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ORIGINAL RESEARCH

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CD3-CD8 immune score associated with a clinical score stratifies PDAC prognosis regardless of adjuvant or neoadjuvant chemotherapy

Coralie Schoumacher[a*], Valentin Derangère[a,b*], Gwladys Gaudillière-Le Dain[a], Titouan Huppe[a], David Rageot[a,b], Alis Ilie[a], Angélique Vienot[c], Christophe Borg[c], Franck Monnien[d], Frederic Bibeau[d], Caroline Truntzer[a,b*], François Ghiringhelli[a,b,e,f], and For the CGE-Pancreas investigators[g*]

[a]Cancer Biology Transfer Platform, Centre Georges-François Leclerc, Dijon, France; [b]INSERM LNC-UMR1231 Research Center, TIRECS Team, Dijon, France; [c]Department of Medical Oncology, CHU Besançon, Besançon, France; [d]Department of Pathology, CHU Besançon, Besançon, France; [e]Genetic and Immunology Medical Institute, GIMI, Dijon, France; [f]Department of Medical Oncology, Centre Georges-François Leclerc, Dijon, France; [g]Please see list of CGE-Pancreas collaborators in supplementary methods 1, SM1

ABSTRACT

Stratification of the prognosis of pancreatic cancer (PDAC) patients treated by surgery is based solely on clinical variables, such as tumor stage and node status. The development of biomarkers of relapse is needed, especially to drive administration of adjuvant therapy in this at-risk population. Our study evaluates the prognostic performance of a CD3- and CD8-based immune score. CD3, CD8 and Foxp3 expression were evaluated on whole slides in two retrospective PDAC cohorts totaling 334 patients. For this study, we developed an immune score to estimate CD3 and CD8 infiltration in both tumor core and invasive margin using computer-guided analysis with QuPath software. Variables were combined in a dichotomous immune score. The association between immune and clinical scores, and both PFS and OS was investigated. We observed that a dichotomous immune score predicts both PFS and OS of localized PDAC. By univariate and multivariate analysis, immune score, tumor grade, adjuvant therapy, lymph node status, and adjuvant chemotherapy administration were associated with PFS and OS. We subsequently associated the PDAC immune score and clinical variables in a combined score. This combined score predicted patient outcomes independently of adjuvant or neoadjuvant treatment, and improved patient prognostic prediction compared to clinical variables or immune score alone.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent type of pancreatic cancer accounting for over 90% of all pancreatic malignancies diagnosed.[1] PDAC is a devastating disease with 5-year survival of less than 10%.[2] Its incidence is on the rise, and it is projected to be the second leading cause of cancer-related death worldwide by 2030.[3] The only curative strategy is surgical resection when patients do not present metastasis[4] However, more than 80% of tumors are unresectable at the time of diagnosis, and most patients who benefit from surgery present recurrence.[5,6] To improve survival, adjuvant treatments are proposed. Gemcitabine was the standard adjuvant therapy, but has now been replaced by FOLFIRINOX, which has drastically improved the chances of prolonged survival.[7] With this strategy, neoadjuvant chemotherapy is also currently widely proposed to improve PDAC resectability.[8]

There is still an unmet need for prognostic markers to predict PDAC recurrence. It is known that PDAC is widely invaded by fibroblastic tissue with its characteristics which are linked to outcome.[9] Although immunotherapy has not led to improvements in PDAC treatment,[10–12] recent studies have nonetheless underlined that the tumor microenvironment is of major importance in PDAC.[13] PDAC is infiltrated with immune cells, and the tumor immune microenvironment is mainly thought to be immunosuppressive, with a high proportion of Foxp3 regulatory T cells and tumor-associated macrophages.[14,15] However, previous studies have underlined that high CD3 or CD8 infiltration could be associated with better prognosis in various tumor types,[16] notably PDAC.[17] In the field of colorectal cancer, Galon *et al.* proposed the Immunoscore® concept, which studies CD3 and CD8 tumor infiltration in the tumor core (TC) and invasive margin (IM). The Immunoscore® allows a more accurate definition of patient prognosis than the TNM stage in this tumor type.[18] These CD3-CD8 infiltrates are associated with a lower risk of tumor dissemination and improved survival.[19]

In the present study, using two series of PDAC patients treated by surgery, with or without neoadjuvant or adjuvant chemotherapy, we assessed the prognostic impact of CD3, CD8 and Foxp3 staining in the TC or IM of formalin-fixed paraffin-embedded (FFPE) slides using a single slide per patient, and a multi-scale quantitative assay combining sequential immunohistochemistry (IHC) imaging.

CONTACT Valentin Derangère vderangere@cgfl.fr Cancer Biology Transfer Platform, Centre Georges-François Leclerc, Dijon F-21000, France
*Contributed equally.
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Patients and Methods

Study design and population

Cohort 1 comprised patients with histologically confirmed PDAC, who underwent complete surgical resection at the university teaching hospital of Besançon between January 1998 and December 2018. The database was registered with the French National Commission for bioinformatics data and patient liberty (CNIL; declaration number 1,906,173 v 0). The study methodologies conformed to the standards laid down in the Declaration of Helsinki. Written informed consent for research was signed by all patients with cancer at the time of their first visit to the Department of Medical Oncology. The experiments were undertaken with the understanding and written consent of each subject. Samples were provided by the regional tumor bank of Franche-Comté (University Hospital Besançon, France; registration number BB-0033–00024). The project was approved by the scientific board of the biobank (#F1860-PAC-MA).

Cohort 2 comprised patients with histologically confirmed PDAC, who underwent complete surgical resection between January 2016 and December 2021 in 8 teaching hospitals in France and who were included in the observational Pancreas CGE study (NCT02818907 – investigators are listed in Supplementary Methods 1) (Table 1).

Multiple AEC staining

We performed Multiplexed Immunohistochemical Consecutive Staining on Single Slide (MICSSS) as previously described.[20] Briefly, after EDTA-based antigen retrieval in a PT-Link apparatus at 95°C for 20 minutes, slides were first counterstained with hematoxylin, coverslipped in permanent medium and digitalized at 20X with a Nanozoomer HT2.0 slide scanner. Next, slides were unmounted using xylene and rehydrated in ethanol. Slides were then incubated with Foxp3 antibody using AEC chromogen, counterstained and aqueously mounted. Slides were once again digitalized at 20X and unmounted in xylene. Xylene and rehydration ethanol baths were used for AEC bleaching. By repeating the same procedure, slides were consequently incubated with CD8 antibody, CD3 antibody and Cytokeratin-7 antibody. All reagents used, incubation, and dilution are reported in Supplementary Methods 2.

Multiple staining analysis using computer-guided alignment and QuPath software

Once all staining was performed, CK-7 staining was used as the reference to annotate the region of interest (ROI), i.e. the tumor core (TC) and the invasive margin (IM), with

Table 1. Demographic and clinical characteristics of the two cohorts.

Variable	Cohorte1, $N = 205$[1]	Cohorte2, $N = 129$[1]	p-value[2]
Sex			.6
F	103 (50%)	61 (47%)	
M	101 (50%)	68 (53%)	
NA	1	0	
Age	67 (40, 86)	70 (46.8, 85.2)	**.020**
NA	1	1	
Neoadjuvant treatment			**<.001**
No	194 (95%)	85 (67%)	
Yes	10 (4.9%)	42 (33%)	
NA	1	2	
Adjuvant treatment			.068
No	46 (24%)	15 (15%)	
Yes	146 (76%)	86 (85%)	
NA	13	28	
Resection			.081
R0	161 (80%)	81 (72%)	
R1	37 (18%)	32 (28%)	
R2	2 (1.0%)	0 (0%)	
NA	5	16	
Histological grade			.4
Well differentiated	42 (21%)	27 (23%)	
Moderatly differentiated	125 (61%)	67 (58%)	
Poorly differentiated	32 (16%)	21 (18%)	
Mucinous differentiated	5 (2.5%)	0 (0%)	
NA	1	14	
Tumor status (AJCC 2017)			**<.001**
1	24 (14%)	10 (8.1%)	
2	110 (64%)	52 (42%)	
3	39 (23%)	56 (45%)	
4	0 (0%)	6 (4.8%)	
NA	32	5	
Node status (AJCC 2017)			.6
0	49 (24%)	35 (29%)	
1	83 (41%)	47 (39%)	
2	72 (35%)	40 (33%)	
NA	1	7	

[a]Median (min,max); n (%).
[b]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test.

NDPview software. When it was too difficult to determine whether stained area was neoplastic or not on CK-7 staining, H&E staining was checked to decide if the ROI should be kept for analysis or discarded. As all slides had cross shapes on the right distal corners, these were used as landmarks to register slides. Briefly, for each patient, after automated cross-shape detection with contrast algorithm on CK-7, Foxp3, CD8 and CD3 staining, their centroids were calculated using QuPath software (v.0.3.0).[21] Centroids of the ROI (TC and IM) annotated on CK-7 were calculated and the polygon points' coordinates exported. Distances between the CK-7 ROI and the landmarks' centroid were calculated, and these distances were transferred to each staining for each patient. Polygon points were then transferred as well, allowing the ROI to be drawn and aligned automatically on every staining for a given patient. Cell segmentation was performed on hematoxylin counterstain and transferred to each staining with QuPath. Positive cell detection was then applied using QuPath on each staining (Supplementary Methods 2).

Statistical analysis

For survival analysis, the prognostic value of the different variables was tested using univariate or multivariate Cox regression models for progression-free (PFS) or overall survival (OS). Survival probabilities were estimated using the Kaplan-Meier method, and survival curves were compared using the log-rank test. Continuous variables, including CD8-TC, CD8-IM, CD3-TC, and CD3-IM variables, were dichotomized using the methodology of Lausen et al. via the maxstat R library[22], respectively, for training and validation cohorts. Percentiles for each cutoff are implicitly given through number at risk tables filled out under Kaplan-Meier curves. Using a maximally selected log-rank statistic, this method provides a statically proven optimal cut-point that corresponds to the most significant relation with the outcome. Immune variables that were significantly associated with overall survival in univariate models were used to compute an immune score (IS) as the sum of the corresponding dichotomous variables. The clinical and the combined IS-clinical scores were estimated based on the linear predictor of the corresponding multivariate Cox models. Areas Under ROC curves (AUC) were estimated using survivalROC R library (https://cran.r-project.org/web/packages/survivalROC/index.html) and corresponding distributions were estimated for each model by randomly splitting the whole cohort into a training (2/3 of the data) and a validation set (1/3), 100 times. Nested multivariate models were compared using analysis of deviance based on the log partial likelihood of the Cox models. Statistical analyses were performed using the R software (http://www.R-project.org/) (version 4.2.2, accessed on 22 June 2020) and graphs were drawn using GraphPad Prism version 7.03.

Results

Description of the patient population

We used two patient data sets. The first cohort was a retrospective cohort of patients treated at Besancon teaching hospital for localized PDAC treated by surgery, with or without adjuvant chemotherapy, between 1998 and 2018. It included 205 patients. The second cohort consisted of 129 patients treated in 8 hospitals in an observational prospective study of localized PDAC between 2016 and 2021. Combining the two cohorts, we included a total of 169 (51%) males and 164 (49%) females. Mean age was 67 years. Overall, 52 patients (16%) received neoadjuvant chemotherapy (mainly FOLFIRINOX), and 232 patients (79%) received adjuvant chemotherapy (mainly gemcitabine). The median OS was 22.3 months (95% CI 20.4; 26.1 months), and the median PFS was 10.8 months (95% CI 10.1; 12.7 months). The demographic and clinical characteristics of the study population are summarized in Table 1.

Description of the PDAC immune population and associated prognostic value

Using the MICSSS IHC strategy detailed above, we performed Foxp3, CD3 and CD8 staining on single slides, and first describe the presence of these immune populations. Representative pictures and stained area recognition are shown in Figure 1a. We retained tumor core (TC) and invasive margin (IM) for all analyses, while the stromal area was not analyzed due to tissue fragility over multiple AEC stainings. We observed no significant difference in lymphocyte distribution between the TC and IM for any of the markers evaluated, *i.e*. Foxp3, CD8 and CD3. Only cytokeratin-7 staining, used as a landmark for epithelial cells, was greater in the TC compared to the IM (Supplementary Figure S1A). We then tested the impact of each staining on overall survival (OS). High CD3 T-cell infiltration in the IM (HR: 0.70 [0.53–0.92]; p =.02) and in the TC (HR: 0.74 [0.56–0.98]; p =.04) was associated with better OS (Figure 1b–c). Similarly, high CD8 T-cell infiltration in the IM (HR: 0.68 [0.51–0.90]; p <.01) and in the TC (HR: 0.66 [0.50–0.86]; p <.01) was significantly associated with better OS (Figure 1d–e). High Foxp3 in the TC was negatively associated with OS (HR: 1.49 [1.07–2.08]; p =.02), but we observed no significant impact of Foxp3 in the IM on OS (HR: 0.88 [0.66–1.17]; p =.36) (Supplementary Figure S1B). Similar results were found for PFS, with a high CD8 count in IM (HR: 0.65 [0.50–0.85]; p =.009) and in TC (HR: 0.60 [0.45–0.80]; p <.0001), and a high CD3 count in IM (HR: 0.73 [0.56–0.95]; p =.036) and in TC (HR: 0.67 [0.51–0.89]; p-value =.01) all associated with improved PFS. Foxp3 was not significantly associated with PFS, either in the TC or the IM (Supplementary Figure S1C). We tested correlation between immune markers. We observed that all immune variables were significantly correlated except CD3-TC and CD8-TC (Supplementary Figure S1D). We finally tested whether spatial repartition of CD8 T-cells impacted PDAC prognosis. We showed that when CD8 T-cells were close to tumor cells (*i.e* in a radius of 20 µm from CD8 T-cells centroid) and the ratio of the shared area between CD8 T-cells and tumor cells (Supplementary Figure S1E for details) both significantly positively impacted PDAC prognosis (HR: 0.58 [0.42–0.80]; p =.001; HR: 0.64 [0.46–0.88]; p =.006 respectively) (Supplementary Figure S1E-G). Together these data underline the prognostic impact of CD3 and CD8 in PDAC TC and IM.

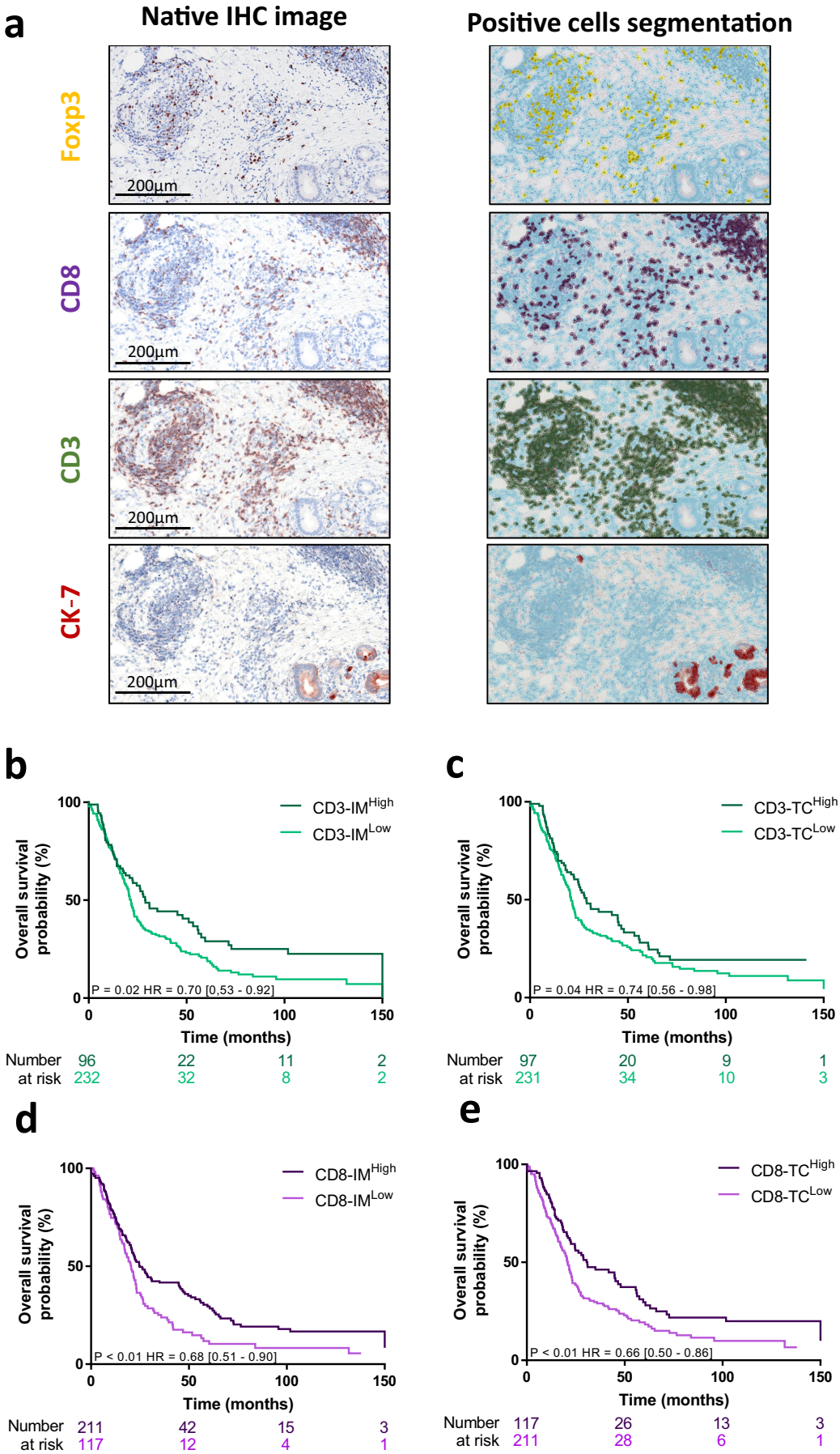

Figure 1. Description of PDAC immune populations and their associated prognostic value. Representative images of PDAC infiltrates (left panel) and their detection using QuPath (right panel). Scale bar is 200 µm (a). Kaplan-Meier survival curves for overall survival (OS) according to CD3-IM (b), CD3-TC (c), CD8-IM (d) and CD8-TC (e). Log-rank test; HR = hazard ratio, 95% CI in square brackets.

Immune score combining CD3 and CD8 IHC markers is associated with PDAC prognosis

We generated an immune score to assess the prognostic role of CD8-TC, CD8-IM, CD3-TC, CD3-IM pooled in a single variable. We split the cohort into a training and a validation set, using random sampling of 2/3 of the cohort for the training set, and the remaining 1/3 for the validation set. We combined the four immune variables into a five-modality immune score scaled from 0 to 4, built by summing the low (0 points) or high (1 point) infiltrate score for each variable (CD8-TC, CD8-IM, CD3-TC, CD3-IM) (Figure 2a). We tested the prognostic value of this immune score in our training cohort. We observed that the immune score was associated with PDAC prognosis in terms of OS (Figure 2b) and PFS (Figure 2c). A similar, albeit non-significant tendency was observed in the validation cohort in terms of OS (Supplementary Figure S2A) but not in terms of PFS (Supplementary Figure S2B). To simplify the model, we split the population into patients harboring a higher immune score (score 3 or 4) versus patients with a lower immune score (score 0 to 2). This binary analysis in the training dataset again highlighted the prognostic value of the immune score in terms of both OS (HR: 0.44 [0.31–0.64]; p <.001) (Figure 2d) and PFS (HR: 0.50 [0.36–0.70]; p <.001) (Figure 2e). The prognostic value of the binary immune score was confirmed in the validation dataset in terms of OS (HR: 0.54 [0.32–0.91]; p <.01) (Supplementary Figure S2C) but not PFS (Supplementary Figure S2D). Together, these results demonstrate the prognostic role of PDAC immune score to predict patient survival following PDAC resection.

Improved prognostic value for PDAC outcome with a combination of clinical features and immune score

Using Cox univariate models in the whole cohort, we observed that histological grade, lymph node status according to the 8th TNM classification, absence of adjuvant chemotherapy, and female status were associated with worse PFS and OS, while PDAC immune score was associated with better PFS and OS. In a multivariable model, we observed that all variables remained significantly associated with OS (Table 2), and all but gender were associated with PFS (Table 3). Harrell's C statistic was 0.65 for PFS and 0.67 for OS, indicating good fit in the multivariate models.

We next explored the prognostic capacity of a composite model associating both the immune score, and clinical features. Using our training dataset, we built a multivariate model using the clinical features that were significantly in the univariate model, as listed above. This model, called the clinical score, was strongly associated with PDAC prognosis in the training dataset in terms of OS (HR: 2.63 [1.78–3.84]; p <.0001) (Figure 3a) and similar results were observed in the validation dataset (HR: 2.63 [1.67–4.67]; p <.001) (Figure 3b). In terms of PFS, the clinical score was also associated with PDAC prognosis in the training dataset (HR: 2.13 [1.53–3.05]; p <.001) (Supplementary Figure S3A) and in the validation dataset (HR: 2.64 [1.67–4.10]; p <.0001) (Supplementary Figure S3B).

Next, we generated a combined score pooling the immune score and the clinical score. We observed a major impact of this score on PDAC prognosis in terms of OS in the training cohort (HR: 3.03 [2.12–4.34]; p <.0001) (Figure 3c) and similarly in the validation cohort (HR: 2.87 [1.64–5.00]; p < 0.001) (Figure 3d). Comparison of the AUC between all models showed that the combined score had the best predictive capacity (Figure 3e) in both the training and validation datasets. Similar results were observed in terms of PFS in both the training (HR: 2.26 [1.65–3.11]; p <.0001) and validation (HR: 2.91 [1.85–4.56]; p <.0001) datasets (Supplementary Figure S3C and S3D respectively). AUC comparison between all models for PFS showed that the combined score had also the best predictive capacity for PFS (Supplementary Figure S3E). Together, these data demonstrate that the PDAC immune score improved the prognostic value when added on top of clinical variables, to predict both PFS and OS in resected PDAC.

Combined score associating immune score and clinical score is associated with PDAC prognosis regardless of neoadjuvant or adjuvant chemotherapy

A subgroup analysis was performed to determine whether the immune score provided prognostic information for patients who received adjuvant or neoadjuvant chemotherapy regimens. A high immune score was associated with better outcome in the subgroup of patients treated with adjuvant therapy (HR: 0.57 [0.40–0.82]; p <.001) (Figure 4a) while no significant difference was observed in the group with neoadjuvant therapy (Figure 4b). To increase the number of patients analyzed, we pooled patients who received neoadjuvant chemotherapy from the two cohorts and reestimated IS. In this setting a high immune score was associated with better outcome (PFS and OS) in both adjuvant and neoadjuvant strategies (Supplementary Figure S4A-D). Analysis of the combined score showed that for the subgroup who received adjuvant chemotherapy, a low combined score was significantly associated with better OS (HR: 2.94 [2.00–4.35]; p <.001) (Figure 4c). We observed similar results (HR: 6.39 [2.24–18.20]; p <.01) in the neoadjuvant subgroup (Figure 4d). Comparison of the AUC showed that for both adjuvant ($AUC^{clinical} = 0.67$, $AUC^{combined} = 0.71$; likelihood ratio p <.0001) and neoadjuvant therapy ($AUC^{clinical} = 0.74$, $AUC^{combined} = 0.77$; likelihood ratio p <.0001), the combined score outperformed the clinical score alone. Results of our combined score on PFS showed a significant difference in both subgroups (HR: 2.87 [1.99–4.13]; p <.001; HR: 8.15 [2.27–29.24]; p <.01 respectively) (Supplementary Figure S4E and F).

Discussion

The tumor microenvironment in PDAC is still the subject of intense scrutiny, notably regarding several immune aspects, including T-cells, cancer associated fibroblasts or myeloid cells. In the present study, we used IHC staining on whole slide tissue, limiting the bias of restricted counting and lack of representativeness of tissue microarray.[23] We retrospectively analyzed two French cohorts, and studied the lymphoid components, namely CD3-T cells, CD8-cytotoxic T-cells and Foxp3 regulatory T-cells located in the tumor core (TC) or the invasive margin (IM), mimicking the well-known Immunoscore® strategy developed in colorectal cancer.[19] We

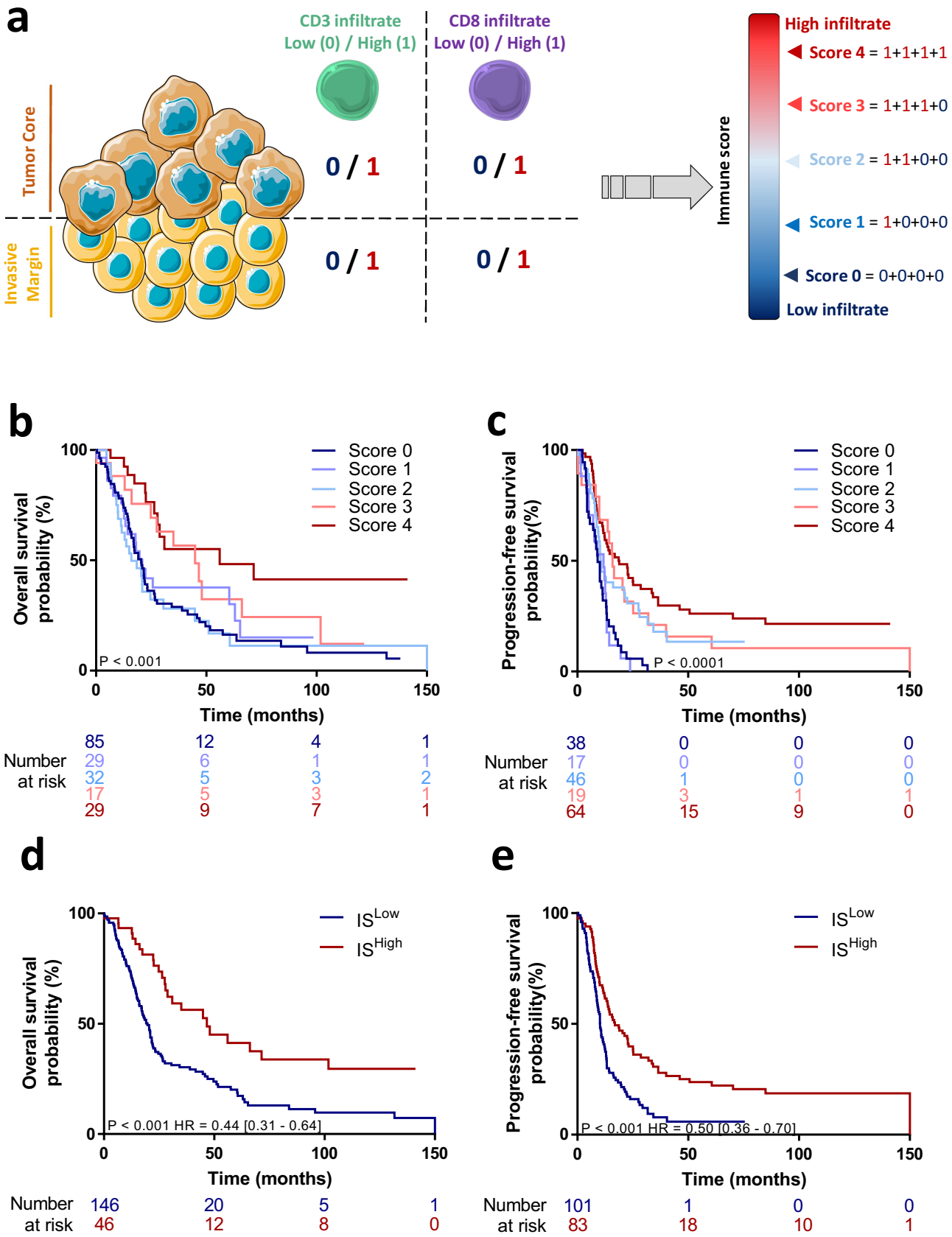

Figure 2. Immune score combining CD3 and CD8 IHC markers is associated with PDAC prognosis. (a) Graphical abstract showing immune score generation. Kaplan-Meier curves for the immune score (IS) in 5 modalities (score 0 to 4) in terms of OS (b) and PFS (c) in the training dataset. Kaplan-Meier curves for low (0–2) vs high (3–4) immune score in terms of OS (d) and PFS (e) in the training dataset. Log-rank test; HR = hazard ratio, 95% CI in square brackets.

Table 2. Univariate and multivatiate Cox models for overall survival in the whole cohort (complete data for $n = 280$ patients).

	Univariate			Multivariate		
Variables	HR	95% CI	p-value	HR	95% CI	p-value
Adjuvant therapy						
No	—	—		—	—	
Yes	0.61	0.44, 0.84	**.003**	0.49	0.35, 0.69	**<.001**
Histological Grade						
Well	—	—		—	—	
Moderatly	1.23	0.86, 1.77	.3	1.21	0.84, 1.75	.3
Poorly	2.65	1.68, 4.20	**<.001**	2.50	1.57, 3.98	**<.001**
Mucinous	4.34	1.54, 12.2	**.005**	5.42	1.84, 16.0	**.002**
Sex						
Female	—	—		—	—	
Male	0.80	0.60, 1.05	.10	0.72	0.55, 0.96	**.023**
Node status (AJCC 2017)						
0	—	—		—	—	
1	1.36	0.95, 1.97	.10	1.73	1.18, 2.54	**0.005**
2	2.22	1.53, 3.22	**<.001**	2.33	1.57, 3.44	**<.001**
Immune Score (IS)						
Low	—	—		—	—	
High	0.60	0.45, 0.80	**<.001**	0.60	0.44, 0.81	**<.001**

HR : Hazard Ratio ; CI : Confidence Interval.

Table 3. Univariate and multivatiate Cox models for progression-free survival in the whole cohort (complete data for $n = 273$ patients).

	Univariate			Multivariate		
Variables	HR[1]	95% CI[1]	p-value	HR[1]	95% CI[1]	p-value
Adjuvant therapy						
No	—	—		—	—	
Yes	0.69	0.51, 0.94	**.020**	0.61	0.44, 0.85	**.003**
Histological Grade						
Well	—	—		—	—	
Moderatly	1.10	0.79, 1.53	.6	1.16	0.83, 1.62	.4
Poorly	2.30	1.50, 3.52	**<.001**	2.14	1.39, 3.28	**<.001**
Mucinous	4.78	1.71, 13.4	**.003**	6.13	2.14, 17.5	**<.001**
Sex						
Female	—	—		—	—	
Male	0.87	0.67, 1.12	.3	0.80	0.61, 1.04	.091
Node status (AJCC 2017)						
0	—	—		—	—	
1	1.19	0.85, 1.66	.3	1.51	1.06, 2.15	**.021**
2	1.84	1.31, 2.59	**<.001**	2.10	1.47, 3.01	**<.001**
Immune Score (IS)						
Low	—	—		—	—	
High	0.54	0.40, 0.74	**<.001**	0.56	0.40, 0.76	**<.001**

HR : Hazard Ratio ; CI : Confidence Interval.

analyzed 334 patients, which is one of the largest PDAC cohorts reported in the literature to date.[24] Our study revealed that this strategy was valuable to predict outcome of PDAC patients treated with surgery, with or without adjuvant or neoadjuvant chemotherapy.

The multiple AEC staining we used is a strategy to perform sequential staining on a single slide in a cost-effective manner. However, such staining or other multiplex tissue imaging remains difficult to apply in routine pathology because these procedures are complex and time consuming.[25] Multiple single labeling on serial slides might be easier, but would still require systematic slide scanning followed by automated tissue registration.[26] Consequently, it is important to note that major improvements in wet and dry labs are needed to consider these kinds of approaches in routine practice.

Our study corroborates previous works, notably from Tahkola et al.[23,27] who showed that the number of CD3 T-cells and CD8 T-cells is linked to PDAC prognosis. We demonstrated that the prognostic value was found in the IM and TC for both CD8 and CD3. For the T-reg components, in line with the abundant existing literature,[28] we confirmed that a high number of Foxp3 positive cells located in the TC was linked to prognosis, at least in OS analysis. Surprisingly, no impact on prognosis was found for Foxp3 positive cells in the IM. The role of peritumoral T-regs in PDAC remains elusive, as only two studies previously tested this variable, and with heterogeneous results, suggesting that the prognostic role of peritumoral Treg is probably less important.[29,30] We combined our immune variables (CD3 and CD8 in the TC IM) to mimic the Immunoscore® strategy. In order to assess the performance of our immune score, we chose to split our dataset into training and validation set. When the PDAC immune score was split into a dichotomous variable (high versus low immune score), we validated its prognostic role in the two datasets in terms of OS. This result, showing more relevance for OS compared to PFS, is in agreement with previous work from Takhola.[23] We also observed a strong prognostic stratification when using immune score in 5 modalities (score 0 to 4 inclusive), in terms of both OS and PFS, but the power of our study precluded validation of this stratification in the validation dataset.

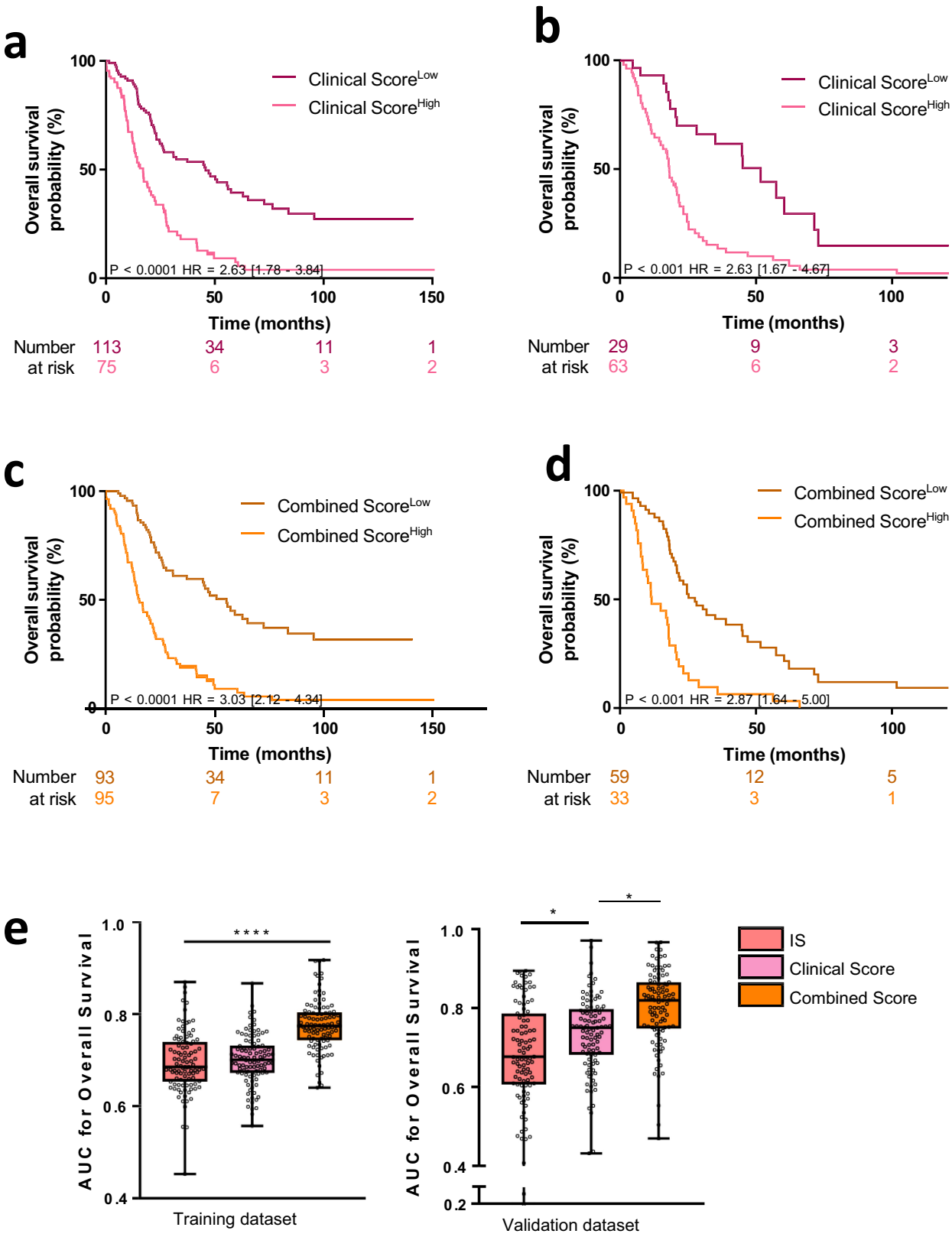

Figure 3. Improved prognostic value for PDAC outcome with a combination of clinical features and immune score. Kaplan-Meier curves for low vs high clinical score in the training (a) and validation datasets (b) in terms of OS. Kaplan-Meier curves for low vs high combined (clinical + immune) score in the training (c) and validation datasets (d) in terms of OS. AUC for predictive value of the different models (e). Log-rank test; HR = hazard ratio, 95% CI in square brackets.

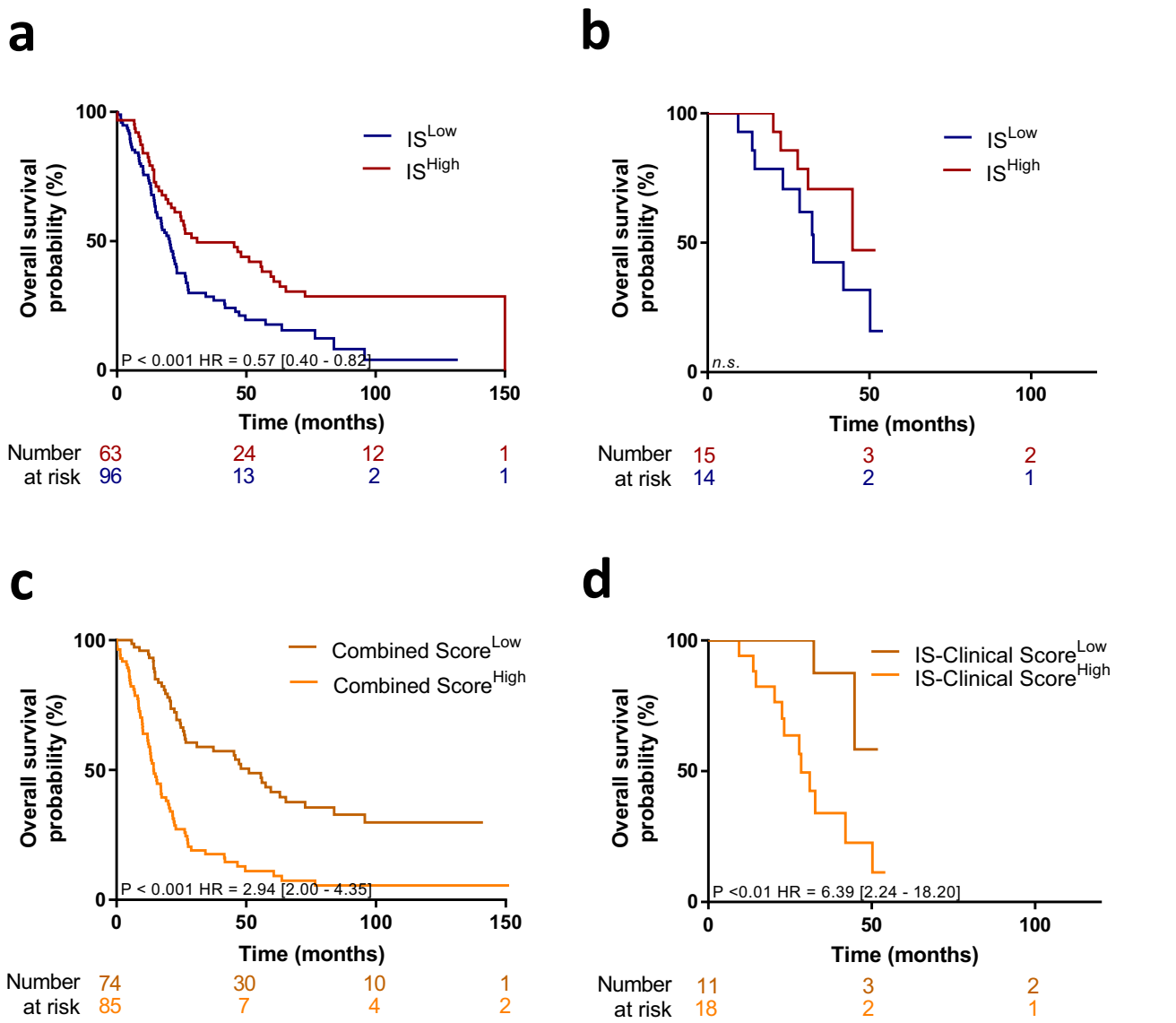

Figure 4. Combined score associating immune score and clinical score is associated with PDAC prognosis regardless of neoadjuvant or adjuvant chemotherapy. Kaplan-Meier curves for low vs high immune score (IS) in patients with adjuvant chemotherapy (a) and in patients with neoadjuvant chemotherapy (b) in the training dataset in terms of OS. Kaplan-Meier curves for low vs high combined score in patients with adjuvant chemotherapy (c) and in patients with neoadjuvant chemotherapy (d) in the training dataset in terms of OS.

Finally, beyond its prognostic value, we need to question how immune infiltrates could impact on patient care. Our study suggests that the PDAC immune score is of value for patients treated with both adjuvant and neoadjuvant strategies. With the development of the FOLFIRINOX adjuvant strategy[7], it might be interesting to determine whether patients with a high immune score could avoid administration of this effective, but toxic regimen. Adaptive dynamic strategies following neoadjuvant therapy are also emerging in PDAC.[31] We suspect that assessing the PDAC immune score could be of value to better select candidates for adjuvant therapy. Besides adjuvant strategies, it is also important to recall that PDAC is one of the cancers in which immune checkpoint (ICP) inhibitors have been disappointing so far.[32] It is plausible that an enhanced understanding of the PDAC tumor microenvironment, especially regarding infiltration of CD3 and CD8 positive cells, could be predictive of ICP inhibitors' efficacy. As a result, IHC analysis could be a prerequisite to better select patients for clinical trials evaluating ICP inhibitors in PDAC in the future.

Conclusion

Despite recent improvements in the management of localized PDAC, the discovery of new biomarkers related to patient prognosis is a crucial need for oncologists, to guide decision-making for chemotherapy administration and patient follow-up. Our study provides proof-of-concept that an immune score, incorporating both CD3 and CD8 infiltration in different tumor areas, is a valuable strategy to predict patient survival, as previously shown in colorectal cancer. If confirmed, these results could be a new tool to better predict PDAC outcomes and to improve decision-making for adjuvant strategies.

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Data availability statement

Date are available on reasonable request.

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