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Precision Oncology in Surgery
Patient Selection for Operable Pancreatic Cancer

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Objective: We aimed to define preoperative clinical and molecular characteristics that would allow better patient selection for operative resection.

Background: Although we use molecular selection methods for systemic targeted therapies, these principles are not applied to surgical oncology. Improving patient selection is of vital importance for the operative treatment of pancreatic cancer (pancreatic ductal adenocarcinoma). Although surgery is the only chance of long-term survival, 80% still succumb to the disease and approximately 30% die within 1 year, often sooner than those that have unresected local disease.

Method: In 3 independent pancreatic ductal adenocarcinoma cohorts (total participants = 1184) the relationship between aberrant expression of prometastatic proteins S100A2 and S100A4 and survival was assessed. A preoperative nomogram based on clinical variables available before surgery and expression of these proteins was constructed and compared to traditional measures, and a postoperative nomogram.

Results: High expression of either S100A2 or S100A4 was independent poor prognostic factors in a training cohort of 518 participants. These results were validated in 2 independent patient cohorts (Glasgow, n = 198; Germany, n = 468). Aberrant biomarker expression stratified the cohorts into 3 distinct prognostic groups. A preoperative nomogram incorporating S100A2 and S100A4 expression predicted survival and nomograms derived using postoperative clinicopathological variables.

Conclusions: Of those patients with a poor preoperative nomogram score, approximately 50% of patients died within a year of resection. Nomograms have the potential to improve selection for surgery and neoadjuvant therapy, avoiding surgery in aggressive disease, and justifying more extensive resections in biologically favorable disease.

Keywords: biomarkers, genomics, pancreatic cancer, personalized medicine

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Although molecular markers are being used more frequently to select patients for systemic targeted agents, only imaging modalities are used to stage patients and assess suitability for operative resection. Decisions on primary surgery or a neoadjuvant approach are made without biological measures of tumor aggressiveness, or the risk of occult metastatic disease. This is exemplified in pancreatic ductal adenocarcinoma (PDAC), which has overtaken breast cancer to become the third most common cause of cancer death in the USA.[1] Surgical resection offers the only chance of cure, with chemotherapy adding modest benefit, but surgery can be associated with significant morbidity and mortality risk. Even with complete resection and adjuvant chemotherapy, the 5-year survival rate is only approximately 20%,[2–6] with approximately 30% succumbing within the first year (mostly due to distant metastatic disease).[7,8] This high metastatic recurrence rate indicates current staging modalities for PDAC cannot identify patients with occult metastases and aggressive biology. For these patients, surgical resection brings uncertain benefit. Whipple pancreaticoduodenectomy can be associated with significant mortality risk and morbidity that leads to long postoperative recovery periods of 3 to 6 months, which presents significant implications on patients' quality of life.[9] Hence, better selection methods are urgently needed.

Prognosis prediction tools such as nomograms have been developed for many cancer types to better inform treatment decisions. The most widely used tool in resectable PDAC is the prognostic nomogram developed at the Memorial Sloan-Kettering Cancer Center (MSKCC).[10–13] These, however, can only be applied after resection as they include clinicopathological variables only available following assessment of the resected specimen.

Numerous molecular biomarkers with potential clinical utility have been studied in PDAC, but few have been independently validated.[14–16] Our group and others have demonstrated that aberrant expression of S100A2 and S100A4 calcium-binding proteins, both of which function to accentuate tumor aggressiveness and metastasis, are associated with poor survival in PDAC.[17–19] Using RNA sequencing and methylation arrays, we recently reported hypomethylation of S100A2 is associated with the prognostic "Squamous" subtype of PDAC.[20] This poor prognostic subtype is consistently defined in molecular classifications of PDAC (also termed Quasi-Mesenchymal[21] or Basal[22]) and S100A2 remains a highly significant gene in each classifier.[20–22]

Here, we assess and validate the prognostic value of these 2 molecules in 1184 patients. Aberrant expression of these biomarkers stratify patients with resectable pancreatic cancer into 3 distinct prognostic groups in a training set (n = 518) and form the basis of a biomarker-based preoperative nomogram aimed at identifying those at high risk of early recurrence. This nomogram was validated in 2 further cohorts (n = 198 and 468), and the proof-of-concept feasibility of its preoperative use was assessed using preoperative endoscopic ultrasound fine-needle aspiration (EUS-FNA) biopsies.

METHODS

Patients and Tissue Specimens

Detailed clinicopathological and outcome data were obtained for 3 cohorts of consecutive unselected patients, totaling 1184, with primarily resected PDAC (Fig. 1). None of the patients received neoadjuvant chemo- or radiotherapy. The training cohort of 518 patients were accrued prospectively through the Australian Pancreatic Cancer Genome Initiative (APGI) (www.pancreaticcancer.net.au), for the International Cancer Genome Consortium (ICGC; www.icgc.org).[23] The 2 independent validation cohorts of 198 and 468 patients were from the West of Scotland Pancreatic unit, Glasgow Royal Infirmary, United Kingdom; and the Technical University of Dresden, Dresden, Germany, respectively (Table 1). Some patients from these cohorts were used for previous studies.[19,20,24–29] The current study was conducted in accordance with the TRIPOD type 3 model development approach, and REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) criteria (Supplementary Material, http://links.lww.com/SLA/B541).[30] All patients were treated after 1998 with more modern approaches such as multimodality therapy, and some were part of Phase III randomized-controlled trials such as ESPAC-3.[2] All cohorts displayed clinical and pathological features consistent with the behavior of PDAC and are similar to published PDAC cohorts worldwide.[31,32] The diagnosis and all pathological features were reviewed centrally by at least 1 specialist pancreatic histopathologist, and the date and cause of death were obtained from Central Cancer Registries or treating clinicians. RNA sequencing data were generated as part of the APGI's contribution to the ICGC, and sample processing and data analysis was performed as previously described.[20]

Ethical Statement

Ethical approval for the acquisition of data and biological material was obtained from the Human Research Ethics Committee at each participating institution (Supplementary Material, http://links.lww.com/SLA/B541).

Tissue Microarray and Immunohistochemistry (IHC)

Detailed methods for tissue microarray (TMA) construction and immunostaining assessment were described previously.[33] In brief, immunostaining on resection specimens were performed using TMAs constructed from 3 distinct areas per tumor. TMA sections were incubated with anti-S100A2 mouse monoclonal antibody, 1:50 dilution (clone DAK-S100A2/1; Dako Corporation, Glostrup, Denmark) and anti-S100A4 rabbit polyclonal antibody (Neo-Markers, Cat. No. RB-1804, Fremont, CA) with a dilution of 1:100 for 60 minutes. As part of a standardized biomarker discovery and development process, initial cut-offs were generated using earlier training/discovery cohorts, then validated using independent validation cohorts. S100A2 and S100A4 expression analysis was performed in an early training cohort of PDAC to define the optimal expression for analysis.[19] High S100A2 expression was defined as cytoplasmic staining with intensity 3+ in >30% of cells and positive S100A4 expression was defined as either nuclear and/or cytoplasmic staining of any intensity in >1% of cells.[19] Aberrant expression of S100A2 and S100A4 was correlated to survival after pancreatectomy.

Statistical Analysis

The influence of clinicopathological variables on survival was assessed with Cox proportional hazards regression, and the differences in outcome between predefined subgroups was evaluated using the log-rank test.[34] Where multiple cohorts were included in a single model, baseline hazard was always stratified by cohort throughout the procedure. On the basis of exploratory analysis, age was modeled with a cohort interaction term in the combined models; no other substantive variable to cohort interactions were identified. *P* values of <0.05 were considered statistically significant. Statistical analysis was performed using SPSS (Version 22.0; IBM SPSS Statistics, IBM Corporation, Armonk, NY). Model fitting and nomogram generation was performed using R 3.4.0 (The R Project for Statistical Computing, Vienna, Austria). Disease-specific survival was used as the primary endpoint for the APGI and Glasgow cohorts. Patients succumbing to other causes were right censored in the analysis. As the majority of patients with PDAC unfortunately succumb to disease, even after seemingly curative resection,[35] overall survival

Biomarker Validation (*n* =1184)

APGI Cohort
n = 518
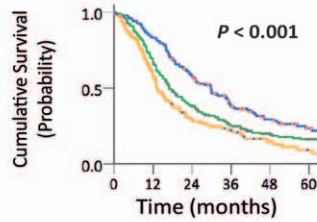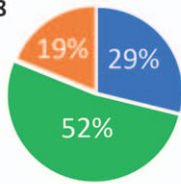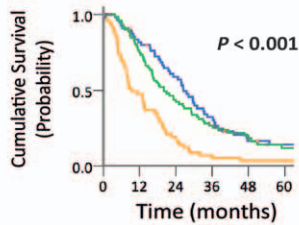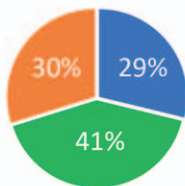
Glasgow Cohort
n = 198

German Cohort
n = 468
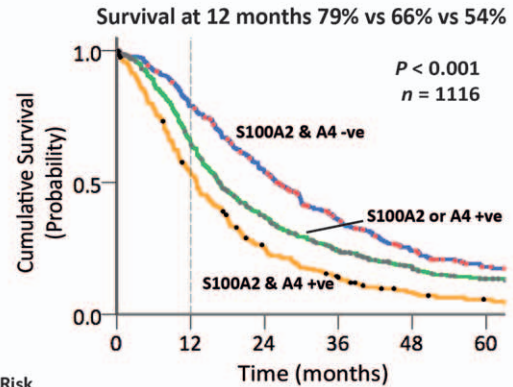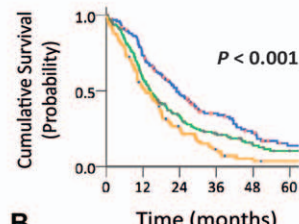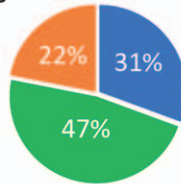
Survival at 12 months 79% vs 66% vs 54%
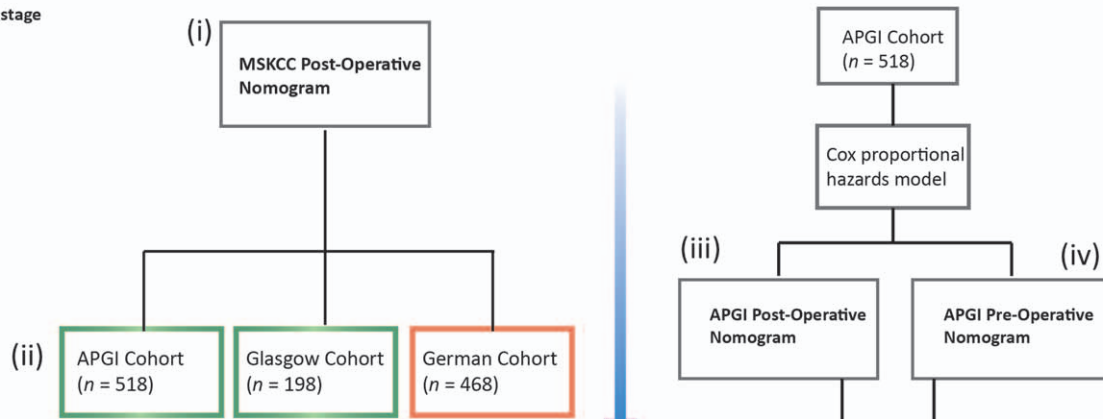
FIGURE 1. Methodology for biomarker validation and nomogram construction and validation. A, The expression of biomarkers S100A2 and S100A4 was determined using immunohistochemistry in 3 independent cohorts of PDAC and correlated with survival after pancreatectomy. Biomarker expression prevalence is presented as individual pie charts. B and C, Survival following pancreatectomy for all 3 cohorts (b) individually and (c) combined, stratified by biomarker expression (both negative, 1 positive, both positive), is represented by Kaplan-Meier survival curves. Patients with both biomarkers positive had a survival rate of only 54%, 26%, and 6% at 1, 2, and 5 years, respectively. This was found to be 79%, 54%, and 18% in the biomarker negative and 66%,

TABLE 1. Patient Characteristics for the Australian Pancreatic Cancer Genome Initiative, Glasgow, and the German Cohorts

	APGI Cohort			Glasgow Cohort			German Cohort		
Variables	n = 518 No. (%)	Median DSS, mo	*P* (Logrank)	n = 198 No. (%)	Median DSS, mo	*P* (Logrank)	n = 468 No. (%)	Median OS, mo	*P* (Logrank)
Sex									
Male	258 (49.8)	18.7		105 (53.0)	17.8		255 (54.5)	17.4	
Female	260 (50.2)	21.0	0.573	93 (47.0)	20.4	0.547	213 (45.5)	15.2	0.455
Age, yr									
Mean	66.7			62.5			64.2		
Median	68.0			63.9			66.0		
Range	28.0–88.0			37.4–86.0			31.0–84.0		
Outcome									
Follow-up, mo	18.0–163.8			35.0–84.0			0.0–137.8		
Median follow-up, mo	47.0			48.0			31.9		
Death PDAC	394 (76.1)			170 (85.9)					
Death other	31 (6.0)			19 (9.6)					
Death unknown	3 (0.6)			0 (0.0)			380 (81,2)		
Alive	89 (17.2)			9 (4.5)			88 (18.8)		
Lost to FU	1 (0.1)			0 (0.0)					
Stage (AJCC 7th)									
I	32 (6.2)	51.9		1 (0.5)	—		25 (5.4)	26.0	
II	474 (91.5)	18.8		193 (97.5)	19.1		408 (87.3)	16.8	
III	2 (0.4)	20.0		4 (2.0)	11.3		8 (1.7)	6.8	
IV	10 (1.9)	6.0	<0.001	0 (0.0)	—	0.249	26 (5.6)	10.8	0.001
T Stage (AJCC 7th)									
T1	21 (4.1)	34.4		2 (1.0)	11.1		11 (2.4)	24.8	
T2	48 (9.3)	24.3		14 (7.1)	33.5		60 (12.8)	18.2	
T3	447 (86.3)	18.3	0.033	178 (89.9)	18.0		385 (82.4)	16.4	
T4	2 (0.4)	20.0		4 (2.0)	11.3	0.119	11 (2.4)	6.8	0.010
N Stage (AJCC 7th)									
N0	169 (32.8)	22.4		36 (18.2)	31.0		146 (31.5)	22.9	
N1	347 (67.2)	18.7	0.008	162 (81.8)	18.5	0.001	318 (68.5)	15.4	0.008
Grade/differentiation									
I/well	41 (7.9)	35.4		12 (6.1)	23.2		18 (4.0)	24.7	
II/moderate	340 (65.9)	21.0		122 (61.6)	20.4		196 (43.9)	20.9	
III/poor	130 (25.2)	17.0	0.039	64 (32.3)	13.4	0.039	229 (51.2)	13.7	
IV/undifferentiated	5 (1.0)	13.0					4 (0.9)	34.4	<0.001
Tumor size									
≤20 mm	100 (19.5)	34.4		30 (15.2)	28.1		34 (16.6)	18.4	
>20 mm	414 (80.5)	17.0	<0.001	168 (84.8)	18.4	0.052	171 (83.4)	17.1	0.621
Margins									
Clear	338 (65.3)	23.7		52 (26.3)	26.6		340 (74.7)	18.4	
Involved	180 (34.7)	15.4	<0.0001	146 (73.7)	16.8	0.002	115 (25.3)	12.9	0.002
Tumor location									
Head	426 (82.2)	22.0		198 (100.0)	19.1		372 (91.2)	18.2	
Others	92 (17.8)	12.1	0.001	0 (0.0)	—	—	36 (8.8)	16.9	0.414
Perineural invasion									
Negative	114 (22.5)	26.0		14 (7.1)	18.2		134 (42.0)	19.2	
Positive	393 (77.5)	18.3	0.015	184 (92.9)	19.5	0.505	185 (58.0)	16.5	0.144
Vascular invasion									
Negative	234 (46.9)	23.0		100 (50.5)	23.1		187 (73.3)	20.0	
Positive	265 (53.1)	17.0	< 0.001	98 (49.5)	16.3	0.006	68 (26.7)	14.6	0.016
S100A2 Expression									
Low	392 (77.3)	21.0		135 (68.2)	24.7		282 (70.5)	18.2	
High	115 (22.7)	15.0	0.023	63 (31.8)	13.0	<0.001	118 (29.5)	11.9	<0.001
S100A4 expression									
Negative	169 (32.9)	29.9		61 (30.8)	26.4		142 (33.0)	22.0	
Positive	345 (67.1)	16.2	< 0.001	137 (69.2)	16.2	0.011	288 (72.0)	14.6	0.013
Adjuvant chemotherapy									
≥3 Cycles	206 (39.8)	27.1	<0.001	85 (42.9)	21.8	0.142	153 (32.7)	20.6	0.015
<3 Cycles	310 (59.8)	15.1		113 (57.1)	14.8		315 (67.3)	15.1	

DSS indicates disease-specific survival; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma.

38%, and 14% in the single biomarker positive groups respectively. D, Clinicopathological variables for all 3 cohorts were independently entered into the MSKCC postoperative nomogram to validate its performance in the patient cohorts. The MSKCC nomogram predicted survival in the APGI ($P = 5.0 \times 10^{-5}$) and Glasgow cohorts ($P = 0.025$) (green), but not the German cohort ($P = 0.31$) (red). E, The APGI training cohort was used to construct 2 Cox proportional hazard models, 1 was termed the APGI postoperative prognostic nomogram, and 1 the APGI preoperative prognostic nomogram. These were assessed and validated against the Glasgow ($P = 1.7 \times 10^{-3}$) and German ($P = 1.2 \times 10^{-5}$) validation cohorts with excellent fit in both cohorts.

was used for the German cohort, as disease-specific survival was not available.

Prognostic Nomograms

Memorial Sloan-Kettering Cancer Center Nomogram Evaluation

The published MSKCC nomogram[10] was applied to the APGI, Glasgow, and German cohorts, yielding per-patient estimates of linear risk score and 6-, 12-, and 24-month survival probabilities (Fig. 1) (Supplementary Material, http://links.lww.com/SLA/B541). Some variables in the MSKCC nomogram were not collected in the current cohorts and were imputed to the mean value of that variable reported for the MSKCC nomogram derivation cohort (Table S2, http://links.lww.com/SLA/B541).

Nomogram Construction

Two Cox proportional hazard models were fit to the APGI training cohort data (Fig. 1), 1 containing conventional clinicopathological variables available postoperatively (age, tumor size, T-stage, tumor location, vascular invasion, perineural invasion, margin status, presence of lymph node metastases, and differentiation), and the other containing only variables assessable preoperatively (age, tumor size, tumor location, and S100A2 and S100A4 status). Patient sex was not included in models due to its known poor prognostic value in PDAC.[10] To improve the clinical utility of the nomogram, follow-up was truncated at 24 months to focus prognosis prediction in this most clinically critical period after surgery. To simplify generation and application of the predictive nomograms, violations of the proportional hazards assumption were addressed by stratifying the baseline hazard by predictive variables, rather than introducing interaction with a time-dependent stratum.

Nomogram Testing

Nomograms were tested for discrimination and calibration against validation cohorts (Glasgow and Germany) using established methods.[36,37] Variability of the Brier score assessment of overall fit was estimated using 5000 bootstrap rounds.[36,37]

Endoscopic Ultrasound Fine-needle Aspiration and Cell Block Construction

EUS-FNA samples were collected and processed as per the standard diagnostic pathway using endoscopic and cytohistological techniques according to local practice. Formalin-fixed EUS-FNA tissue fragments or cell block preparations were embedded in paraffin, sectioned (4 μm) and H&E stained as standard. Staining for S100A2 and S100A4 were performed as described above and compared with corresponding resection specimen from 17 consecutive patients undergoing both EUS-FNA and pancreatectomy.

RESULTS

Patient characteristics are summarized in Table 1, with detailed descriptions provided in the Supplementary Material, http://links.lww.com/SLA/B541.

High S100A2 and S100A4 Expression Is Associated With Poor Survival After Pancreatectomy

In all 3 cohorts, high expression of S100A2 calcium-binding protein was associated with poor survival after pancreatectomy. In the APGI cohort, expression of S100A2 was high in 115 of 507
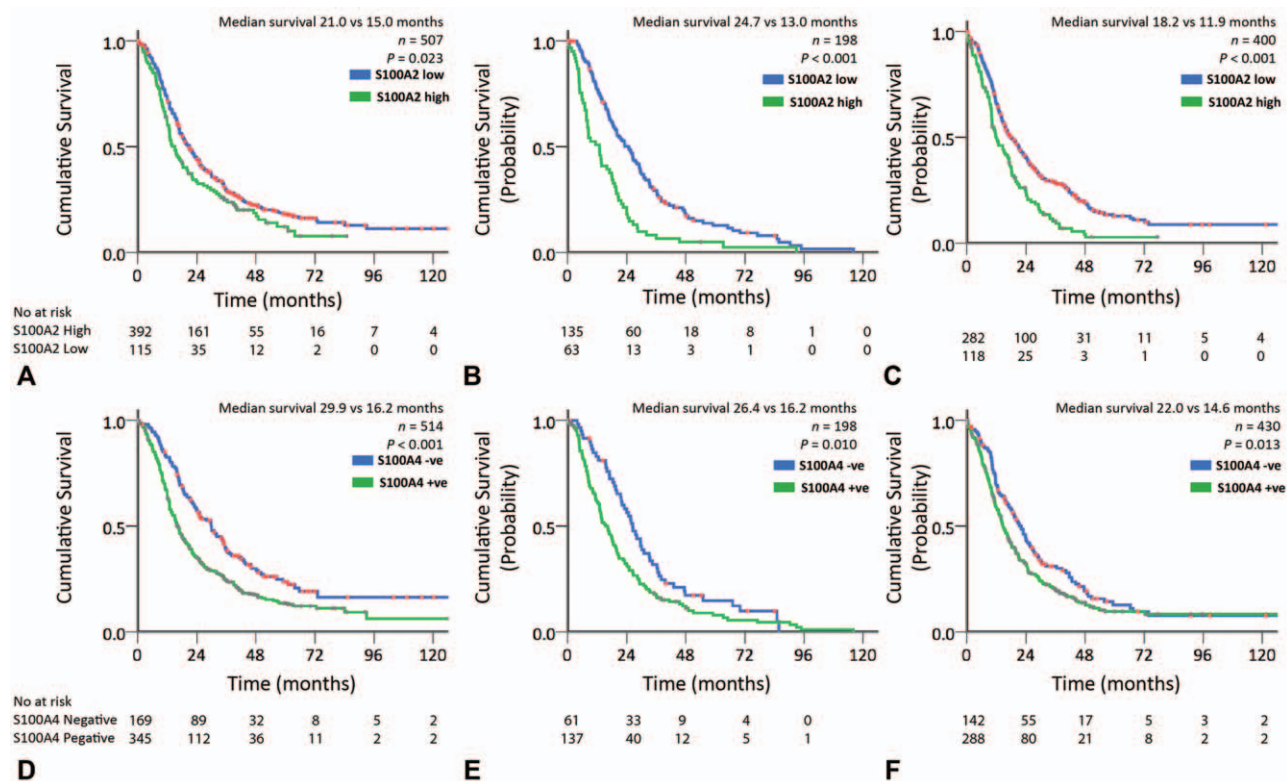

FIGURE 2. Kaplan-Meier survival curves for S100A2 expression in the (A) APGI, (B) Glasgow, (C) German cohorts; S100A4 expression in (D) APGI, (E) Glasgow, (F) German Cohorts.

patients (22.7%) (median survival 21.0 vs 15.0 mo; $P = 0.023$) (Table 1, Fig. 2). S100A2 expression was high in 63 out of 198 patients (31.8%) in the Glasgow cohort (median survival 24.7 vs 13 mo; $P < 0.001$) and in 118 out of 400 patients (29.5%) in the German cohort (median survival 18.2 vs 11.9 mo; $P < 0.001$) (Table 1, Fig. 2).

High S100A2 expression remained a significant independent prognostic factor in a combined multivariate model of all 3 cohorts [Table 2; hazard ratio (HR) = 1.64, 95% confidence interval (CI) 1.33–2.02 $P < 0.001$]. This was also the case in the APGI (Table S3, http://links.lww.com/SLA/B541; HR 1.32, 95% CI 0.97–1.80, $P < 0.001$) and Glasgow (Table S4, http://links.lww.com/SLA/B541; HR 2.00, 95% CI 1.36–2.90, $P < 0.001$) cohorts, but not the German (Table S5, http://links.lww.com/SLA/B541; HR 1.48, 95% CI 0.95–2.29, $P = 0.076$) cohort. It is likely that this reflects reduced power or cohort-specific variable collinearities, as the influence of S100A2 expression on survival was not significantly different between cohorts (Likelihood ratio test, $\chi^2 = 4.86$, df = 2, $P = 0.09$). Furthermore, S100A2 was associated with poor survival in the German cohort (univariate Cox regression (HR 1.69, 95% CI 1.34 – 2.14, $P < 0.001$) and log rank survival analysis (Fig. 2C). When considered alongside a previous study with more than 400 (200 nonredundant) patients this provides a total of more than 1300 cases supporting the association of high S100A2 expression and poor survival.[19]

Positive expression of S100A4 was associated with poor survival after pancreatectomy in all 3 cohorts. In the APGI cohort, 345 out of 514 patients (67.1%) had positive expression of S100A4, which was associated with a significantly worse outcome (median survival 29.9 vs 16.2 mo; $P < 0.001$) (Table 1, Fig. 2). These findings were recapitulated in the Glasgow cohort with 137 out of 198 patients (69.2%) with analyzable tissue demonstrating positive expression of S100A4 that was again associated with poor outcome (median survival 26.4 vs 16.2 mo; $P = 0.010$) (Table 1, Fig. 2). In the German cohort, 288 out of 430 patients (72.0%) with positive S100A4 expression demonstrated significantly worse outcome (median survival 22.0 vs 14.6 mo, $P = 0.013$) (Table 1, Fig. 2).

In a combined multivariate model, S100A4 remained strongly prognostic in a time-dependent manner with its effect on prognosis decreasing after 24 months in both the combined (HR 2.06, 95% CI 1.30–3.28, $P < 0.001$ at 12 mo), and individual models (Table 2, S3–

S5, http://links.lww.com/SLA/B541). This data suggest that S100A4 is a strong predictor of disease recurrence in the first 24 months after surgery, with its effect decreasing after this period. S100A4 was an independent prognostic factor in multivariate models in the APGI (Table S3, http://links.lww.com/SLA/B541, HR 2.13, 95% CI 1.08–4.17, $P = 0.018$ at 12 mo) and Glasgow (Table S5, http://links.lww.com/SLA/B541, HR 2.37, 95% CI 0.97–5.79, $P = 0.048$ at 12 mo) cohorts. Similar to S100A2, S100A4 was not significant in a multivariate model in the German cohort (Table S5, http://links.lww.com/SLA/B541), however, was predictive in univariate cox regression (HR 1.33, 95% CI 1.06–1.67, $P = 0.013$) and log rank survival analysis (Fig. 2).

Expression of S100A2 and S100A4 Stratifies Patients Into 3 Prognostic Groups

The combined prognostic effects of the 2 biomarkers were assessed independently in all 3 cohorts (Fig. 1). In all 3 cohorts, tumors with high S100A2 expression were more likely to be associated with positive S100A4 expression (Table S6 in Supplementary Material, http://links.lww.com/SLA/B541, $P \leq 0.001$). In the APGI cohort, patients with low or no expression of either S100A2 or S100A4 had the best prognosis, followed by patients with either high S100A2 or positive S100A4 expression, and patients with both biomarkers positive had the worst prognosis (median survival 29.8 vs 17.0 vs 13.2 mo, respectively, $P < 0.001$) (Fig. 1). These findings were validated in the Glasgow (median survival 26.5 vs 20.1 vs 9.3 mo; $P < 0.001$) and the German (median survival 22.9 vs 14.3 vs 12.9 mo; $P < 0.001$) cohorts (Fig. 1). When combining all 3 cohorts, patients with both biomarkers positive had a 12-month survival rate of only 54% after pancreatectomy, compared to 79% and 66% in the biomarker negative or single biomarker positive groups respectively ($P < 0.001$) (Fig. 1).

There was no difference in the use of adjuvant chemotherapy between patients who were biomarker negative and those with a single biomarker positive in all 3 cohorts (Table S7 in Supplementary Material, http://links.lww.com/SLA/B541). In the APGI cohort, patients with both biomarkers positive had lower adjuvant therapy use rate (Table S7, http://links.lww.com/SLA/B541, $P = 0.025$). There were no significant differences in adjuvant therapy use amongst biomarker groups in the Glasgow and German cohorts

TABLE 2. Multivariate Cox Model: All Cohorts Combined (Baseline Hazard Stratified by Cohort)

Variable	Coefficient	Hazard Ratio (95% CI)	P
Differentiation (reference value: well)			0.046
Moderate	0.057	1.06 (0.68–1.66)	
Poor/undifferentiated	0.200	1.26 (0.76–1.96)	
Size along longest axis (cm, relative to 3.0 cm)	0.127	1.14 (1.06–1.22)	<0.001
pT Stage T3 or T4 (reference value: T1 or T2)	0.441	1.55 (1.13–2.14)	0.003
Age (decades, relative to 65)	0.210	1.23 (1.07–1.43)	0.271
Lymph nodes positive	0.024	1.02 (0.82–1.28)	0.127
Resection margin involved	0.512	1.67 (1.36–2.04)	<0.001
Location tail (reference: head)	0.110	1.12 (0.79–1.57)	0.254
Perineural invasion	0.290	1.34 (1.03–1.73)	0.009
Vascular invasion	0.166	1.18 (0.97–1.43)	0.062
S100A2 positive	0.494	1.64 (1.33–2.02)	<0.001
S100A4 positive			<0.001
0–6 mo postresection	0.707	2.03 (1.08–3.80)	
6–12 mo postresection	0.724	2.06 (1.30–3.28)	
12–24 mo postresection	0.520	1.68 (1.19–2.38)	
Over 24 mo postresection	−0.122	0.88 (0.63–1.25)	
Age: cohort interaction (reference: APGI)			<0.001
Glasgow	0.647	0.65 (0.52–0.81)	
German	0.945	1.06 (0.70–1.28)	

CI indicates confidence interval.

(Table S7 in Supplementary Material, http://links.lww.com/SLA/B541).

Expression of S100A2 and S100A4 Cosegregates With the Squamous Subtype of Pancreatic Ductal Adenocarcinoma

In 96 patients who underwent whole transcriptome sequencing analysis as part of the APGI (ICGC) cohort, S100A2 and S100A4 mRNA expression was significantly associated with the recently described squamous subtype[20] ($P < 0.01$; Fig. 3, Table S8 in Supplementary Material, http://links.lww.com/SLA/B541). This was recapitulated in the 235 patients who underwent microarray mRNA expression analysis, as described by Bailey et al.[20] Using both mRNA and protein expression, patients with high S100A2 ($P = 0.002$) and positive S100A4 ($P < 0.001$) expression were associated with the squamous subtype, with the strongest correlation in those with both biomarkers positive (Fig. 3, Table S9 in Supplementary Material, http://links.lww.com/SLA/B541). Squamous subtype tumors[20] were associated with a significantly higher mean nomogram score than other subtypes (140 vs 103; $P = 0.004$).

Memorial Sloan-Kettering Cancer Center Prognostic Nomogram Validation for Resectable Pancreatic Ductal Adenocarcinoma

After demonstrating the association of S100A2 and S100A4 expression with the prometastatic squamous subtype and poor prognosis in PDAC, the potential clinical utility of a prognostic nomogram incorporating molecular biomarkers was explored. The overall fit of the published MSKCC nomogram to all 3 cohorts was assessed by stratifying patients using predicted survival score and comparing observed and MSKCC predicted survival [Fig. 1D(i)]. Relative to the true outcome, MSKCC nomogram predictions were optimistic, particularly at later time points, this was especially true for the German cohort (Fig. S1, Supplementary Material, http://links.lww.com/SLA/B541). The MSKCC nomogram risk score was prognostic in the APGI and Glasgow cohorts (Cox regression coefficients 0.79 and 1.35, likelihood ratio test for coefficient not zero, $P = 5.0 \times 10^{-5}$ and 0.025, respectively), but not the German cohort (coefficient 0.15, $P = 0.31$). The MSKCC risk score was well-calibrated against the APGI and Glasgow cohorts (Likelihood-ratio test for coefficient not unity, $P = 0.28$ and 0.56, respectively), but less well against the German cohort ($P = 2.6 \times 10^{-9}$) [Figs. 1D(ii) and S1, Supplementary Material, http://links.lww.com/SLA/B541].

Preoperative Molecular Nomogram Predicts Survival After Pancreatectomy as Accurately as Postoperative Clinicopathological Nomogram

A molecular nomogram incorporating S100A2 and S100A4 expression was then constructed. The APGI cohort was used to construct 2 prognostic nomograms based on the Cox proportional hazards model: 1 employing traditional postoperatively available variables (''Post-operative Prognostic Nomogram'') [Figs. 1E(iii) and S2, Supplementary Material, http://links.lww.com/SLA/B541], and 1 employing only variables that can be measured preoperatively (''Preoperative Prognostic Nomogram'') [Figs. 1E(iv) and 4). To improve the clinical utility of predicting early recurrence and to incorporate the prognostic value of S100A4 over the initial 24 months after surgery, follow-up was truncated at 24 months. Both models included tumor location (pancreatic head vs body/tail), after exploratory analysis in the APGI cohort indicated differences in baseline hazard between these patient groups (Table S10, Supplementary Material, http://links.lww.com/SLA/B541).[38]

Risk stratification accuracy of the APGI preoperative and APGI postoperative nomograms were assessed in the Glasgow and German validation cohorts. The preoperative prognostic nomogram displayed good discrimination on both validation cohorts (risk score Cox coefficients 0.59 and 0.66, $P = 1.7 \times 10^{-3}$ and 1.2×10^{-5} for Glasgow and German, respectively) [Fig. 1E(v)]. Notably, the APGI preoperative nomogram was superior to the MSKCC postoperative prognostic nomogram in both its spread of risk scores, and the accuracy of its absolute survival estimates (Figs. S1 and S3, Supplementary Material, http://links.lww.com/SLA/B541).

The overall accuracy of the APGI preoperative nomogram in predicting patient survival was assessed and compared to the APGI postoperative nomogram. Although the preoperative nomogram was slightly optimistic by predicting marginally better outcome probabilities than those observed in the Glasgow and German cohorts, it was more accurate than the MSKCC postoperative nomogram (Figs. S1 and S3, Supplementary Material, http://links.lww.com/SLA/B541). Brier scores was used to formally evaluate the relative performance of the APGI preoperative and postoperative nomograms with more than 5000 bootstrap draws of each validation cohort. This demonstrated the APGI preoperative nomogram was more accurate than the MSKCC postoperative nomogram, and as accurate as the APGI postoperative nomogram in outcome predictions (Figs. S4 and S5, Supplementary Material, http://links.lww.com/SLA/B541).
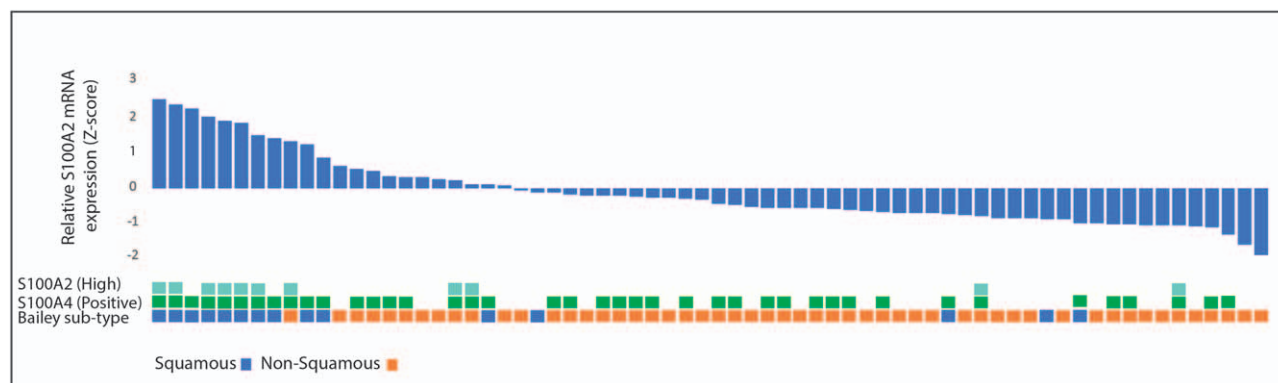

FIGURE 3. High S100A2 and positive S100A4 expression correlates with the squamous subtype of PDAC. Patients are ranked according to S100A2 mRNA expression and the relative expression z-score is represented by a waterfall plot, IHC staining and Bailey subtype is shown below. High S100A2 and positive S100A4 expression associated strongly with the squamous subtype ($P < 0.001$).
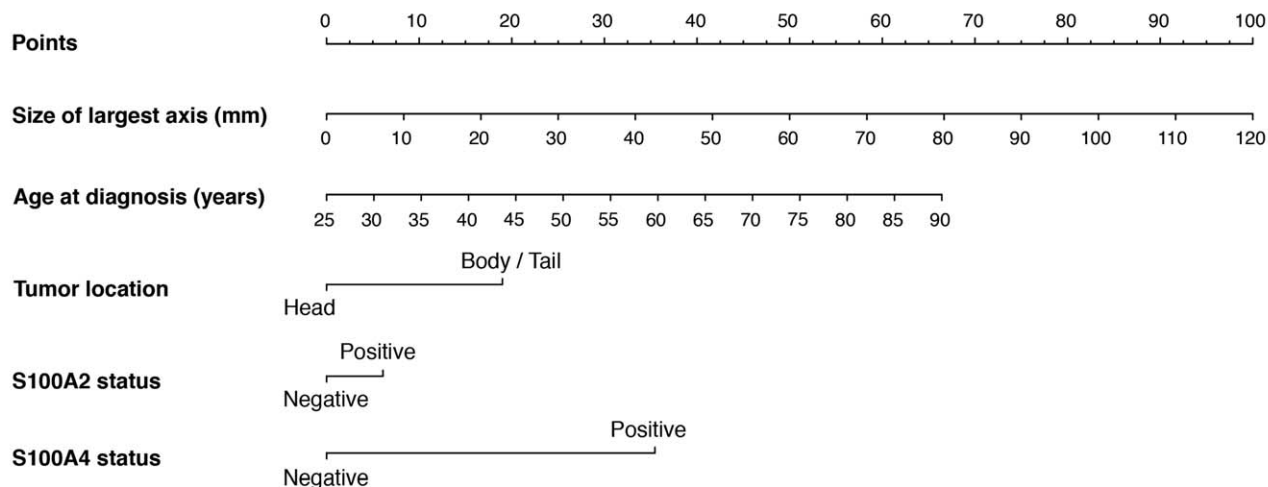
RISK FACTORS

PROGNOSIS

FIGURE 4. A preoperative molecular prognostic nomogram for resectable pancreatic cancer.

Preoperative Assessment of Biomarker Expression in Endoscopic Ultrasound Fine-needle Aspiration Samples

A pilot study was performed on 17 consecutive patients to compare biomarker expression status between preoperative EUS-FNA cell blocks and the corresponding surgical resection specimen (Table S11, Supplementary Material, http://links.lww.com/SLA/B541). S100A2 and S100A4 expression correlated in 15 (88%) and 14 (82%) out of 17 patients, respectively, based on the EUS-FNA cell block and the surgical specimen for both biomarkers examined (representative images are shown in Fig. 5). This demonstrates that biomarker status can be measured preoperatively using immunohistochemistry, and it is likely that EUS biopsy assessment will improve with the current development of more effective biopsy needles and standardization of processing techniques.

DISCUSSION

Despite our increasing understanding of the molecular heterogeneity in morphologically identical cancers, and the advances in molecularly guided targeted therapy selection, the impact of these findings in surgical decision-making has not been addressed. Perioperative mortality for pancreatectomy has improved dramatically over the last 30 years and the definition of "resectability" has been expanded over the last decade with increasingly aggressive surgery being performed.[39,40] Early recurrence, however, remains the Achilles' heel of surgical resection, making better patient selection for surgery a priority area of research.

In this study, the expression of 2 molecules, S100A2 and S100A4, which functionally promote carcinogenesis and metastasis, were validated as prognostic biomarkers in multiple independent cohorts of patients with resectable PDAC (n = 1184), in keeping with earlier studies (n = ∼400).[19] These 2 biomarkers were used to stratify patients with resectable PDAC into distinct prognostic phenotypes after pancreatectomy. Patients with both biomarkers positive are at significant risk of early recurrence, with almost half of these patients succumbing within 12 months after pancreatectomy (12-month survival rate = 54%). Suggesting that disease recurrence occur at around 6 months or earlier for the majority of this group. The risk and pattern of disease recurrence following pancreatectomy is not proportional and early recurrence has recently been defined as within 12 months after surgery.[35,41] We
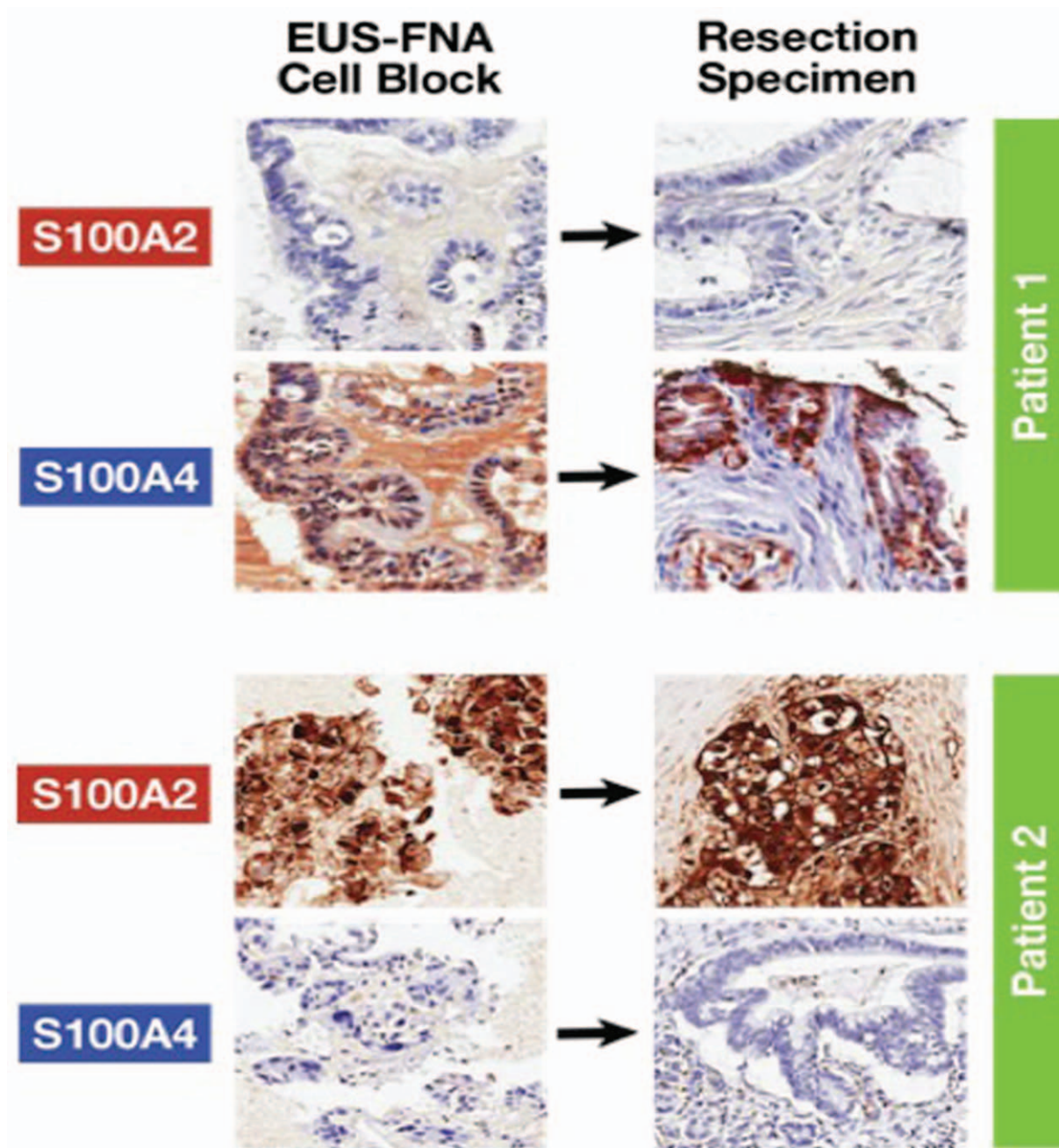
FIGURE 5. Immunohistochemistry of EUS-FNA versus resection specimen in 2 patients.

focused on outcome in the first 24 months postoperatively for nomogram construction since the majority of patients that develop early recurrence will succumb to the disease by this point, and thus improve its clinical utility.[41] A preoperative prognostic nomogram incorporating these 2 biomarkers, and preoperatively determined variables including age, tumor size, and location was developed and independently validated. This preoperative prognostic nomogram performed as well as the published MSKCC postoperative prognostic nomogram, which is the most widely used, and currently considered the criterion standard. In our study, a number of variables

used in the MSKCC nomogram were missing and thus comparing the performance of both nomograms in these cohorts is not optimal. The APGI preoperative nomogram, however, uses less variables and all are obtainable before surgical resection to aid decision making. Finally, as a proof-of-concept, biomarker expression status was assessed using immunohistochemical staining of the EUS-FNA cell blocks.

However, due to the retrospective nature of the study, and the cohorts acquired were mature, with long-term follow-up, there are a few limitations with the study. First, a proportion of the

recurrence pattern data were not available; therefore, the association between recurrence patterns and biomarker expression was not assessed. Secondly, preoperative CA19-9 measurements were not available for a significant proportion of the patients. Increased serum levels of CA19-9 has been shown to be associated with early recurrence, and may improve the performance of a preoperative nomogram.[41] Thirdly, only a small number of preoperative EUS samples had sufficient material available for comparison with postoperative S100A2 and S100A4 immunostaining. Therefore, to further validate the clinical utility of the preoperative nomogram, its use should be tested in parallel with trials in PDAC comparing upfront resection and neoadjuvant therapy. Finally, this study was not powered nor designed to assess S100A2 and S100A4 expression and response to adjuvant chemotherapy. Chemotherapy response in the adjuvant setting is difficult to reliably assess as survival difference is dependent on many factors including residual occult metastatic disease and performance status, requiring multicenter personalized medicine trials (such as *PRECISION-Panc* in the UK and *Precision Promise* in the USA) to further delineate this relationship. Interestingly, in the APGI cohort, patients with both biomarkers positive were less likely to be administered adjuvant therapy, possibly due to an aggressive disease phenotype leading to more early recurrence and declining performance status and subsequently reduced adjuvant chemotherapy use.

Aberrant S100A2 and S100A4 expression correlated to the recently described poor prognosis "squamous" (also termed QM or Basal)[21,22] subtype of PDAC, which is enriched for transcriptional programs associated with proliferation, inflammation, and metastasis.[20] Mechanistically, S100A2 hypomethylation and associated increased expression is a feature of the squamous subtype. The regulation of S100A4 expression is more complex, potentially involving both tumor and microenvironment factors, and will require further investigation.[42,43] Interestingly, the squamous subtype was associated with a higher mean nomogram score and demonstrates the potential clinical utility of the currently presented molecular prognostic nomogram in identifying patients with aggressive tumor biology and a prometastatic phenotype. These patients are at high risk of early recurrence and are unlikely to benefit from pancreatectomy and are perhaps better treated with neoadjuvant therapy (an increasingly popular approach to PDAC in many centers), as occult metastatic disease that is not detected by current staging modalities will likely manifest itself during this period. However, the use of neoadjuvant therapy is not universal and a significant proportion of patients may not respond to this approach.[44] Thus, patients predicted to have a favorable prognosis may be better served with upfront surgery and adjuvant therapy with median survival up to 54 months reported using adjuvant modified FOLFIRINOX in patient cohorts with favorable postoperative prognostic features.[45] Furthermore, the growing interest in more aggressive and extensive surgery in the setting of borderline resectable or locally advanced disease could be justified by prognostic indicators before initiating therapy.[39,40] Accurate prognostication can assist multidisciplinary and shared decision making, especially in patients with borderline fitness for surgery, which is a significant proportion of patients with PDAC, delivering a more personalized treatment plan. This approach has the potential to improve the overall outcomes and quality of life for patients with PDAC.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin.* 2018;68:7–30.
2. Neoptolemos JP, Stocken DD, Bassi C, et al. Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. *JAMA.* 2010;304:1073–1081.
3. Regine WF, Winter KA, Abrams RA, et al. Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: a randomized controlled trial. *JAMA.* 2008;299:1019–1026.
4. Oettle H, Post S, Neuhaus P, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. *JAMA.* 2007;297:267–277.
5. Dreyer SB, Chang DK, Bailey P, et al. Pancreatic cancer genomes: implications for clinical management and therapeutic development. *Clin Cancer Res.* 2017;23:1638–1646.
6. Neoptolemos JP, Palmer DH, Ghaneh P, et al. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. *Lancet.* 2017;389:1011–1024.
7. Barugola G, Falconi M, Bettini R, et al. The determinant factors of recurrence following resection for ductal pancreatic cancer. *JOP.* 2007;8:132–140.
8. 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.
9. Schniewind B, Bestmann B, Henne-Bruns D, et al. Quality of life after pancreaticoduodenectomy for ductal adenocarcinoma of the pancreatic head. *Br J Surg.* 2006;93:1099–1107.
10. Brennan MF, Kattan MW, Klimstra D, et al. Prognostic nomogram for patients undergoing resection for adenocarcinoma of the pancreas. *Ann Surg.* 2004;240:293–298.
11. De Castro SM, Biere SS, Lagarde SM, et al. Validation of a nomogram for predicting survival after resection for adenocarcinoma of the pancreas. *Br J Surg.* 2009;96:417–423.
12. White RR, Kattan MW, Haney JC, et al. Evaluation of preoperative therapy for pancreatic cancer using a prognostic nomogram. *Ann Surg Oncol.* 2006;13:1485–1492.
13. Ferrone CR, Kattan MW, Tomlinson JS, et al. Validation of a postresection pancreatic adenocarcinoma nomogram for disease-specific survival. *J Clin Oncol.* 2005;23:7529–7535.
14. Garcea G, Neal CP, Pattenden CJ, et al. Molecular prognostic markers in pancreatic cancer: a systematic review. *Eur J Cancer.* 2005;41:2213–2236.
15. Jamieson NB, Carter CR, McKay CJ, et al. Tissue biomarkers for prognosis in pancreatic ductal adenocarcinoma: a systematic review and meta-analysis. *Clin Cancer Res.* 2011;17:3316–3331.
16. Ansari D, Rosendahl A, Elebro J, et al. Systematic review of immunohistochemical biomarkers to identify prognostic subgroups of patients with pancreatic cancer. *Br J Surg.* 2011;98:1041–1055.
17. Ai KX, Lu LY, Huang XY, et al. Prognostic significance of S100A4 and vascular endothelial cell growth factor expression in pancreatic cancer. *World J Gastroenterol.* 2008;14:1931–1935.
18. Huang S, Zheng J, Huang Y, et al. Impact of S100A4 expression on clinicopathological characteristics and prognosis in pancreatic cancer: a meta-analysis. *Dis Markers.* 2016;2016:8137378.
19. Biankin AV, Kench JG, Colvin EK, et al. Expression of S100A2 calcium-binding protein predicts response to pancreatectomy for pancreatic cancer. *Gastroenterology.* 2009;137:558–568. 568.e1–11.
20. Bailey P, Chang DK, Nones K, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature.* 2016;531:47–52.
21. Collisson EA, Sadanandam A, Olson P, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. *Nat Med.* 2011;17:500–503.
22. Moffitt RA, Marayati R, Flate EL, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. *Nat Genet.* 2015;47:1168–1178.
23. Hudson TJ, Anderson W, Artez A, et al. International network of cancer genome projects. *Nature.* 2010;464:993–998.
24. Biankin AV, Waddell N, Kassahn KS, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature.* 2012;491:399–405.
25. Waddell N, Pajic M, Patch AM, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature.* 2015;518:495–501.

26. Jamieson NB, Mohamed M, Oien KA, et al. The relationship between tumor inflammatory cell infiltrate and outcome in patients with pancreatic ductal adenocarcinoma. *Ann Surg Oncol.* 2012;19:3581–3590.

27. Mann KM, Ward JM, Yew CC, et al. Sleeping Beauty mutagenesis reveals cooperating mutations and pathways in pancreatic adenocarcinoma. *Proc Natl Acad Sci U S A.* 2012;109:5934–5941.

28. Chang DK, Johns AL, Merrett ND, et al. Margin clearance and outcome in resected pancreatic cancer. *J Clin Oncol.* 2009;27:2855–2862.

29. Murphy NC, Scarlett CJ, Kench JG, et al. Expression of LMO4 and outcome in pancreatic ductal adenocarcinoma. *Br J Cancer.* 2008;98:537–541.

30. Collins GS, Reitsma JB, Altman DG, et al. Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD). *Ann Intern Med.* 2015;162:735–736.

31. Schnelldorfer T, Ware AL, Sarr MG, et al. Long-term survival after pancreatoduodenectomy for pancreatic adenocarcinoma: is cure possible? *Ann Surg.* 2008;247:456–462.

32. Cameron JL, Riall TS, Coleman J, et al. One thousand consecutive pancreaticoduodenectomies. *Ann Surg.* 2006;244:10–15.

33. Biankin AV, Kench JG, Colvin EK, et al. Expression of S100A2 calcium-binding protein predicts response to pancreatectomy for pancreatic cancer. *Gastroenterology.* 2009;137:558–568.

34. Bland JM, Altman DG. The logrank test. *BMJ.* 2004;328:1073.

35. Groot VP, Rezaee N, Wu W, et al. Patterns, timing, and predictors of recurrence following pancreatectomy for pancreatic ductal adenocarcinoma. *Ann Surg.* 2018;267:936–945.

36. Altman DG, Royston P. What do we mean by validating a prognostic model? *Stat Med.* 2000;19:453–473.

37. Gerds TA, Schumacher M. Consistent estimation of the expected Brier score in general survival models with right-censored event times. *Biom J.* 2006;48:1029–1040.

38. Dreyer SB, Jamieson NB, Upstill-Goddard R, et al. Defining the molecular pathology of pancreatic body and tail adenocarcinoma. *Br J Surg.* 2018;105:e183–e191.

39. Isaji S, Mizuno S, Windsor JA, et al. International consensus on definition and criteria of borderline resectable pancreatic ductal adenocarcinoma 2017. *Pancreatology.* 2018;18:2–11.

40. Gemenetzis G, Groot VP, Blair AB, et al. Survival in locally advanced pancreatic cancer after neoadjuvant therapy and surgical resection. *Ann Surg.* 2018 [Epub ahead of print].

41. Groot VP, Gemenetzis G, Blair AB, et al. Defining and predicting early recurrence in 957 patients with resected pancreatic ductal adenocarcinoma. *Ann Surg.* 2018 [Epub ahead of print].

42. Mahon PC, Baril P, Bhakta V, et al. S100A4 contributes to the suppression of BNIP3 expression, chemoresistance, and inhibition of apoptosis in pancreatic cancer. *Cancer Res.* 2007;67:6786–6795.

43. Fei F, Qu J, Zhang M, et al. S100A4 in cancer progression and metastasis: a systematic review. *Oncotarget.* 2017;8:73219–73239.

44. Murphy JE, Wo JY, Ryan DP, et al. Total neoadjuvant therapy with FOLFIRINOX followed by individualized chemoradiotherapy for borderline resectable pancreatic adenocarcinoma: a phase 2 clinical trial. *JAMA Oncol.* 2018;4:963–969.

45. Conroy T, Hammel P, Hebbar M, et al. Unicancer GI PRODIGE 24/CCTG PA.6 trial: A multicenter international randomized phase III trial of adjuvant mFOLFIRINOX versus gemcitabine (gem) in patients with resected pancreatic ductal adenocarcinomas. *J Clin Oncol.* 2018;36:LB10.