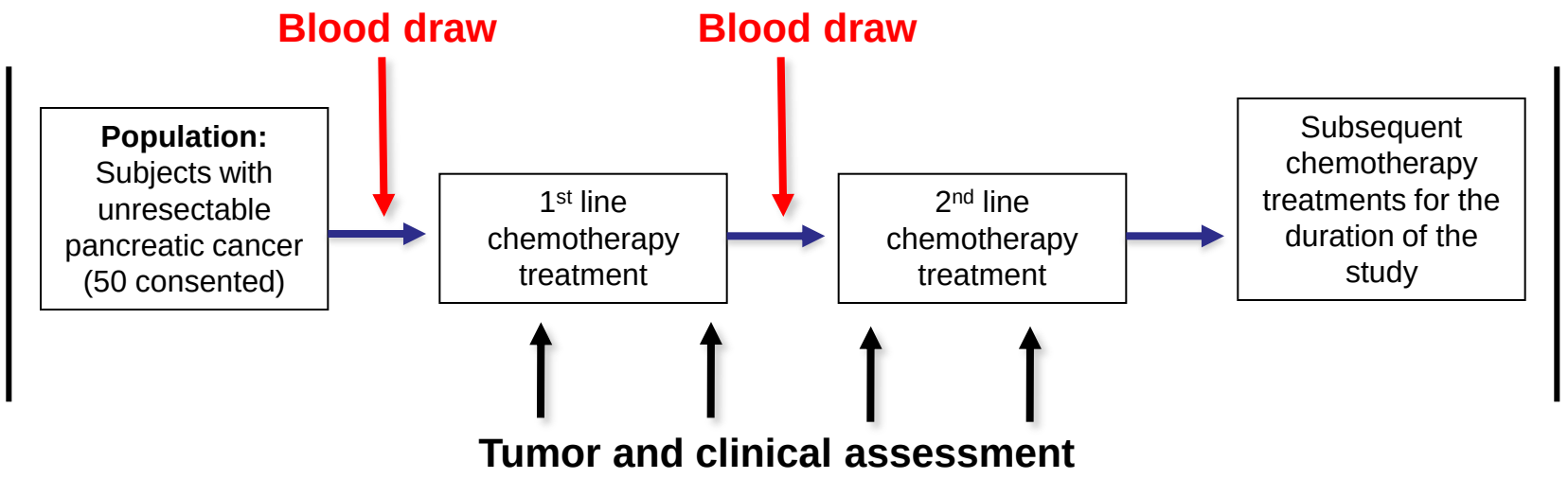
Blue deletion (e.g. *PTEN*)

Red amplification (e.g. *c-MYC*)

Overall Survival
Follow up is 2-14 months



MSKCC Clinical Trial Design



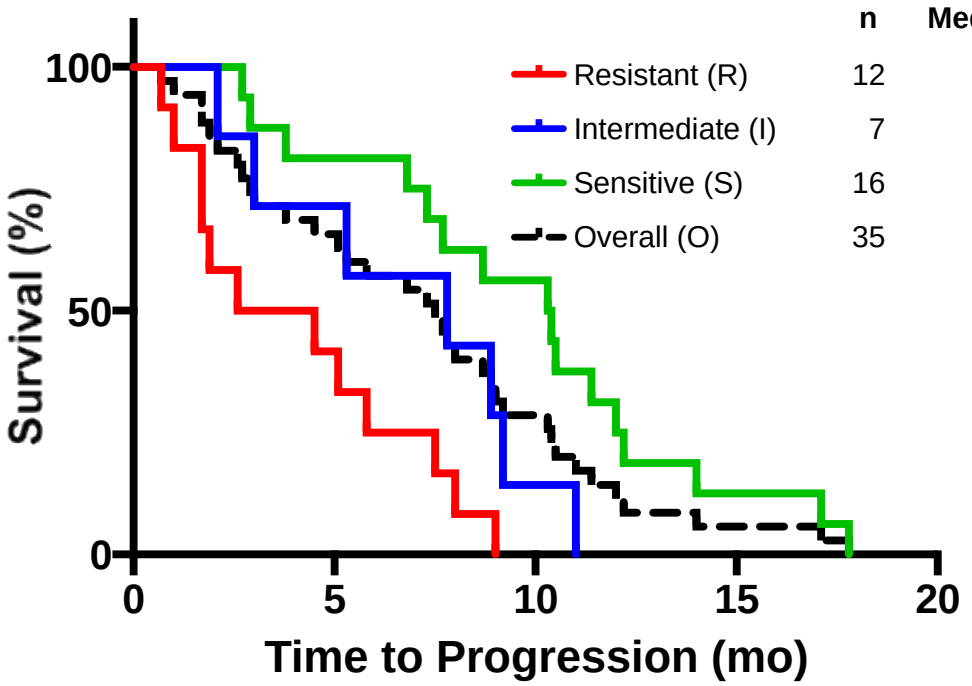
Clinical questions:

1)Does PGx model predict treatment response?
2)Does PGx profile change when cancer progresses?

Individual blood samples sequentially processed to:

1)Isolate/expand circulating tumor/invasive cells
2)Extract total RNA
3)Gene expression profile
4)PGx profile
5)Perform Gene and Network analysis

Clinical Trial in Advanced PDAC with FOLFIRINOX



Logrank test for trend $p < 0.0001$

	S v R	S v I	I v R
HR (95% CI)	0.14 (0.05 to 0.39)	0.34 (0.11 to 1.08)	0.39 (0.15 to 1.04)

	S v O	O v R
HR (95% CI)	0.62 (0.34 to 1.12)	0.30 (0.12 to 0.74)

Conclusions

- Genetically complex, unstable and heterogenous disease.
- No biomarker for personalize medicine available yet.
- Incipient opportunities for personalize medicine are emerging.

GI Group at CNIO

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