Neoadjuvant Chemotherapy Generates a Significant Tumor Response in Resectable Pancreatic Cancer Without Increasing Morbidity

Results of a Prospective Phase II Trial

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Objective: To evaluate the morbidity of pancreaticoduodenectomy after neoadjuvant chemotherapy for resectable pancreatic cancer and to assess its histologic and metabolic response.

Background: Adjuvant chemotherapy improves the outcome of pancreatic cancer, but 25% of patients remain unfit after surgery. Neoadjuvant chemotherapy can be offered to all patients in a multimodality approach, but its efficacy and surgical morbidity are unknown.

Methods: Patients with resectable, cytologically proven adenocarcinoma of the pancreatic head received 4 bi-weekly cycles of gemcitabine (1000 mg/m^2) and cisplatin (50 mg/m^2) in this prospective phase II trial. Staging and restaging included chest x-ray, abdominal computed tomography (CT), positron emission tomography (PET)/CT, endoscopic ultrasound, and laparoscopy. Fluorodeoxyglucose uptake was quantified by the standard-uptake value (SUV) on baseline and restaging PET/CT. Immunohistochemistry for GLUT-1 and Ki-67 was performed. The histologic response, cytopathic effects, and surgical complications were graded by respective scores.

Results: Twenty-four of 28 patients had resection for histologically confirmed adenocarcinoma. The surgical morbidity was low without perioperative death and one pancreatic fistula. Histologic response was documented in 54% and cytopathic effects in 83% of the patients. A significant SUV decrease occurred during chemotherapy ($P = 0.031$), which correlated with the baseline SUV ($P = 0.001$), Ki-67 expression ($P = 0.016$), and histologic response ($P = 0.01$). Neither the metabolic nor the histologic response was predictive of the median disease-free (9.2 months) or overall survival (26.5 months).

Conclusion: Neoadjuvant chemotherapy induced a significant metabolic and histologic response, which was best predicted by PET. Most importantly, surgery after neoadjuvant chemotherapy for pancreatic cancer was safe.

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Pancreaticoduodenectomy (PD) has become a safe surgical procedure for resectable pancreatic cancer with a mortality rate below 5% in specialized centers.[1,2] However, long-term prognosis remains poor with a reported median survival of 12 months after PD.[1,2] Furthermore, long-term survival of patients after PD for confirmed pancreatic cancer is rare.[3] This poor outcome is mainly attributed to the aggressive biology of this disease with early lymphatic tumor spread, infiltration of perineural sheaths, and undetected distant metastases at the time of surgery. Optimal patient selection and effective multimodality treatments are the mainstays to improve the long-term survival after curative resections. One recently introduced means to refine the patient selection by detecting previously unrevealed metastases is the integrated positron emission tomography/computed tomography (PET/CT), which improved the staging over standard staging alone.[4]

Although the benefit of adjuvant chemoradiation therapy (CRT) remains unclear,[5,6] adjuvant chemotherapy has resulted in prolonged recurrence-free[7] and overall[8] survival of patients with resected pancreatic cancer. The major disadvantage of any adjuvant approach is that at least 25% of the patients will never receive such treatments due to a prolonged postoperative course after PD.[5] For this reason, neoadjuvant (preoperative) regimens have gained an increasing clinical interest.[9] These have mainly been based on CRT but, although CRT resulted in major histologic response,[10,11] they failed to improve long-term survival.[11–13] Considering the recent improvements in overall survival by adjuvant treatments based on systemic chemotherapy, neoadjuvant chemotherapy without radiation also seems attractive. However, the current experience with neoadjuvant chemotherapy is very limited.

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The histologic assessment of tumor response represents the gold standard to assess the cytotoxic effects of oncologic treatments. Because this requires repeated biopsies, tumor response is usually estimated noninvasively by CT or equivalent imaging.[14] Alternatively, the course of specific tumor markers can be used to monitor tumor response to chemotherapy. The best established tumor marker for pancreatic cancer is CA 19-9, which has successfully been used to determine the tumor response to palliative therapy for pancreatic cancer.[15] Furthermore, the response to oncologic treatments can be determined by the metabolic activity of the tumor, which is assigned to the uptake of 18-fluorodeoxyglucose (FDG) by PET. This ability of PET to predict a histologic response even before a morphologic change is detectable on other imaging modalities has been demonstrated for several tumor entities.[16]

We have recently performed a prospective phase II trial on neoadjuvant chemotherapy for resectable pancreatic cancer. This treatment was well tolerated and resulted in an improved nutritional status and quality of life. Furthermore, we observed a significant histologic response in the resected specimen.[17] The aims of the current analysis were to assess the potential of the tumor marker CA 19-9 and PET to predict the histologic response to neoadjuvant chemotherapy and to assess the surgical morbidity of PD after neoadjuvant chemotherapy.

PATIENTS AND METHODS

Study Protocol

A prospective phase II trial on resectable cancer of the pancreatic head was performed between August 2001 and April 2007 at the University Hospital of Zurich. The cytologic proof of adenocarcinoma was mandatory for study inclusion. Tumors with distant metastases and/or vascular infiltration of the superior mesenteric or celiac arteries were excluded, whereas the infiltration of the portal vein was not considered as contraindication for resection. Baseline staging included contrast-enhanced abdominal CT, PET/CT, endoscopic ultrasound with fine needle aspiration cytology, diagnostic laparoscopy, and CA 19-9 serum levels. Chemotherapy included 4 bi-weekly cycles of gemcitabine (1000 mg/m^2) and cisplatin (50 mg/m^2), before which cholestasis had to be controlled (bilirubin <100 μmol/L). Surgery was scheduled 2 weeks after the last chemotherapy, and all staging procedures were repeated during the week before surgery (Fig. 1). All patients had to give written informed consent before study inclusion. This protocol was approved by the local ethics committee.

Surgery

The baseline diagnostic laparoscopy was performed in French position through a subumbilical incision. Two additional 5-mm trocars were inserted as needed. The abdominal cavity was assessed with particular interest to the surface of the liver and the entire parietal peritoneum, but the lesser sac was not opened because biopsies of the primary tumor were not allowed in the study protocol. Biopsies were taken from any lesion suspicious for cancer. The restaging laparoscopy was performed at the beginning of the PD in supine position according to the baseline laparoscopy. All patients received subcutaneous octreotide 0.1 mg 3 times per day from the evening before PD until postoperative day 7 to decrease the risk of pancreatic fistula.[18] A standard PD was always performed. The reconstruction was done by a retrocolic side-to-end pancreatico-jejunostomy and a side-to-end hepato-jejunostomy. This jejunal loop was used for the side-to-side gastro-jejunostomy, and a feeding jejunostomy for enteral nutrition was inserted distally. The enteral nutrition was started on postoperative day 1 with 10 mL/h and adapted according to the clinical course.

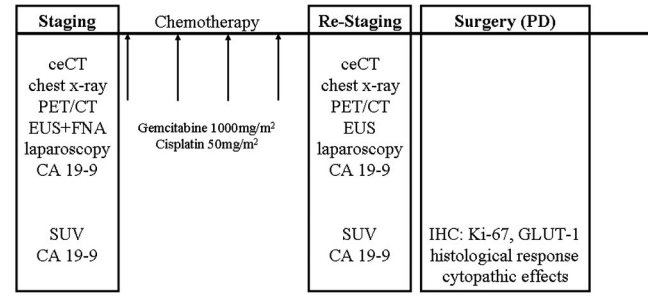

FIGURE 1. Schematic description of the study protocol. After staging excluded contraindications, patients received neoadjuvant chemotherapy with consequent restaging followed by PD.

Surgical Complications

Complications were classified and graded according to a validated complication classification,[19,20] in which grade I and II complications describe minor deviations from a normal postoperative course that can be observed or treated with drugs, blood transfusion, physiotherapy, and nutritional supply. Grade III complications require interventions under local (IIIa) or general anesthesia (IIIb), and grade IV complications require ICU management due to single (IVa) or multiorgan failure (IVb). Grade V means death of the patient (for more details, please consult www.surgicalcomplication.info).

PET/CT and Quantification of FDG-Uptake

FDG PET/CT was performed by a standardized protocol as described previously.[4] The FDG-uptake of the pancreatic lesion was quantified by the maximum standard uptake values (SUV) of FDG on staging and restaging PET, which was normalized for body weight of the patient.[21] The cut-off levels were determined by posthoc analyses (see below).

Histologic Assessments

Because the tumor node metastasis classification has changed during the study, all resected specimen were reassessed by a staff pathologist (A.W.) after study completion, and all tumor stages are provided according to the 6th edition of tumor node metastasis classification.[22]

The histologic tumor response was assessed by a validated score[10]: grade I describes a limited response with up to 10% of tumor destruction; grade II and III defined 10% to 90% and $>$90% of destruction, respectively. Tumors without viable tumor cells are graded as IV. Grade II changes are further subdivided in IIa and IIb, reflecting 10% to 50% and 51% to 90% of tumor destruction, respectively. Furthermore, we developed a score to assess cytopathic effects on the

tumor cells[17]: the mildest changes include swelling of the tumor cells (grade 0). More severe reactions include cytoplasmic vacuolation and clearing (grade I), whereas maximal cell damage includes nuclear condensation and marginalization (grade II) in addition to grade 0 and I reactions.

Immunohistochemistry for the proliferation marker Ki-67 and the GLUT-1 transporter was performed on standardized protocols using monoclonal mouse-antihuman Ki-67 (Dako A/S Glostrup, Denmark, 1:20) and polyclonal rabbit-antihuman antibodies (Chemicon International Inc., 1:1000). All slides were pretreated with cell-conditioning solution and analyzed using the Ventana Benchmark automated staining system (Ventana Medical Systems, Tucson, AZ). Because of the inhomogenous expression, GLUT-1 expression was semi-quantitatively analyzed: grade 0 reflected no expression, grade 1 slight, grade 2 intermediate, and grade 3 strong expression. According to international standards, Ki-67 expression was quantified as percentage of positive tumor cells. The maximum value was taken for all histologic analyses, which were performed by the same pathologist blinded for clinical information (A.W.).

Statistics

The primary study end point was a resectability rate >70% after neoadjuvant chemotherapy. According to the Simon 2-stage phase II design, 28 patients had to be included to achieve a power of 80% ($P < 0.05$). Dichotomous variables are compared using the χ^2 test (McNemar if appropriate). Continuous variables are expressed by mean (±SD) and compared using the (paired) student *t*-test. In case of nonparametric variables, median (range) is provided and compared by the Mann-Whitney *U* test. Correlations between different parameters were tested by the Spearman rank correlation. Survival data are calculated from the date of study inclusion until the event according to the Kaplan-Meier method (Log-rank test). For all analyses, $P < 0.05$ is considered significant. The SPSS 12.2.2 software was used for all analyses.

RESULTS

Patient Characteristics

Twenty-eight patients were included and received neoadjuvant chemotherapy. Of these, 25 patients had complete staging and restaging procedures, showing no contraindication for surgery. Although 1 patient underwent PD after 2 cycles without restaging PET/CT due to persistent emesis, laparoscopy revealed peritoneal metastases in 2 patients. Consequently, 26/28 patients (93%) were resectable upon restaging examinations. Another patient was locally unresectable due to an infiltration of the superior mesenteric artery (SMA) found during surgical exploration, and 1 patient was excluded from the current analysis because he had high-grade dysplasia without invasive cancer in the final histology. Therefore, surgical specimens were available from 24 patients (female/male, 12/12) with a median age of 59 years (range, 39–77). Twenty-three tumors were T3 and 1 T4, with lymph node metastases (N+) in 19/24 patients (79%). The portal vein was resected in 3 patients due to suspected tumor infiltration, which was histologically confirmed in one patient.

Histologic Response to Neoadjuvant Chemotherapy

The gold standard for the determination of the efficacy of an oncologic treatment is the histologic response.

As reported previously, 11 patients (46%) revealed grade I, and 13 (54%) grade II histologic response, of which 8 patients had IIb and 5 IIa responses. No grade III–IV responses occurred. Furthermore, we found significant cytopathic effects (grade I–II) in the surgical specimen in 83% of the patients: grade I effects were found in 15/24 patients (62%), and grade II effects in 5/24 patients (21%).[17]

In summary, neoadjuvant chemotherapy induced significant cytopathic effects and histologic response in the majority of the patients.

CA 19-9 Response to Neoadjuvant Chemotherapy

The tumor marker CA 19-9 is expressed by pancreatic cancer cells and predicts response to palliative chemotherapy.[15] Therefore, this marker also appears appealing for the response assessment to neoadjuvant chemotherapy.

CA 19-9 serum levels were available from all patients before and after chemotherapy. Seven patients (26%) had normal CA 19-9 levels at baseline, and all of them remained normal during chemotherapy. The mean baseline CA 19-9 level of the entire group was 325.9 kU/L (±438) and decreased to 171.1 kU/L (±232) during chemotherapy ($P = 0.015$). Among patients (n = 20) with pathologic CA 19-9 levels, the mean CA 19-9 level decreased from 418.7 kU/L to 215.3 kU/L ($P = 0.014$). Both patients with peritoneal metastases had normal CA 19-9 levels before and after chemotherapy, whereas the patient with local infiltration of the SMA revealed a CA 19-9 decrease from 269.5 kU/L to 168 kU/L.

Taken together, the data suggest a response to neoadjuvant chemotherapy, although a CA 19-9 decrease did not predict resectability.

Metabolic Response to Neoadjuvant Chemotherapy

The primary tumor was FDG-positive in 23/27 patients (85%). Because of the strong FDG-uptake of pancreatic cancer, we evaluated whether neoadjuvant chemotherapy resulted in a decrease in the metabolic activity of the tumors.

The median SUV at baseline was 4.4 (range, 2.1–14.1) and decreased to 3.3 (range, 2.1–18.6) during chemotherapy ($P = 0.03$) (Fig. 2). Therefore, we used SUV of 4.4 and 3.3 as cut-off levels for baseline and postchemotherapy SUV, respectively. We defined 30% as cut-off level for the metabolic response due to its mean decrease, which highly correlated with the baseline SUV ($P = 0.001$, Fig. 3). The median baseline SUV was 6.6 (range, 3.6–14.1) in patients with a metabolic response >30% and 3.1 (range, 2.1–11.3) in patients below <30% ($P = 0.003$). The median postchemotherapy SUV of patients with a metabolic response >30% was 3.6 (range, 2.1–18.6) and 3.2 (range, 2.3–13.4) in the remaining patients ($P = 0.72$).

Summarizing the information gained from this analysis, we observed a significant metabolic response, which was

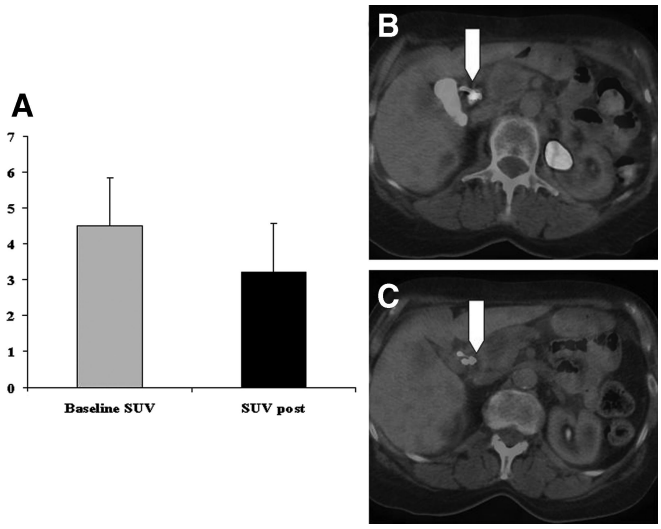

FIGURE 2. Representative PET/CT images of a female patient with cancer of the pancreatic head demonstrating the metabolic response to chemotherapy. The baseline SUV (B) of this patient was 9.1 and decreased to 3.6 (C) during neoadjuvant chemotherapy (arrows indicate focal FDG accumulation in the pancreatic head). (A) Underlines the SUV decrease during chemotherapy.

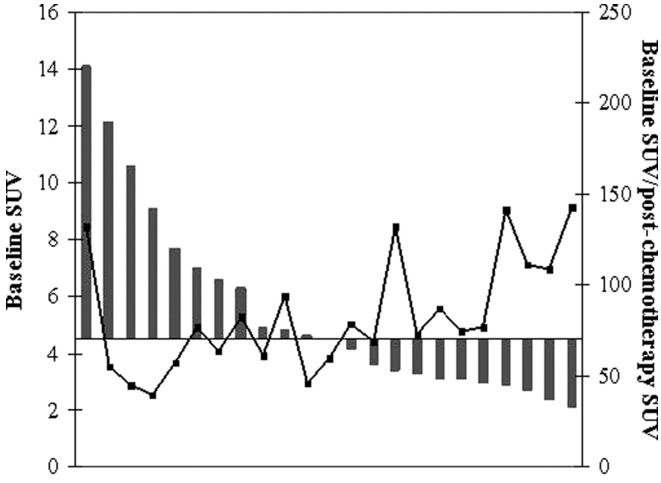

FIGURE 3. The waterfall plot of the baseline SUV and the remnant SUV after chemotherapy demonstrates that a high baseline SUV results in a metabolic response during chemotherapy (the metabolic response is presented as the ratio of postchemotherapy/baseline SUV, and an SUV increase during chemotherapy therefore resulted in values >100%).

most pronounced in tumors with a high metabolic activity at baseline.

Mechanism of Metabolic Response in Pancreatic Cancer

Because we observed a significant metabolic response, we further evaluated putative mechanisms linking the SUV response to effective chemotherapy. After cellular uptake by the GLUT-1 transporter, FDG accumulates in the cell be-

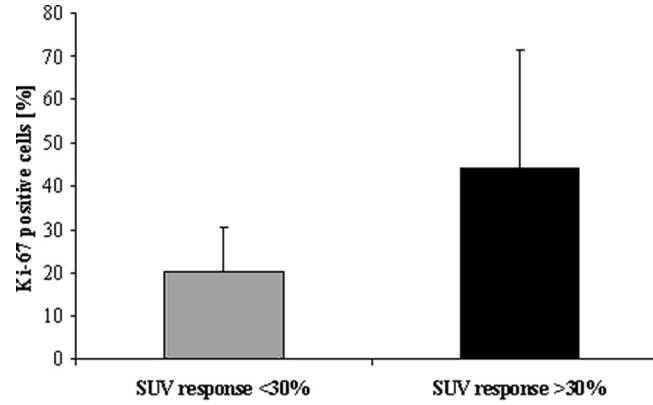

FIGURE 4. Differences in the Ki-67 expression of tumors with a high (>30%) and low (<30%) SUV response.

cause it is not metabolized.[16] Also, the magnitude of SUV correlated with the proliferation rate in breast cancer.[23] Therefore, we performed IHC for the GLUT-1 transporter and the proliferation marker Ki-67 to evaluate the impact of these 2 parameters on the metabolic response.

All tumors expressed the GLUT-1 transporter and were positive for Ki-67 on IHC to a variable amount. GLUT-1 expression was of grade I in 8 patients (36%), grade II in 11 patients (50%), and grade III in 3 patients (14%). The GLUT-1 expression neither correlated with baseline, nor restaging SUV, nor with the histologic response.

An average of 30% (±22.4) of cancer cells stained positive for Ki-67, which did not correlate with postchemotherapy SUV, histologic response, or cytopathic effects. However, tumors with a baseline SUV above 4.4 had higher Ki-67 (40.8%, ±25.7) expression than tumors with a lower baseline SUV (19.5%, ±11.2) ($P = 0.025$). Also, tumors with high metabolic response had higher Ki-67 expression ($P = 0.016$; Fig. 4).

As expected, pancreatic cancer over-expressed the GLUT-1 transporter, but the low SUV after chemotherapy is independent of the intensity of GLUT-1 expression. Also, the SUV-response seems independent of a change in the intensity of GLUT-1 expression. In contrast, tumors with a high proliferative activity in the resected specimen had a high metabolic activity at baseline and revealed a better metabolic response to neoadjuvant chemotherapy.

Prediction of Histologic Response

We observed a significant histologic response to neoadjuvant chemotherapy, and both CA 19-9 and FDG-uptake strongly suggested a response to this treatment. Therefore, we evaluated whether these 2 parameters were predictive of the histologic response.

Neither histologic response nor cytopathic effects revealed a correlation with CA 19-9 serum levels. The baseline SUV revealed a negative correlation with the histologic response ($P = 0.01$): the median baseline SUV was 6.6 (range, 2.4–14.1) in patients without, and 3.5 (range, 2.1–7.0) in patients with a histologic response >10% ($P = 0.016$). Similarly, the postchemotherapy SUV correlated negatively

with the histologic response ($P = 0.01$): the median postchemotherapy SUV was 4.4 (range, 2.6–18.6) in patients without and 2.9 (range, 2.1–5.4) in patients with a histologic response of more than 10% ($P = 0.02$). The metabolic response correlated significantly with cytopathic effects ($P = 0.02$), but the differences in the median baseline (4.9 vs. 3.6, $P = 0.15$) and postchemotherapy (4.2 vs. 2.7, $P = 0.086$) SUV did not reach significance in patients with cytopathic effects below grade II compared with those with grade II, respectively.

From this analysis, we conclude that CA 19-9 serum levels are not suitable to predict tumor response to neoadjuvant chemotherapy. In contrast, low baseline and postchemotherapy SUVs are highly predictive for a histologic response and suggest cytopathic effects.

The Value of PET/CT for Restaging After Neoadjuvant Chemotherapy

PET/CT was performed to exclude patients with distant metastases before study inclusion and was repeated after neoadjuvant chemotherapy to exclude extrapancreatic disease progression and to evaluate the metabolic response to neoadjuvant chemotherapy.

The baseline PET/CT depicted FDG-positive regional lymph nodes in 2 and FDG-negative lymph nodes in 5 patients. All were N+ cancers upon final histology. The tumor, which infiltrated the SMA, was FDG-negative, whereas both patients with peritoneal metastases after chemotherapy had FDG-positive primary tumors at baseline. Moreover, PET/CT revealed several findings unrelated to cancer by the CT portion. Five patients had small pulmonary lesions, which remained unclear due to the small size and were classified as benign lesions. Restaging PET/CT diagnosed liver metastases in 2 patients, of which 1 had 2 FDG-negative lesions in the liver on the CT, and another patient had an FDG-positive hepatic lesion. Intraoperative wedge resections revealed benign hamartoma in both cases. All other findings remained stable during neoadjuvant chemotherapy.

Thus, in addition to the detection of distant metastases,[4] the staging PET/CT depicted several benign and unclear findings by the CT portion in addition to distant metastases. After chemotherapy, PET/CT could not differentiate benign from malignant disease.

Morbidity and Mortality of PD After Neoadjuvant Chemotherapy

One of the most important concerns against neoadjuvant approaches is that the pretreatment could increase the surgical morbidity. This is an important issue because PD alone is a procedure with a high morbidity.[20]

The median hospital stay after PD was 16 days (range, 8–36 days) including a median postoperative stay on the intensive care unit of 1 day (range, 1–4 days). The mean intraoperative blood loss was 736 mL (±476.9 mL), and 13 patients (46%) received blood transfusions (median, 1 unit; range, 1–3). The in-hospital and 30-day mortalities were 0%. One patient died 39 days after PD from massive bleeding from the hepatic artery due to an abscess confirmed by autopsy. Most complications were of low grade (I–II). We observed 1 pancreatic fistula, and none of the patients developed a DGE (Table 1).

TABLE 1. Complications of PD After Neoadjuvant Chemotherapy

	Complications	
Grade	**N**	**%**
I	74	49
II	67	44.4
IIIa	4	2.6
IIIb	3	2
IVa	—	—
IVb	2	1.3
V	—	—
	151	100

Total number of each complication and proportion of all complications, left; number of patients experiencing at least 1 of each complication grade and proportion of patients who underwent resection, right (for more details, please consult www.surgicalcomplication.info).

Therapy-relevant complications, specific complications of PD, and the mortality rate were very low after neoadjuvant chemotherapy and PD.

Survival Prediction

We speculated that the observed histologic response to chemotherapy would impact on the patient outcome. Therefore, we evaluated whether parameters of the current analysis were predictive for survival after neoadjuvant chemotherapy.

The median overall survival of the 24 patients with resected adenocarcinoma was 26.5 months (95% CI, 16.1–36.8 months) after study inclusion, and the median recurrence-free survival in these patients was 9.2 months (95% CI, 7.6–10.9). Results of the univariate analysis for potentially predictive factors are summarized in Table 2.

From this analysis, we learned that none of the tested parameters was predictive of survival.

DISCUSSION

The main finding of this study is that PD after neoadjuvant chemotherapy was safe and associated with low morbidity and mortality rates. Furthermore, neoadjuvant chemotherapy using gemcitabine and cisplatin is an effective treatment considering its histologic response and cytopathic effects. The magnitude of histologic response was best predicted by a low metabolic activity of the tumor at baseline and after chemotherapy. Moreover, tumors with a high proliferative activity revealed the highest baseline SUV and the strongest metabolic response, which was, however, not reflected in the histologic response.

Optimal patient selection and effective multimodality regimens are pivotal to improve the long-term prognosis of patients with resected pancreatic cancer. Adjuvant chemotherapy is increasingly considered as standard treatment after PD after promising results of recent randomized trials on adjuvant chemotherapy.[7,8] But, a high proportion of patients (>25%) will not receive adjuvant treatments in a timely manner due to complications from surgery.[5] Neoadjuvant

TABLE 2. Univariate Analysis of Clinical Factors Potentially Predictive for Disease-Free and Overall Survival

			Disease-Free Survival		Overall Survival	
	Cut-Off	**n**	**Median (95% CI) (mo)**	***P***	**Median (95% CI) (mo)**	***P***
Histological response	≤1	11	9.2 (7.6–10.9)	0.97	32.4 (15.3–49.5)	0.42
	>1	13	9 (2.8–15.3)		19.1 (13.6–24.5)	
Cytopathic effects	≤1	19	9 (8.1–10)	0.71	26.5 (13.2–39.7)	0.42
	>1	5	12 (5.6–18.3)		13 (3–23.1)	
CA 19-9	<37 kU/L	5	12 (3.2–20.7)	0.81	34.2 (nb)	0.82
	>37 kU/L	19	9 (8.1–10)		26.5 (13.8–39.1)	
	<100 kU/L	9	8.8 (3.7–13.9)	0.96	34.2 (22.2–46.2)	0.43
	>100 kU/L	15	9.2 (6.84–11.6)		18.7 (15–22.4)	
SUV baseline	<4.4	11	9.8 (5.9–13.8)	0.91	27.5 (11.3–43.6)	0.89
	≥4.4	12	7.8 (3.3–12.4)		19.1 (7.1–31.1)	
SUV post	<3.3	13	9 (6.7–11.4)	0.93	27.5 (12.7–42.2)	0.53
	≥3.3	10	8.8 (6.6–10.9)		19.1 (8.3–29.9)	
SUV response	<30%	14	9.2 (7.5–10.9)	0.49	18.7 (16.1–21.3)	0.43
	≥30%	10	8.8 (0–20.2)		27.5 (21.9–33)	

regimens are applicable to all patients and may achieve a tumor down-sizing and treatment of circulating tumor cells preoperatively through a physiologic blood supply. However, neoadjuvant treatment also harbors the risks of disease progression in case of an ineffective treatment and a higher morbidity due to inflammatory reactions. Similar to the treatment of other gastrointestinal malignancies, various neoadjuvant regimens have been tested for pancreatic cancer, which were based on CRT except for 1 recent trial.[9,24]

CA 19-9 is an established tumor marker for pancreatic cancer, which can be used to assess the response to palliative chemotherapy,[15] and has predictive value for resectable pancreatic cancer.[25] Because patients required a bilirubin level below 100 μmol/L before neoadjuvant chemotherapy, most of our patients underwent internal biliary drainage. Hence, the observed decrease of CA 19-9 levels during chemotherapy may rather reflect the variable relief of cholestasis than a true tumor response.

In chemo-naive patients, PET and PET/CT have been shown to improve patient selection for surgery.[4] The value of the unclear pulmonary nodules upon staging PET/CT remains unclear, in particular because they did not effect overall survival. However, restaging PET/CT did not depict any of the 2 histologically confirmed peritoneal metastases and was false-positive in 2 benign lesions related to chronic cholestasis. These findings underline the necessity to obtain histologic confirmation of all suspicious lesions and implies that PET/CT should not be considered a standard follow-up tool after neoadjuvant chemotherapy outside controlled trials.

In addition to its value in detecting distant metastases, PET is increasingly used to determine the metabolic response to treatments. FDG enters the cell through the GLUT-1 transporter and accumulates there because it is not completely metabolized. Consequently, FDG-PET primarily measures the magnitude of FDG uptake and the glycolytic activity of tissue.[17,21] Various parameters can be used to quantify the FDG-uptake and metabolic response of which the SUV is the most frequently used due to its easy determination. We applied a highly standardized scanning protocol and normalized the SUV for body weight to minimize variations related to eg, weight loss due to chemotherapy.[21] Our data suggest that the magnitude of baseline SUV in pancreatic cancer is independent of the GLUT-1 expression but related to the proliferation rate of the tumor after chemotherapy. As described for lung cancer, the baseline SUV was highly predictive for the extent of metabolic response.[26] Although an additional decrease in the proliferative activity during chemotherapy cannot be excluded, the primary mechanism of the metabolic response to neoadjuvant chemotherapy is presumably a decrease in the mitochondrial activity, considering that cisplatin induces apoptosis in cancer cells through a selective effect on mitochondria.[27] However, we failed to detect a relevant caspase-3 release or decrease in the mitochondrial mass by IHC (data not shown), probably because apoptosis is an early event, and PD was performed 2 weeks after the last chemotherapy cycle.

Although PET has been increasingly used to predict survival and histologic response, the relation of metabolic activity/response, histologic response, and outcome is not uniform for all types of cancer. Although a high baseline SUV predicted better histologic response and survival to neoadjuvant treatment of lung cancer,[26] it was a negative predictive factor for histologic response and outcome in lymphoma[28,29] and cancer of the oropharynx.[29] Also, we found a stronger histologic response in tumors with lower baseline and postchemotherapy SUV in response to neoadjuvant chemotherapy. Although this heterogeneity in the literature may be related to different scanning protocols and treatments, it mainly reflects differences in the biology of the diseases. Furthermore, it underlines that FDG is a marker for metabolic activity rather than a specific tumor marker. Because no baseline histology was available in our study due to the study design, we can only speculate on the magnitude of the baseline proliferative activity of the tumors and its effect

on tumor response. Also, it may be assumed that the lack of histologic response in tumors with a strong metabolic response indicates that these tumors require a longer or more intensive treatment to reveal a histologic response similar to tumors with lower baseline SUV. In accordance with the literature, a low post-therapy SUV correlated with a low remnant tumor mass in our study.[26]

Several clinical factors are considered predictive for survival after PD in large cohorts of patients, and a histologic and metabolic response would be expected to result in improved outcome. However, none of these parameters were predictive for the patient outcome in our study population. Most probably, the lack of significance is due to the sample size of the respective groups, as this study was not designed to define predictive factors for outcome. Because no information was available about neoadjuvant chemotherapy without radiation when this study was planned, safety (resectability rate) was chosen as primary end-point. This end-point was achieved and was even higher than previously reported resectability rates after CRT. Despite the limited sample size, we were, however, able to explore the mechanisms of metabolic response due to the prospective character and meticulous data management of this study.

Because all patients had resectable tumors at study entry, which were potentially curable by surgery, we specifically evaluated the morbidity of PD in this setting. Importantly, neoadjuvant chemotherapy did not increase the morbidity of PD after neoadjuvant chemotherapy compared with the literature.[20] We documented a fair number of grade I and II complications, which might be related to the neoadjuvant treatment. However, prospective data on these types of complication (eg, atelectasis or hypertension) are rare. On the other hand, complications requiring invasive treatments (eg, abscess, fistula) or ICU management seem less frequent than in a recent analysis of a large data-base for pancreas surgery by us.[20] A similar observation was reported from Cheng et al. who found less intra-abdominal abscesses and pancreatic fistulae after PD after neoadjuvant CRT.[30] Whether this is a real effect of the pretreatment or due to patient selection needs to be evaluated in a randomized trial.

In conclusion, neoadjuvant chemotherapy is an effective treatment for pancreatic cancer resulting in a high degree of histologic and metabolic response. Furthermore, we demonstrate for the first time that PET is predictive of this response to neoadjuvant chemotherapy for pancreatic cancer. Based on our results, the baseline FDG uptake is related to the proliferative activity of the tumor, and the metabolic response is primarily related to a decreased metabolic activity. Most importantly, PD after neoadjuvant chemotherapy is safe. However, a large randomized trial is necessary to investigate the value of neoadjuvant chemotherapy in pancreatic cancer, to define the exact role of PET for the response prediction, and to further evaluate the surgical morbidity in this setting.

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Discussions

M. ROTHMUND: In the period between 2001 and 2006, you included 28 patients in the study. It is likely that more than 28 patients with carcinoma of the head of the pancreas were operated on in that time period in your institution. What were the inclusion criteria for patients in this study? What patients were excluded?

My second question relates to your end point. You mentioned that your primary end point was a resectability rate of more than 70%, and you mentioned this in the statistical portion of your manuscript as well. In the manuscript discussion you say, "Safety was chosen as (the) primary end point." What was your actual primary end point?

The third question is on morbidity. You said "neoadjuvant chemotherapy did not increase surgical morbidity," but you did not refer to morbidity due to chemotherapy. Therefore, what was the morbidity caused by chemotherapy?

Finally, what happened to the patients who progressed during neoadjuvant chemotherapy?

S. HEINRICH: In answer to your first question, because of the strict staging protocol, we excluded several patients from this study because of previously unknown metastatic disease. Also, we lost several patients because of false negative fine needle aspirations and due to patients' refusal to participate in this trial. However, all consecutively eligible patients were included in the study.

Regarding the end point of our study, phase II trials in general are safety trials. When we started this trial, information about the risk of disease progression to an unresectable stage under neoadjuvant chemotherapy was not available. Consequently, a safe treatment would be one that achieves a resectability rate equal to the baseline. Therefore, we chose the resectability rate after neoadjuvant chemotherapy as the primary end point of this study.

Your third question focused on the side-effects of chemotherapy. These results were published in a separate article in the *Journal of Clinical Oncology*.[17] Briefly, most patients developed only grade 1 and 2 side-effects according to the National Cancer Institute, most of which were hematological or gastrointestinal. In the current analysis, we focused on the surgical morbidity.

Regarding your last question, patients who progressed to a locally unresectable or metastatic disease reached the primary study end point, and therefore did not undergo surgery, but did receive palliative treatment.

H. FRIESS: As mentioned by Professor Rothmund, I also have concerns regarding a patient selection bias. By looking at the time period when these patients were included and how few patients were finally enrolled in this study, I would have expected that many more patients with resectable pancreatic cancer were treated in your center in Zurich.

The second and third questions address whether a reliable response evaluation to neoadjuvant treatment can be done in pancreatic cancer as it is reported in other GI malignancies. I expect that you took small biopsy samples before you started neoadjuvant treatment, which served as a control for the histologic response evaluation in the resected pancreatic cancer specimens. Because pancreatic cancer is characterized by a strong desmoplastic reaction, I doubt that a small tissue biopsy is sufficient for a reliable histologic response evaluation in such a setting. We know from experience, if you do fine needle biopsy in pancreatic cancer, you often find much fibrotic tissue, but no or only small areas with cancer cells. Therefore, I doubt whether an adequate histologic response evaluation can be done in this disease.

The same might be true for the role of PET in the response evaluation in this study. We know from a number of clinical studies that PET has its limitations in the differentiation of pancreatic cancer from chronic pancreatitis and many patients with chronic pancreatitis are PET positive. Therefore, in pancreatic cancer, you cannot be sure whether your PET signals come from the cancer cells or from fibrotic or desmoplastic tissue.

S. HEINRICH: I discussed the reasons for the long patient accrual time previously. When we initiated this trial, we had just launched our pancreas program, which grew dramatically during recent years. Moreover, all of our patients had locally advanced disease with T3/4 and nearly all having N+ disease. In addition, we included 1 patient each with simultaneous rectal cancer, echinococcus disease, and after kidney transplantation. These patients' characteristics clearly do not represent a positive selection.

We fully agree with you that pancreatic cancer has a difficult histologic appearance including a wide range of desmoplastic reactions. Pancreatic cancer was proven before study inclusion by fine needle aspiration cytology. This does not allow

a histologic diagnosis or grading of the tumor nor an assessment of the desmoplastic reaction. We assessed the tumor response with an established score from the M.D. Anderson group (Evans et al). This score is based on histologic changes attributable to the treatment and quantifies the percentage of tumor destruction in the resected specimen. However, these changes clearly differ from a desmoplastic reaction.

Different degrees of chronic pancreatitis or desmoplastic reactions are potential explanations for the variability of FDG (radioactive glucose fluorodeoxyglucose) uptake at study inclusion. Furthermore, there is growing evidence in the literature that the degree of FDG uptake is related to the expression of different oncogenes. However, the mechanism of SUV (standard uptake value) response to chemotherapy in this setting remains unclear.

C. Bassi: The neoadjuvant approach is one to explore, but before we understand an approach, we have to stratify our patients in the best way possible. We have to understand the philosophy underlying what we intend to look for. Are we looking for a strict policy to apply for all patients suffering from pancreatic cancer who are resectable today and who we will see in 3 months, or will we apply this policy in probable borderline, unresectable cases? Hence, are we looking for a strict neoadjuvant with a sandwich treatment or down staging? In this setting, I would ask if you absolutely need that laparoscopy before neoadjuvant treatment and before surgery.

Also, which kind of chemotherapy; the same therapy before and after, or different chemotherapy?

Finally, we are all aware that about 15% to 30% of patients who underwent radical surgery as reported in the literature will die about 1 year after surgery and this is true everywhere in the world. With the neoadjuvant approach, we can perhaps avoid surgery and for patients in whom it does not play a role because the same results are achievable with modern chemotherapy.

S. Heinrich: Thank you, Dr. Bassi, for your question. The indication for neoadjuvant chemotherapy is, of course, unknown. We cannot conclude from our study, which patients would benefit the most. Looking at other gastrointestinal cancers, like gastric cancer, liver metastases from colorectal cancer, rectal cancer, and esophageal cancer, there is more evidence that multimodality and preoperative treatments are beneficial. Consequently, the question arises why this should be different in pancreatic cancer. We only included patients with resectable tumors including those with portal vein infiltration, which would be considered borderline resectable by others.

We will soon launch a randomized trial to further evaluate the indication of neoadjuvant chemotherapy and to determine which patients might benefit the most. At the moment, we do not know. We currently believe that all patients should receive neoadjuvant chemotherapy.

In the setting of a prospective study, we had to use diagnostic laparoscopy because a peritoneal carcinomatosis at the time of the Whipple procedure would always be considered as a treatment failure of neoadjuvant chemotherapy, although it might have been there before chemotherapy. Since this fact would impact on the primary end point of this study, we insisted on a prechemo laparoscopy. Indeed, we detected previously undetected liver metastases and peritoneal carcinomatosis by laparoscopy in some patients. However, we probably do not need a laparoscopy outside clinical trials because most surgeons will perform a double bypass procedure in case of unresectability in symptomatic patients anyway.

At the moment, the adjuvant treatment with the best effect is gemcitabine. This was shown recently (Oettle et al *JAMA* 2007; 297:267–277) and therefore we consider this as the standard adjuvant chemotherapy. Neoadjuvant chemotherapy is still experimental, and therefore no standard regimen exists. The treatment should be based on a combination of treatments with high response rates in the palliative setting. Currently, these are the platins in combination with gemcitabine.

What becomes obvious with neoadjuvant chemotherapy is that we are not treating a localized disease that can simply be treated by surgery, but a systemic disease. Therefore, we believe that patients should receive chemotherapy with a maximal systemic effect.

J. Schmidt: Many patients suffer from cholestasis and have a prosthesis in place. Did you observe any cholangitis in addition to chemotherapy problems?

The low FDG uptake patients' histopathological response was the best, which is somewhat surprising. How do you explain it and what is your conclusion? Do you exclude the patients with high uptake from neoadjuvant treatment?

S. Heinrich: Among the study inclusion criteria, we used a bilirubin level below 100. This was necessary for chemotherapy. All patients with a bilirubin level below 100 did not receive stenting, and all patients with a bilirubin level above 100 at study inclusion received a stent. Cholangitis occurred in 4 patients.

Regarding the FDG uptake and histologic response, it is a difficult question that requires further analysis. We cannot conclude from this study that the patients with a high FDG uptake cannot receive chemotherapy. This would be unfair. We do not have a good explanation. In the literature, if you look at several cancer types (eg, gynecologic cancer, and head and neck cancer), there are patients with a low FDG uptake whose prognosis is better, and this would fit with our data. Looking at esophageal cancer, for example, patients with PET-negative tumors are often excluded from the analysis, so we look at high FDG positive tumors. This makes a difference, of course, and we do not know if highly FDG-positive tumors in our series would show a correlation to the histologic response. We did not have enough patients for these subgroup analyses.