Subtyping of pancreatic cancer patient-derived xenograft tumors and implications for anti--cancer agent testing

Peter Bronsert[1], Tim Kees[2], Bruno Zeitouni[2], Anne-Lise Peille[2], Manuel Landesfeind[2], Heinz-Herbert Fiebig[2], Simon Kuesters[3], Vincent Vuaroqueaux[2]
[1]Institute of Pathology, University Medical Center Freiburg, Germany; [2]Oncotest GmbH, Freiburg, Germany;
[3]Clinics for General and Visceral Surgery, University Medical Center Freiburg, Germany

1 INTRODUCTION

Despite improvements in treatment, pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal cancers with a continuous increase in incidence, emphasizing the need for further research and therapeutic development. Facing the need to test new anti-cancer agents, we report here the development of 42 patient--derived xenograft (PDX) models of PDAC for pharmacogenomics investigations. In order to show the suitability of these models for pre-clinical studies, we performed an integrative analysis to highlight model features and subtypes by combining histology, genomic and transcriptomic profiles.

2 PDAC-PDX ESTABLISHMENT

Table1. Patient's clinical characteristics and PDX implantation rates

	All implanted tumors		Established PDX		
Parameters	Class	Number (%)	Number (%)	Implantation rate (%)	Significance (P-value)
Total amount of models		65	42	65	
Gender					
	male	33 (50.8)	23 (54.8)	69	
	female	32 (49.2)	19 (45.2)	59	N.S
Age (in years)	<50	8 (12.)	4 (10)	50	
	>50	57 (88)	38 (90)	67	N.S
Primary tumor status	pT1	1 (1.5)	1 (2.4	100	
	pT2	4 (6.2)	3 (7.1)	75	
	pT3	59 (90.8)	37 (88.1)	62	
	pT4	1 (1.5)	1 (2.4)	100	N.S
Lymph node status	pN0	17 (26.2)	7 (16.7)	41	
	pN+	48 (73.8)	35 (83.3)	73	0.03
Metastasis status	pM0	55 (84.6)	36 (85.7)	65	
	pM1	10 (15.4)	6 (14.3)	60	N.S
Differentiation	well	1 (1.5)	0	0	
	moderate	43 (66.2)	28 (66.7)	65	
	poor	21 (32.3)	14 (33.3)	67	N.S

- PDAC from 65 patients were implanted into female, 4-6 weeks old, NMRI nu/nu (Harlan, The Netherlands) immuno-compromised mice.
- 42 PDAC-PDX were stably established (success rate 65%).
- Patient survival (Fig 1) and lymph node invasion were associated with model establishment (P=0.008 and P=0.03, respectively).
- The resulting PDAC-PDX were further characterized for whole exome mutations (Agilent SureSelect human all exons kits Hiseq2000/2500), chromosome rearrangements, gene copy number variations (Affymetrix SNP6) and gene expression (Affymetrix HGU133 Plus2.0).
- PDAC-PDX were also evaluated for *in vivo* sensitivity towards gemcitabine (240 mg/kg/day iv Days 0, 7, 14), 5-Fluorouracil (75 or 100 mg/kg/day ip Days 0, 7, 14), erlotinib (50 mg/kg/day po Days 0-20) and everolimus (10 mg/kg/day po Days 0-4, 7-11, 14-18).

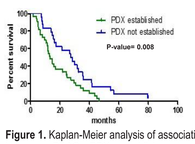

Figure 1. Kaplan-Meier analysis of association between PDAC PDX establishment and patient survival.

3 RESULTS

PDAC-PDX MORPHOLOGY FEATURES

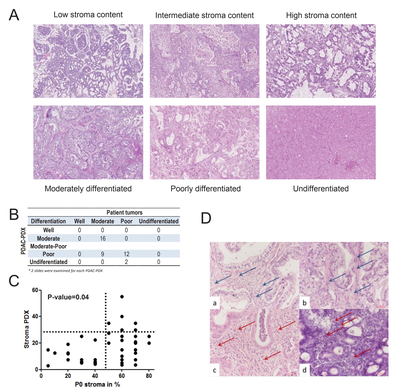

Figure 2 A. Representative pictures of PDAC-PDX with different differentiation and stroma content levels. **B** Contingency table comparing PDAC-PDX grade with those of parental tumors and **C.** Correlation between the stroma content of the patient tumor and the PDAC-PDX. **D.** Activated stroma in patient tumors (a, c) and PDX tumors (b, d) according to Ha *et al.* 2014 classification. Activated tumor stroma is defined by fibroblasts with small spindle cell morphology, a thin and wavy body-structure and a symmetric/parallel orientation (a, b; blue arrows). By comparison, inactivated tumor stroma features fibroblasts with plump spindle-shaped cell morphology, a prominent nucleus and nucleoli and with randomly spatial orientation (c, d; red arrows). *Reference: Ha et al., (PLOS One 2014). "The prognostic significance of cancer-associated fibroblasts in esophageal squamous cell carcinoma".*

- As with patient tumors, grades and stroma content of PDAC-PDX were heterogeneous, both correlating inversely ($p<0.0001$; Fig 2A).
- PDAC-PDX grades usually correlated with those of parental tumors, however some models shifted to a higher grade (Fig 2B).
- PDAC-PDX demonstrated the capability to develop low to high murine stromal content. Part of PDAC-PDX models, correlated with corresponding parental tumors (Fig 2C).
- As recently described in patient tumors, parts of PDAC-PDX showed stromal activation consisting of fibroblasts with small spindle cell morphology, a thin and wavy body-structure and a symmetric/parallel orientation (Fig 2D).

PDAC-PDX GENOMIC ALTERATION PROFILES

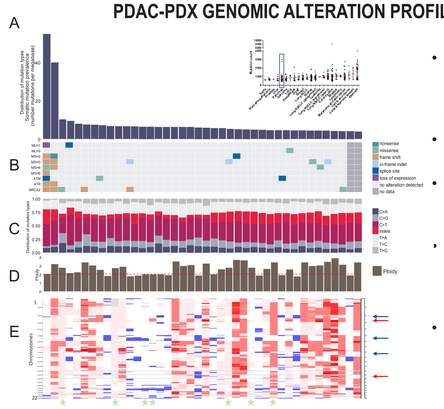

Figure 3. A. Barplot showing mutation prevalence (WES) in PDAC-PDX and scatter plot comparing the mutation loads in PDAC-PDX to other PDX histotypes**. B.** Alterations in repair genes. **C.** Proportion of substitutions and small indels (WES). **D** & **E.** Gene copy number variations (SNP6.0, PICNIC analysis).

- Hypermutation due to mismatch repair deficiency was seen in two models, while the others had on average a lower mutation load compared to other histotypes such as colon, lung or melanoma PDX (Fig 3 A and B).
- Depending on mutation loads, PDAC-PDX showed distinct mutational signatures (Fig 3 C).
- With the exception of MMR deficient tumors, an increased proportion of SNV (compared to indels) were seen in tumors with high mutation loads.
- PDAC-PDX were characterized by frequent chromosomal instability, with 62% of the models presenting a moderate polyploidy while the 38% remaining models were normal or aneuploid (Fig 3 D).
- GISTIC2 analysis revealed recurrent amplifications in the 3q and 14q (red arrows) and deletions in the 3, 6, 9p (blue arrows; Fig 3 E).

PDAC-PDX TRANSCRIPTOME SUBTYPE

The 62-gene expression signature established by Collisson *et al.*, classified PDAC-PDX and patient tumors into distinct transcriptomic subtypes. 22 models were of the classical subtype (52%), 15 of the quasi-mesenchymal (QM, 36%) and 5 of the exocrine-like subtype (12%).

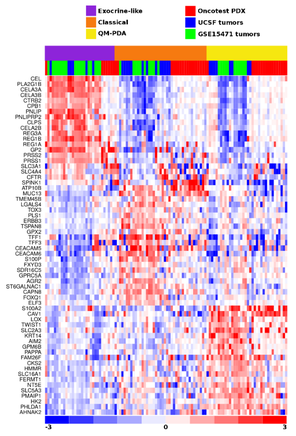

Figure 4. Subtyping of PDAC patient and PDX tumors. Heatmap showing the three subtypes of pancreatic tumors in a DWD-merged microarray data analysis that includes clinical datasets (from UCSF and GSE15471) and the 42 PDAC-PDX using the PDAssigner 62-gene set from Collisson *et al.* (2011). *Reference: Collisson et al., (Nature Medicine 2011) "Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy".*

PDAC-PDX INTEGRATIVE ANALYSIS

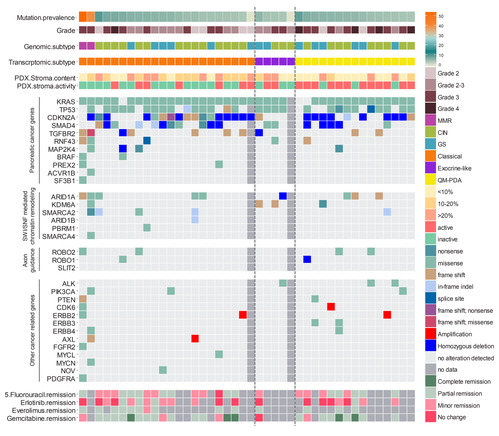

Figure 5. Heatmap showing the mutation load, grades, genomic subtypes, stroma content and activation, the genomic alterations of cancer-related genes and the response to *in vivo* therapies. (*in vivo* activity: Complete remission: T/C < 10%; partial remission: 10% ≥ T/C > 50 %; minor remission: 50% ≥ T/C > 75 %; no change: 75% ≥ T/C > 125 %)

- PDAC-PDX grades ($p=0.13$), stroma content ($p=0.02$) and activation ($p=0.01$) are associated with transcriptomic subtypes. QM PDAC-PDX were mostly of higher grade ($p=0.04$), lower stroma content and activation (both $p=0.01$), whereas the classical PDAC-PDX often presented with stroma inactivation.
- Transcriptomic subtypes do not correlate with tumor mutation loads or genomic alteration pattern. As seen in patient tumors, most of the models had alterations in KRAS (93%), TP53 (74%), CDKN2A (67%) and TGFBR2/SMAD4 (69%) genes.
- Drug sensitivity toward 5-FU and gemcitabine did not appear to be associated with PDAC-PDX stroma content and activation, nor with specific genomic and transcriptomic subtypes.
- While the KRAS mutation explained the lack of sensitivity toward erlotinib, tumor models revealed numerous alterations such as ERBB2 amplification and CDK6, PIK3CA and AXL mutations that are targetable.

4 CONCLUSIONS

Comprehensive characterization of our PDAC-PDX collection revealed similarities with patient tumors with regards to histology features including stroma content and fibroblast activation. At the molecular level, the models demonstrated similar genomic and transcriptomic patterns to those reported for patient tumors, demonstrating the preservation of patient tumor features and the suitability of this collection for pharmacogenomics investigations.