Supplementary Material

Patients and Methods

Based on the lack of dose-limiting toxicity, dose level 4 was selected as the dose for the phase II trial [1]. The phase I design is a contraction of 5GyE x 5 from two weeks to one week. Patients were enrolled in a standard 3+3 design (see **Supplementary Table S1**).

Statistical Analysis

The OS time of a patient still alive at the time of last follow-up was censored. The PFS time was measured until the earliest date of locoregional failure, metastatic progression or death; otherwise the event time was censored at the time of last follow-up for progression-free patients still alive. The time to locoregional failure was measured until a patient had a recurrence or progression documented at the relevant site, or otherwise was treated as a competing event at the date of death or censored at the date of last follow-up if still alive. Survival rates were estimated by the Kaplan-Meier method. We used log-log transformation to obtain the confidence interval (CI) for the median as well as pointwise CIs for the survivor function. The cumulative incidence of local failure was estimated using the method of Prentice *et al*. [2], with death considered as a competing event.

We compared the level of each plasma biomarker between genotype groups using the exact Mann-Whitney-Wilcoxon test. We applied log-transformation to circulating biomarkers prior to regression analysis. CA19-9 measurements below the detection level were imputed with the lower limit of detection. Missing measurements were excluded from analysis. Among the patients with distant progression where the extent of metastatic disease was assessable, Gray's test was used to compare the risk of developing oligometastatic disease between genotype groups in the presence of widely disseminated metastases as a competing risk [3]. Statistical analysis was computed using SAS 9.2 (SAS Inst Inc, Cary, NC) and R version 2.6.2 (R Found Stat Comput).

Results and Discussion

Tissue and circulating biomarker studies

The prognosis of resectable PDAC is influenced by a complex interaction between genotype, growth factor levels, and tumor microenvironment [4-6]. PDAC progression is driven by genetic alterations but is facilitated by the activation of the host-derived stroma, in particular tumor-associated desmoplasia and inflammation [7, 8].

We detected *KRAS*^{G12D} mutation in 14/38 resected PDAC patients (37%). *KRAS*^{G12D} mutation—but not any *KRAS* mutation—correlated with elevated circulating levels of the cytokine TNF-α (AUC ROC=0.77; n=8; p=0.036). SMAD4 expression was detectable in 12/32 resected PDAC patients (38%). *SMAD4* status did not correlate with survival. The extent of metastatic disease was assessable in 31/35 patients who progressed in distant sites. In these patients, *SMAD4* deficiency tended to associate with a higher risk of widely disseminated metastases at first relapse, while *SMAD4* preservation tended to correlate with higher risk of developing oligometastatic disease (p=0.051). SMAD4, also known as DPC4, has been identified as a potential biomarker of the clinical course of progression in PDAC [9, 10]. Our study data support the potential utility of *SMAD4* in predicting metastatic spread in resectable patients, although we found no association between *SMAD4* status or disease burden with OS.

In peripheral blood, we found that prior to surgery (~2 weeks after neoadjuvant treatment) there was no significant change in serum CA19-9 or CEA or plasma PlGF, sVEGFR1, sVEGFR2, HGF, s-cMET, IL1β, IL-6, IL-8, TNF-α or circulating progenitor cells (**Supplementary Table S1**). High levels of HGF, TNF-α, CEA and CA19-9 after chemoradiation were associated with inferior survival outcomes (**Supplementary Table S2**). Interestingly, we found that plasma VEGF, SDF1α and bFGF concentrations decreased after chemoradiation. This drop occurred despite the doubling in plasma levels of CAIX (a potential marker of hypoxia, which can stimulate pro-angiogenic molecule expression). Moreover, despite detectable treatment-induced myelosuppression (data not shown), the

number of circulating CD14+ monocytes increased after chemoradiation. Collectively, the histological and biomarker data may indicate a profound primary tumor response after neoadjuvant therapy, accompanied in some patients by tumor hypoxia and inflammation, which could facilitate PDAC progression [6, 11].

In addition, by semi-quantitative immunohistochemistry, we detected high levels of intratumoral expression of both HGF and cMET in the surgical specimens. HGF (a secreted protein) was diffusely detectable in PDAC cells and in the fibrotic stroma of PDAC, while its receptor cMET was almost exclusively expressed on plasma membranes of PDAC cells after treatment (**Supplementary Fig. S1**). In all specimens analyzed, the fibrotic stroma was uniformly positive for HGF although intensity of expression varied among patients.

HGF/c-MET pathway activation is induced by hypoxia and inflammatory factors and has been implicated in cancer cell migration and survival [12, 13]. Preclinical data support the role of HGF/cMET in PDAC progression and resistance to cytotoxics [14-16], and agents targeting the HGF/c-MET axis are currently in clinical trials for various malignancies including advanced PDAC [17].

Finally, *KRAS*^{G12D} mutation correlated with higher plasma levels of TNF-α and both associated with poor outcome. High circulating concentration of the inflammatory cytokine TNF-α is a biomarker of poor prognosis in resected PDAC patients [18]. Of note, TNF-α directly increases HGF expression in mesenchymal cells via activation of the P38MAPK and PI3K/Akt pathways [19].

References

1. Hong TS, Ryan DP, Blaszkowsky LS et al. Phase I study of preoperative short-course chemoradiation with proton beam therapy and capecitabine for resectable pancreatic ductal adenocarcinoma of the head. Int J Radiat Oncol Biol Phys 2011; 79: 151-157.

2. Prentice RL, Kalbfleisch JD, Peterson AV et al. The analysis of failure times in the presence of competing risks. Biometrics 1978; 34: 541-544.

3. Gray RJ. A Class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat 1988; 16: 1141-1154.

4. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011; 473: 298-307.

5. Jain RK. Normalizing tumor microenvironment to treat cancer: bench to bedside to biomarkers. J Clin Oncol 2013; 31: 2205-2218.

6. Bardeesy N, DePinho RA. Pancreatic cancer biology and genetics. Nat Rev Cancer 2002; 2: 897-909.

7. Han H, Von Hoff DD. SnapShot: pancreatic cancer. Cancer Cell 2013; 23: 424-424 e421.

8. Erkan M, Michalski CW, Rieder S et al. The activated stroma index is a novel and independent prognostic marker in pancreatic ductal adenocarcinoma. Clin Gastroenterol Hepatol 2008; 6: 1155-1161.

9. Iacobuzio-Donahue CA, Fu B, Yachida S et al. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. J Clin Oncol 2009; 27: 1806-1813.

10. Crane CH, Varadhachary GR, Yordy JS et al. Phase II trial of cetuximab, gemcitabine, and oxaliplatin followed by chemoradiation with cetuximab for locally advanced (T4) pancreatic adenocarcinoma: correlation of Smad4(Dpc4) immunostaining with pattern of disease progression. J Clin Oncol 2011; 29: 3037-3043.

11. Sanford DE, Belt BA, Panni RZ et al. Inflammatory Monocyte Mobilization Decreases Patient Survival in Pancreatic Cancer: A Role for Targeting the CCL2/CCR2 Axis. Clin Cancer Res 2013; 19: 3404-3415.

12. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev Mol Cell Biol 2003; 4: 915-925.

13. Gherardi E, Birchmeier W, Birchmeier C, Vande Woude G. Targeting MET in cancer: rationale and progress. Nat Rev Cancer 2012; 12: 89-103.

14. Jin H, Yang R, Zheng Z et al. MetMAb, the one-armed 5D5 anti-c-Met antibody, inhibits orthotopic pancreatic tumor growth and improves survival. Cancer Res 2008; 68: 4360-4368.

15. Shah AN, Summy JM, Zhang J et al. Development and characterization of gemcitabine-resistant pancreatic tumor cells. Ann Surg Oncol 2007; 14: 3629-3637.

16. Avan A, Caretti V, Funel N et al. Crizotinib inhibits metabolic inactivation of gemcitabine in c-Met-driven pancreatic carcinoma. Cancer Res 2013.

17. Peters S, Adjei AA. MET: a promising anticancer therapeutic target. Nat Rev Clin Oncol 2012; 9: 314-326.

18. Dima SO, Tanase C, Albulescu R et al. An exploratory study of inflammatory cytokines as prognostic biomarkers in patients with ductal pancreatic adenocarcinoma. Pancreas 2012; 41: 1001-1007.

19. Zhang A, Wang Y, Ye Z et al. Mechanism of TNF-alpha-induced migration and hepatocyte growth factor production in human mesenchymal stem cells. J Cell Biochem 2010; 111: 469-475.

Supplementary Table S1: Phase I design.

Dose Level	Step 1 Lead-in Phase	Dose/fraction	# Tx	Fractionation Schedule	Total Dose	Week 1 Schedule	Week 2 Schedule	Total Days
1	1	3 GyE	10	QD	30	M T W Th Fri	M T W Th Fri	12
	Step 2	**Dose/fraction**	**# Tx**	**Fractionation Schedule**	**Total Dose**	**Week 1 Schedule**	**Week 2 Schedule**	**Total Days**
2	1	5 GyE	5	QD	25	M W F	T Th	11
3	2	5 GyE	5	QD	25	M T Th Fri	M	9
4	3	5 GyE	5	QD	25	M T W Th Fri	-	5

Supplementary Table S2: Analysis of the association between tissue biomarkers with overall survival (OS) and progression-free survival (PFS) after neoadjuvant treatment and surgery in resected PDAC patients. Data are shown as hazard ratios with 95% confidence intervals.

Biomarker	**OS**	**PFS**
***KRAS* mutation (any)** (n=38)	1.04 [0.92,1.18]	1.03 [0.92,1.15]
P value	0.54	0.64
***KRAS*^{G12V} mutation** (n=38)	0.87 [0.74,1.03]	0.88 [0.76,1.01]
P value	0.093	0.066
***SMAD4* status** (n=32)	0.99 [0.87,1.13]	1.00 [0.89,1.14]
P value	0.91	0.94
SDF1α (CXCL12) (intratumoral) (n=32)	1.04 [0.67,1.61]	1.12 [0.76,1.65]
P value	0.86	0.56
SDF1α (CXCL12) (stromal) (n=32)	1.73 [0.85,3.53]	1.14 [0.61,2.14]
P value	0.13	0.68
CXCR4 (intratumoral/cytoplasmic) n=32	0.88 [0.56,1.39]	1.03 [0.69,1.53]
P value	0.59	0.90
CXCR4 (intratumoral/membranous) (n=32)	1.46 [0.80,2.64]	1.35 [0.76,2.42]
P value	0.21	0.30
CXCR4 (stromal) n=31	1.26 [0.67,2.38]	1.31 [0.76,2.27]
P value	0.47	0.33
CXCR7 (intratumoral) n=32	0.93 [0.59,1.44]	1.06 [0.74,1.52]
P value	0.73	0.76
CD68+ macrophage density (tumoral) n=32	1.08 [0.94,1.25]	1.03 [0.90,1.17]
P value	0.29	0.67
CD68+ macrophage density (stromal) n=32	0.96 [0.84,1.10]	0.93 [0.82,1.05]
P value	0.60	0.26

*P values are from Wald test in a univariable Cox regression.

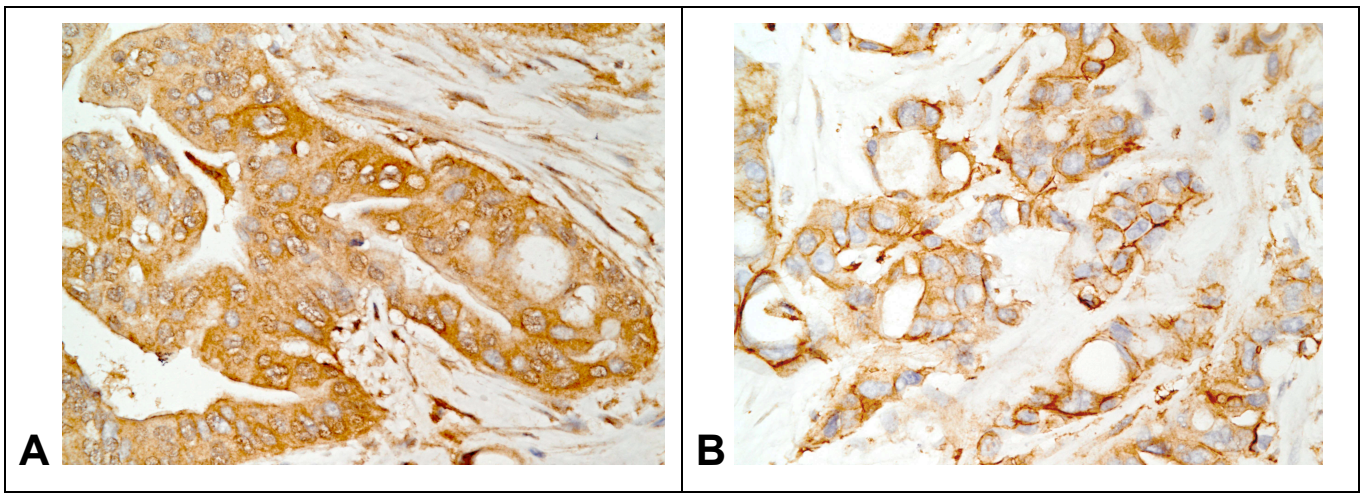
Supplementary Table S3: Kinetics of plasma biomarkers after neoadjuvant chemoradiation in PDAC patients. Data are shown as median concentrations and interquartile ranges for all biomarkers (n=12, except for CA19-9). P values are from Wilcoxon test.

Biomarker/ Time-point	**Pre-Treatment**	**Post-Treatment**	**P value**
Serum CA19-9 (u/ml)	156 [66, 336] n=48	130 [52, 346] n=44	0.089
Serum CEA (ng/ml)	2.50 [1.85, 5.10] n=43	2.60 [1.70, 6.10] n=23	0.61
Plasma s-cMET (pg/ml)	1295 [980, 1424]	1233 [886, 1407]	0.30
Plasma HGF (pg/ml)	1367 [1040, 1602]	1277 [1103, 1608]	0.34
Plasma PlGF (pg/ml)	20 [16, 22]	19 [17, 23]	0.62
Plasma sVEGFR1 (pg/ml)	112 [86, 234]	92 [75, 129]	0.064
Plasma IL-1β (pg/ml)	0.61 [0.45, 0.77]	0.62 [0.45, 0.83]	0.97
Plasma IL-6 (pg/ml)	2.5 [1.4, 4.5]	2.4 [1.8, 4.7]	0.27
Plasma IL-8 (pg/ml)	7.9 [6.6, 10.0]	8.0 [5.1, 10.7]	0.57
Plasma TNF-α (pg/ml)	7.7 [6.1, 11.7]	7.3 [6.2, 10.5]	0.57
CD34+CD133+CD45+ CPCs (% of CD34+CD45+ mononuclear cells)	24.0 [12.3,34.8]	17.5 [8.7,23.3]	0.34

Supplementary Table S4: Significant correlations (shown in bold text) between pre-treatment and post-treatment circulating biomarkers with overall survival (OS) and progression-free survival (PFS) after neoadjuvant treatment and surgery in PDAC patients. Data are shown as hazard ratios with 95% confidence intervals.

Biomarker/ Time-point	Pre-treatment		Post-treatment	
	OS	PFS	OS	PFS
Plasma HGF	**5.56 [1.26,24.64] n=12**	2.57 [0.73,9.11] n=12	**10.12 [0.74,138.3] n=12**	**2.53 [1.03,6.21] n=12**
P value	**0.0057**	0.13	**0.0002**	**0.015**
Plasma TNF-α	3.72 [0.84,16.46] n=12	3.87 [0.92,16.26] n=12	**1.86 [0.95,3.65] n=12**	**3.28 [1.11,9.64] n=12**
P value	0.071	0.054	**0.048**	**0.0074**
Serum CEA	**1.43 [1.08,1.90] n=43**	**1.34 [1.02,1.76] n=43**	**2.02 [1.36,3.01] n=12**	**2.12 [1.37,3.29] n=23**
P value	**0.021**	**0.034**	**0.0001**	**0.0002**
Serum CA19-9	**1.21 [1.04,1.41] n=45**	1.13 [0.97,1.31] n=45	**1.20 [1.06,1.38] n=42**	**1.20 [1.04,1.38] n=42**
P value	**0.014**	0.12	**0.0057**	**0.014**

*P values are from Wald test in a univariable Cox regression using log-transformed covariates for plasma HGF and plasma TNF-α, and rank-transformation for serum CEA and CA19-9. Hazard ratios correspond, to doubling of biomarker concentration (for HGF and TNF-α) and for increase by 10 percentiles (for CEA and CA 19-9).



Supplementary Figure S1: Representative immunohistochemistry in surgical specimen tissues from PDAC patients treated with chemoradiation. A, Expression of HGF is detectable both in the tumor and in the desmoplastic stroma. **B,** Expression of cMET receptor in PDAC cells.