TP53 Mutations Predict Sensitivity to Adjuvant Gemcitabine in Patients with Pancreatic Ductal Adenocarcinoma: Next-Generation Sequencing Results from the CONKO-001 Trial

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ABSTRACT

Purpose: We performed next-generation sequencing (NGS) in the CONKO-001 phase III trial to identify clinically relevant prognostic and predictive mutations and conducted a functional validation in The Cancer Genome Atlas (TCGA) sequencing data.

Experimental Design: Patients of the CONKO-001 trial received curatively intended surgery for pancreatic adenocarcinoma (PDAC) followed by adjuvant chemotherapy with gemcitabine (Gem) or observation only (Obs). Tissue samples of 101 patients were evaluated by NGS of 37 genes. Cox proportional hazard models were applied for survival analysis. In addition, functional genomic analyses were performed in an NGS and RNA-sequencing dataset of 146 pancreatic tumors from TCGA.

Results: The most common mutations in the CONKO cohort were *KRAS* (75%), *TP53* (60%), *SMAD4* (10%), *CDKNA2* (9%), as well as SWI/SNF (12%) complex alterations. In untreated patients, *TP53* mutations were a negative prognostic factor for disease-free survival (DFS; HR mut vs. WT 2.434, $P = 0.005$). With respect to gemcitabine treatment, *TP53* mutations were a positive predictive factor for gemcitabine efficacy [*TP53*mut: HR for DFS Gem vs. Obs, 0.235 (0.130 – 0.423; $P < 0.001$); *TP53*wt: HR for DFS Gem vs. Obs, 0.794 (0.417 – 1.513; $P = 0.483$)] with a significant test for interaction ($P = 0.003$). In the TCGA dataset, *TP53* mutations were associated with shortened DFS.

Conclusions: In CONKO-001, the benefit from adjuvant gemcitabine was confined to the *TP53*mut patient group. This potentially clinical relevant observation needs to be confirmed in independent prospective studies. The sensitivity of TP53mut PDAC to gemcitabine in CONKO-001 provides a lead for further mechanistic investigations.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease with a 5-year survival rate across all stages of about 5% (1). To improve survival for these patients seems to be one of the most challenging tasks in clinical and molecular oncology (2). Surgery remains the only curative therapeutic approach for PDAC, but patients remain at high risk for recurrence even after complete resection. More recently, chemotherapeutic regimen as gemcitabine capecitabine combination (3) or FOLFIRINOX (4) could underline the effectiveness of the combination of surgery and systemic therapies. However, translational research approaches have not been able yet to define relevant predictive or prognostic biomarkers in PDAC; this may be one of the main reasons for the lack of survival improvement by precision medicine so far.

CONKO-001 was a randomized phase III adjuvant trial for patients with pancreatic cancer after curatively intended surgery and established gemcitabine as standard of care (5). Because of the fact that CONKO-001 compared an untreated patient group with a chemotherapy group, biomaterial collected from this trial is an optimal starting point for translational research focused on prognostic and

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Translational Relevance

Prognosis of pancreatic adenocarcinoma remains poor; prognostic and predictive biomarkers are urgently needed to stratify treatment decisions. Data from prospective clinical trials using next-generation sequencing are not available so far. CONKO-001 was an adjuvant trial and established gemcitabine as a standard of care. In this analysis investigating 197 amplicons in 37 genes, we found that patients with TP53-mutated tumors had a significantly improved survival treated in the gemcitabine-group compared with patients with TP53wt tumors. In contrast, in untreated patients from the observation group, TP53 mutations were an adverse prognostic factor. After validation in additional cohorts, the analysis of TP53-mutations might lead to improved personalized therapy strategies in patients with pancreatic adenocarcinoma.

predictive biomarkers. We could recently show for CONKO-001 that up to 20% of patients with pancreatic cancer treated with adjuvant gemcitabine are long-term survivors (6). A characterization of clinical parameters of these long-term survivors showed that they had typically a lower tumor grade and a lower clinical stage, but in the multivariate analysis only grading and adjuvant treatment were significant parameters for improved long-term survival.

The genomic profile of PDAC has been described in several comprehensive sequencing approaches (7–11). In the TCGA cohort of 146 patients (7), mutations in the *KRAS* gene were detected by deep sequencing in 93% of samples. Mutations in *TP53, CDKN2A*, and *SMAD4* were found in 72%, 30%, and 32% of cases, respectively. However, no data from prospective clinical trial cohorts exist so far. Therefore, to verify these investigations in a clearly defined patient cohort and to analyse their clinical utility as markers of prognosis and treatment efficacy in PDAC is of central interest.

It is now feasible to perform targeted next-generation sequencing (NGS) using formalin-fixed paraffin embedded (FFPE) samples from clinical studies. (12) To evaluate the role of the most frequent mutations for therapy efficacy, we performed targeted next-generation sequencing in the CONKO-1 cohort, which allows a separation of prognostic and predictive effects, due to the inclusion of an untreated control arm. The aim of our translational study was to define molecular biomarkers for therapy efficacy with the focus on biologically and clinically relevant mutations.

Materials and Methods

Baseline data of CONKO-001

CONKO-001 (isrctn.org ID: ISRCTN34802808) was a randomized phase III trial to investigate the role of an adjuvant therapy with gemcitabine compared with observation only. 368 patients with PDAC were enrolled after curatively intended surgery (R0 and R1) in this open, multicenter randomized controlled trial between July 1998 and December 2004. 354 patients were defined as intention-to treat (ITT) population. Treatment with gemcitabine (1,000 mg/m^2 d1, 8, 15, q29) was given for 6 cycles in an outpatient setting. The study was conducted according to the ethical principles outlined in the current version of the Declaration of Helsinki and the principles of Good Clinical Practice (GCP). All patients provided written informed consent.The clinical results have been published (5, 13). The translational research programme was approved by the institutional ethic review committee (EA1/139/05, Amendment 2012), the study is reported according to the REMARK criteria (14).

Tumor tissue samples

Active patient recruitment was finished in December 2004, but the translational program for CONKO-001 was started only in 2010. Tumor tissue samples were collected retrospectively from the participating study centers. Because of fact that CONKO-001 started already in the late 1990s and that CONKO-001 was a community-based trial, it was possible to collect only tumor tissue from 183 patients (46%).

NGS panel development and sequencing

A customized sequencing panel for PDAC was designed using Ion AmpliSeq Designer based on mutation data of pancreatic cancer from the COSMIC database. The PDAC panel IAD85732_169 covered 37 genes (for full list of genes see Supplementary Methods). The sequencing panel comprised 197 amplicons with a median length of 123 bp (minimal length: 71 bp, maximal length: 137 bp). It covered 23.9 kbp of the human genome whereof 18.8 kbp were located in the coding sequence. The panel includes the most important driver mutations for PDAC (*KRAS, TP53, SMAD4, CDKN2A*). The genes reflect different molecular motifs like the G_1–S checkpoint machinery (*TP53, CDKN2A*), histone modification (*KDM6A*), genes of the ASCOM–TP53 complex (KMT2C/MLL3, KMT2D/MLL4, TP53), SWI/SNF complex (*ARID1A/BAF250A, ARID1B/BAF250B, ARID2/BAF200, PBRM1/BAF180, SMARCA2/BRM, SMARCA4/BRG1*), BRCA pathway (*ATM*), WNT signaling defects RNF43), and RNA processing (SF3B1). The alterations of the ASCOM complex were mainly driven by *TP53*, therefore the results for this group are not reported in detail.

For microdissection, three serial 5-μm–thick sections were prepared (15). DNA was extracted by a Versant system (Siemens) using the DNAext protocol according to the manufacturer's instructions. Samples were sequenced using Ion 318 chip v2 BC chips (Thermo Fisher Scientific) with an adapted standard protocol using 330 flows (16). For library and template preparation as well as NGS data processing strategy, please refer to Supplementary Methods.

In the retrospectively collected tissue samples from different centers that were initially processed between 1998 and 2004, DNA quantity and quality varied considerably and formalin-induced artifacts were observed (C>T substitutions). Under careful consideration, we chose a more conservative approach to obtain a higher level of confidence in a trade off with a lower sensitivity of mutation detection. Briefly, low-, medium-, and high-quality samples were defined with a required minimal tumor area of 10% to 20% and different variant allele frequencies (VAF), ranging vom 5% to 15% (for details see Supplementary Methods).

Statistical analysis

Disease-free survival (DFS) was defined as time from study entry to local or distant disease relapse or death from any cause, overall survival (OS) as time from study entry to death of any cause. The relation of the different mutations with clinical and pathologic tumor characteristics was evaluated using χ^2 tests. To minimize effects of multiple testing, the survival analysis was focussed on the most common genomic alterations (*KRAS, TP53, SMAD4, CDKNA2*) as well as SWI/SNF complex in the CONKO-001 cohort. The Kaplan-Meier method with log-rank tests was used for univariable survival analyses. The Cox proportional hazard models including adjustment for patient age (reported < 60 years vs. ≥ 60 years), tumor stage (T1-2 vs. T3-4),

nodal status (N+ vs. N−), grading (G1-2 vs. G3), and resection status (R0 vs. R1) were used for multivariate survival analysis. Statistical significance was assessed two-sided and reported in terms of raw *P* values before multiple testing correction. Multiple testing correction with respect to the five investigated biomarkers was performed using the Bonferroni method and *P* values < 0.001 were considered statistical significant. Statistical analysis was performed using SPSS version 24 (IBM Corp.).

Functional genomics analysis of TP53 mutations

Mutation calls, mRNA gene expression data, and clinical data of the TCGA pancreatic cancer cohort (7) were obtained from the cBioPortal (http://www.cbioportal.org). The study cohort included 146 early PDAC, which were profiled by DNA and RNA sequencing after the exclusion of four cases with metastatic disease at the time of diagnosis. Univariate analysis of DFS and OS comparing TP53mut and TP53wt tumors was performed using Kaplan–Meier curves and Cox proportional hazard models to estimate HRs. Differential expression between TP53mut and TP53wt tumors was assessed using Welch *t* test. Resulting *P* values were corrected for multiple testing by the Benjamini–Hochberg (BH) method and a list of 419 differentially expressed genes was obtained controlling the false discovery rate (FDR) at 10%. The gene list was checked for enrichment of gene sets annotated in the Molecular Signatures database (MSigDB) version 6.1 (http://software.broadinstitute.org/gsea/msigdb) using Fisher's exact test.

Results

Baseline factors

354 patients were included in the ITT cohort of the CONKO-001 trial. Of those, 183 FFPE samples (51.7%) could be collected for the NGS study presented here. Seventy-six samples were not suitable for NGS analysis due to insufficient amount of tissue or tumor contents below 10%. NGS was successful in 101 samples, corresponding to 55.2% of the available tissue samples. The baseline clinical data (patient's and tumor characteristics and survival) of the studied subgroup were comparable with the overall study population and balanced between the two groups (gemcitabine treated vs. observation only). There was a slight imbalance in concern of grading: more patients in our cohort had G3 tumors in comparison with the original study population (49.5% vs. 32%, $P = 0.002$). For more details, please refer to the CONSORT diagram in **Fig. 1** and Supplementary Table S1.

Frequency of mutations detected by NGS

An overview of mutation frequencies is shown in **Fig. 2**. The most frequent mutations were found in *KRAS* (76 patients, 75%), followed by *TP53* (61 patients, 60%), *SMAD4* (10 patients, 10%), *CDKN2A* (9 patients, 9%). In 12 patients (12%) at least 1 mutation in one of the genes of the SWI/SNF complex (ARID1A/BAF250A, ARID1B/BAF250B, ARID2/BAF200, PBRM1/BAF180, SMARCA2/BRM, SMARCA4/BRG1) was detected. 33 (33%) had one, 37 (37%) two, 13 (13%) three mutations, and 13 (13%) had four or more different mutations. Only 5 patients (5%) had no detectable mutation in the regions covered with our NGS panel.

For *TP53*, 61 of 101 (60%) patients had at least one *TP53* mutation, in 12 (12%) patients more than one mutation was found. Eighteen patients (17%) had at least one *TP53* mutation that has been characterized as a gain-of-function mutation (refs. 17, 18; 3 x p.R175H, 6x p.R248W, 2x p.R248Q, 2x p.R273C, 1x p.R273H, 1x p.R273S, 3x p.Y220C). The clinical parameters of the *TP53*mut and the *TP53*wt tumors are compared in Supplementary Table S2.

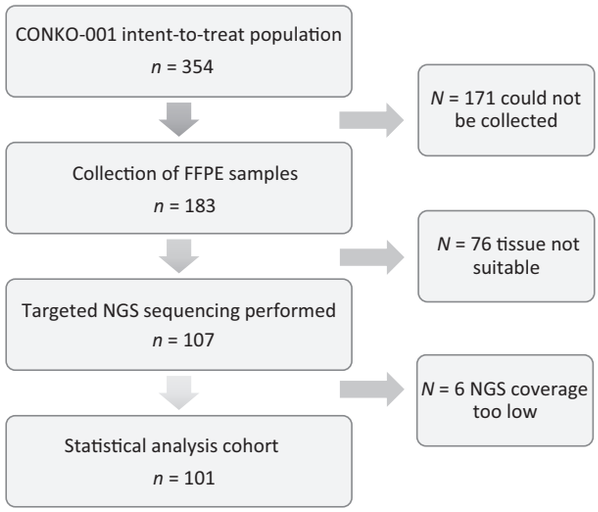

Figure 1.
CONSORT statement.

The five most common genetic alterations: mutations in TP53, KRAS, SMAD4, CDKNA2, and the SWI/SNF complex were further analyzed for association with survival and benefit from gemcitabine. Survival analysis was performed separately in the observation arm, the gemcitabine arm, and the complete cohort (for DFS see **Table 1**, for OS see Supplementary Table S3).

Genetic alterations as prognostic biomarkers

In the observation arm, TP53 alterations were associated with significant changes in disease-free survial (DFS) and overall survival (OS). For TP53 and DFS, we observed an HR of 2.43 (95% CI, 1.32–4.51, $P = 0.005$) in univariate and of 3.74 (95% CI, 1.87–7.46, $P < 0.001$) in multivariate survival analysis.

Genetic alterations as predictive biomarkers

The benefit from gemcitabine was analyzed in patient subgroups defined by the status of the three genes *TP53*, *KRAS*, and *CDKN2A* (**Fig. 3A** and **B**). In the TP53mut subgroup, we observed a strong and significant benefit from gemcitabine (DFS: HR = 0.235, $P < 0.001$; OS: HR = 0.39, $P = 0.0017$), while benefit from gemcitabine was weak and not significant in the TP53wt subgroup (DFS: HR = 0.79, $P = 0.48$; OS: HR = 0.86, $P = 0.65$). A significant interaction of the treatment status with TP53 mutational status was observed for DFS ($P = 0.003$; **Table 1**).

Finally, a stratification of the study cohort into four patient groups defined by TP53 status and by study arm was subjected to Kaplan–Meier analysis (**Fig. 3C** and **D**). A poor survival was observed for patients with TP53-mutated tumors in the observation arm, while the best survival was observed for patients with TP53-mutated tumors that received adjuvant gemcitabine. For patients with TP53-mutated tumors, survival was significantly better in the gemcitabine arm compared with the observation arm (DFS: $P < 0.001$; OS: $P = 0.001$). In contrast, for patients with TP53 wild-type tumors, neither DFS nor OS was significantly different between the gemcitabine arm and the observation arm (DFS: $P = 0.48$; OS: $P = 0.65$).

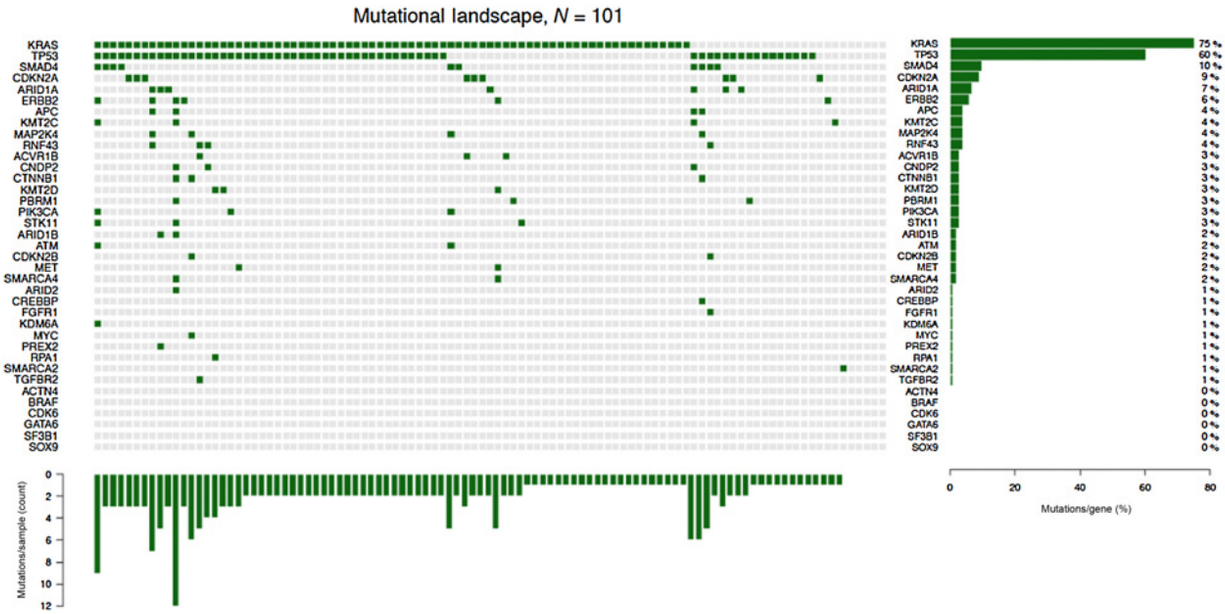

Figure 2.

Mutational landscape of 101 pancreatic adenocarcinomas profiled by targeted NGS sequencing. *KRAS, TP53*, and *SMAD4* were the most frequently observed alterations in 75%, 60%, and 10% of patients, respectively. The mutation frequencies per sample (bar plot on the bottom) ranged from none to 12 mutations.

In addition, the results for *KRAS, SMAD4, CDKNA2*, and the SWI/SNF complex were summarized in **Table 1**, Supplementary Table S2, and Supplementary Fig. S1. For these genes, Cox regression showed no significant survival difference in the complete cohort or in the two study groups (**Table 1**; Supplementary Table S2), the interaction with adjuvant treatment was not significant. In the Kaplan–Meier analysis (Supplementary Fig. S1), the significant differences reflect the generally better survival of the gemcitabine group, that is evident in most subgroups.

To evaluate the impact of multiple mutations, we fit bivariate regression models with interaction term for TP53 and the other most frequently observed mutations (Supplementary Table S4).

TP53 analysis in the TCGA cohort

The clinical analysis in CONKO-1 suggests a relevant role for TP53 in the prediction of adjuvant chemotherapy efficacy. To further validate this proposed influence of *TP53*mut, we analyzed the TCGA cohort of 146 patients with PDAC excluding those with metastatic disease at diagnosis (7). Nonsynonymous *TP53* mutations were detected in 68% of these tumors and correlated significantly negative with DFS (HR = 2.2, $P = 0.0062$), but not significantly with OS (Supplementary Fig. S2A and S2B).

Furthermore, we performed a functional genomics analysis to uncover gene expression programs that were activated in TP53mut tumors compared with TP53wt tumors (Supplementary Fig. S2C). A total number of 419 differential expressed genes was detected when taking into consideration multiple testing for 20,437 genes and FDR control at 10% (data not shown). In a *post hoc* analysis of this pathway with a total number of 67 genes, we detected 23 differentially expressed genes (Supplementary Fig. S2C, FDR = 10%, multiple testing correction within the pathway). In particular, this included an overexpression of PKM (fold change 1.28, $P < 0.001$) and an underexpression of PCK1 (fold change −2.53, $P < 0.001$) in the TP53mut tumors supporting differential regulation of a key control point of glycolysis and a switch to more glycolysis and less glyconeogenesis in TP53mut tumors (Supplementary Fig. S2D and S2E). This suggests that TP53mut tumors have a different metabolic background, which might be partially relevant for the efficacy of adjuvant chemotherapy, as shown in the CONKO cohort.

Discussion

While the genomic profile of resectable PDAC has been described in several comprehensive sequencing approaches, the role of these mutations for treatment efficacy has not been investigated in the context of prospective randomized treatment trials so far.

In the study presented here, we investigated a clinical trial cohort of 101 patients with PDAC treated in CONKO-001 using a NGS panel of 37 genes. The main finding of our analysis is that patients with TP53-mutated tumors had an unfavorable prognosis when randomized to observation and had a clear benefit from adjuvant gemcitabine. In contrast, adjuvant treatment with gemcitabine did not significantly prolong DFS in patients with *TP53*wt tumors. In untreated patients, *TP53* mutations were confirmed as a poor prognostic factor: patients with *TP53*wt tumors had a better prognosis without adjuvant therapy if compared with patients with *TP53*mut tumors. Interestingly, adjuvant gemcitabine did not significantly improve survival in *TP53*wt tumors.

Our data suggest a role for TP53 as a predictive marker in PDAC treated with gemcitabine and that chemotherapy seems to have a relevant effect on survival, in particular, in patients with *TP53*mut tumors. However, before the introduction of these findings into clinical practice and its translation to other chemotherapy regimen, further investigations in additional cohorts will be needed.

To validate our findings, we used the TCGA PDAC cohort to substantiate the impact of TP53 mutations on survival in these patients. We detected a significant negative correlation with DFS, but not OS, in TP53mut tumors. In the TCGA PDAC cohort, information

Table 1. Univariate and multivariate[a] analysis of disease-free survival for the most common mutations: separate analysis for the complete cohort, the gemcitabine arm, and the observation arm.

	Observation arm ($n = 53$)				Gemcitabine arm ($n = 48$)					Complete cohort ($n = 101$)			
	Univ. Cox-regression		Multiv. Cox regression		Univ. Cox-regression		Multiv. Cox regression		Test for interaction[b]	Univ. Cox-regression		Multiv. Cox regression	
Disease-free survival	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
TP53		**0.005**		**<0.001**		ns		ns	**0.003**		ns		ns
WT	**1.00**		**1.00**		1.00		1.00			1.00		1.00	
mut	**2.434 (1.315–4.505)**		**3.738 (1.873–7.459)**		0.699 (0.376–1.301)		0.583 (0.301–1.127)			1.097 (0.723–1.663)		1.052 (0.683–1.620)	
KRAS		ns		ns		ns		ns	Ns		ns		ns
WT	1.00		1.00		1.00		1.00			1.00		1.00	
mut	0.884 (0.450–1.736)		0.941 (0.437–2.027)		0.980 (0.490–1.958)		0.943 (0.437–2.036)			1.050 (0.650–1.694)		1.136 (0.687–1.877)	
SMAD4		ns		ns		ns		ns	Ns		ns		ns
WT	1.00		1.00		1.00		1.00			1.00		1.00	
mut	0.577 (0.204–1.632)		0.665 (0.216–2.044)		1.227 (0.480–3.135)		1.632 (0.586–4.543)			0.856 (0.430–1.704)	ns	1.069 (0.525–2.177)	
CDKN2A		ns		ns		ns		ns	Ns		ns		ns
WT	1.00		1.00		1.00		1.00			1.00		1.00	
mut	0.639 (0.268–1.523)		0.711 (0.267–1.892)		1.713 (0.521–5.633)		1.372 (0.382–4.935)			1.132 (0.566–2.263)		1.151 (0.567–2.337)	
SWI/SNF		ns		ns		ns		ns	Ns		ns		ns
WT	1.00		1.00		1.00		1.00			1.00		1.00	
mut	1.284 (0.395–4.170)		1.421 (0.401–5.031)		0.574 (0.238–1.384)		0.540 (0.197–1.479)			0.555 (0.276–1.115)		0.556 (0.263–1.156)	

[a]Multivariate Cox regression analysis adjusted for patient age (< 60 years vs. >60 years), tumor stage (T1-2 vs. T3-4), nodal status (N+ vs. N−), grading (G1-2 vs. G3), and resection status (R0 vs. R1).
[b]Test for interaction *P* value derived from Cox regression analysis for disease-free survival for all patients, including mutational alterations and treatment arms.

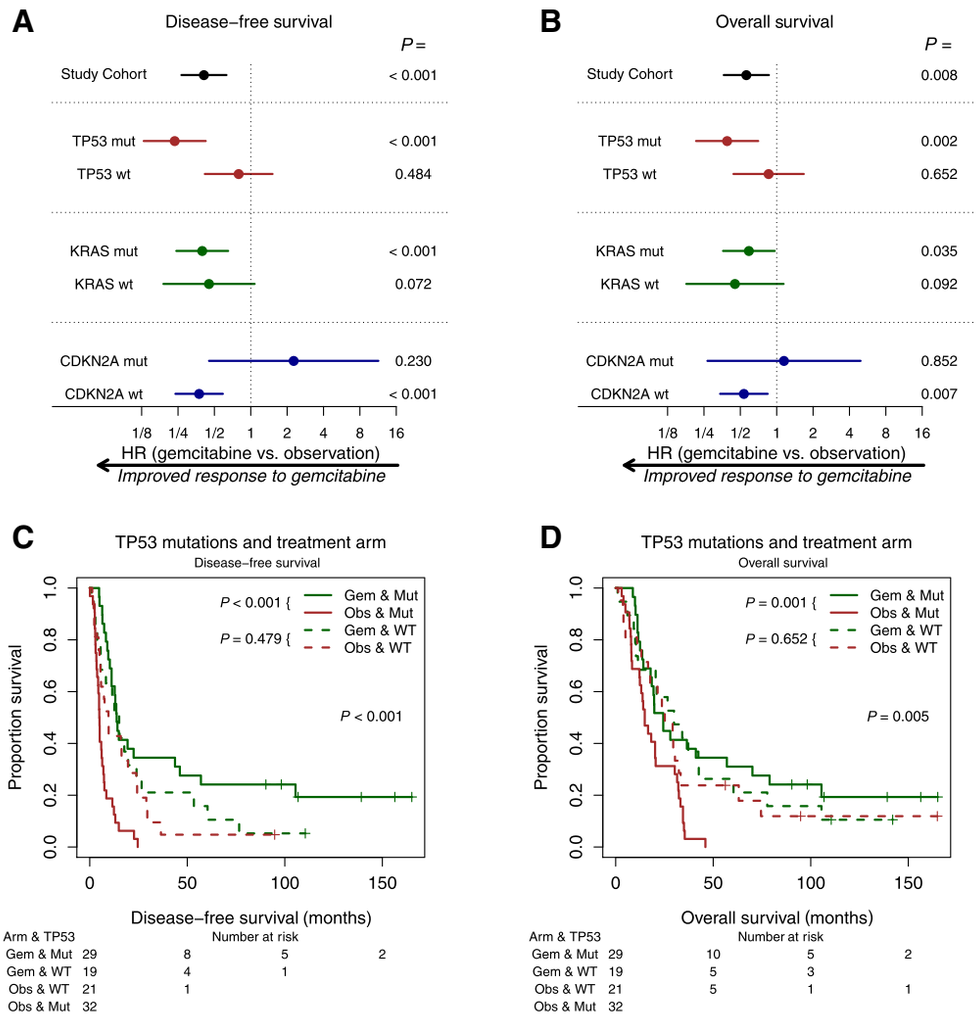

Figure 3.

A significant benefit of gemcitabine treatment is evident in patients with TP53-mutant tumors, whereas patients with wild-type tumors show no difference according to treatment. Forest plot showing the HR for gemcitabine versus observations in the CONKO-001 cohort and mutational subtypes for disease-free (**A**) and overall (**B**) survival. Survival plots for disease-free (**C**) and overall (**D**) survival according to treatment arm stratified for TP53 mutational status.

about adjuvant treatment were not available so the testing of interaction of TP53 with adjuvant treatment could not be validated. Furthermore, TP53 mutations were not predictive for DFS in full CONKO-001, but in TCGA patients. In a functional genomics analysis of the TCGA PDAC cohort, we detected an upregulation of glycolysis in *TP53*mut tumors compared with *TP53*wt tumors mediated by higher mRNA expression of enzymes involved in glycolysis and lower mRNA expression of enzymes involved in glyconeogenesis. Our data suggest a higher flux through glycolysis and a more pronounced Warburg effect in *TP53*mut tumors. Interestingly, a similar result has been recently described in breast cancer (19). These data are in favour of a *TP53* mutation status dependent regulation of the Warburg effect in PDAC that may contribute to the differences in therapy efficacy and survival observed in CONKO-001 and in the TCGA cohort. It should be noted that these findings are only hypothesis-generating and need to be validated in additional independent studies.

To our knowledge, this is the first next-generation sequencing analysis of a clinical trial cohort of primarily resectable PDAC after curatively intended surgery and adjuvant therapy. The strength of our study is the well-characterized study group with comprehensive follow-up data. In concordance with the ESPAC-studies (3, 20), the CONKO-001 study established adjuvant gemcitabine as standard of care in pancreatic cancer (5) and contributed to underline the important role of adjuvant therapy in pancreatic cancer (21). Because of its prospective randomized phase III status and to the fact that the study can provide data for patients treated with adjuvant gemcitabine as well as for untreated patients, the CONKO-001 study is a particularly informative resource for translational research. However, is should be realized that our study has a very limited direct influence on actual decision making in current clinical practice. This is mainly due to the fact that the treatment algorithm changed since CONKO-001 and more effective adjuvant therapies became available with the combination of gemcitabine

and capecitabine (ESPAC-4 trial; ref. 3) and the FOLFIRINOX regimen (PRODIGE 24/CCTG PA.6 trial; ref. 4). Furthermore, due to the still extremely aggressive course of disease and the very good tolerability of gemcitabine, treatment deescalation concepts, even in patients with a bad general condition, actually play no role in the adjuvant decision making of PDAC.

There were several additional limitations of our study: Tumor tissue samples were collected retrospectively from the participating study centers, therefore it was only possible to collect tumor tissue from 183 (52%) of the 354 patients (ITT population), and DNA quantity and quality varied considerably.

Furthermore, probalby due to the self digestion nature of pancreatic cells and the tissue samples processing time beeing between 1998 and 2004, DNA quantity and quality varied considerably. Also many formalin-induced artifacts were observed (C>T substitutions). Altogether only 101 sample were suitable for the NGS data analysis, as another 82 patients had to be excluded because of low DNA quality, corresponding to a rate of 29%. It should be noted that the indicence of the mutations in our cohort was lower than reported in TCGA, which might be due a lower sensitivity of NGS analysis in FFPE samples., This leeds to a major limitation of our study as well as the retrospective approach.

Despite these limitations due to sample age, insufficient sample quality and low tumor cell contents, we were able to confirm the most important driver mutations for PDAC (*KRAS, TP53, SMAD4, CDKN2A*) described in the literature so far (7) applying our customized pancreatic cancer hotspot panel. To overcome these limitations in future clinical trials, the implementation of mandatory prospective tumor sample collection in clinical trials, as suggested in the recent American Society Clinical Oncology position paper (22), seems to be essential. This is particularly important in comparably rare tumor entities such as PDAC.

The role of *TP53* mutations in cancer was redefined several times in the last decades (23). *TP53* mutation is mainly known as a negative prognostic factor in (pancreatic) cancer (24). This effect was confirmed in the CONKO-001 trial for patients without adjuvant treatment, when patients had the natural course of disease after curatively intended resection. Furthermore, *TP53* mutations were found to predict sensitivity to adjuvant gemcitabine as patients in the gemcitabine group with *TP53* mutations had a significantly improved survival if compared with patients with *TP53wt* tumors.

Disclosure of Potential Conflicts of Interest

M. Sinn reports receiving speakers bureau honoraria from Amgen, AstraZeneca, Servier, Sanofi, and Incyte, and reports receiving other remuneration from Servier and Amgen. F. Damm reports receiving other commercial research support from Novartis. H. Riess reports receiving speakers bureau honoraria from Celgene and Servier. C. Denkert is an employee/paid consultant for Amgen and Daiichi Sankyo, reports receiving speakers bureau honoraria from Teva, Novartis, Roche, Amgen, and Pfizer, holds ownership interest (including patents) in Sividon Diagnostics/Myriad, and reports receiving other remuneration from MSD Oncology. No potential conflicts of interest were disclosed by the other authors.

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