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Research paper

Prognostic and predictive value of a five-molecule panel in resected pancreatic ductal adenocarcinoma: A multicentre study

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ABSTRACT

Background: Pancreatic ductal adenocarcinoma (PDAC) has a devastating prognosis. The performance of clinicopathologic parameters and molecules as prognostic factors remains limited and inconsistent. The present study aimed to construct a multi-molecule biomarker panel to more accurately predict post-resectional prognosis of PDAC patients.

Methods: Firstly, a novel computational strategy integrating prognostic evidence from omics and literature on the basis of bioinformatics prediction (CIPHER) to generate the network, was designed to systematically identify potential high-confidence PDAC-related prognostic candidates. After specimens from 605 resected PDAC patients were retrospectively collected, 23 candidates were detected immunohistochemically in tissue-microarrays for the development cohort to construct a multi-molecule panel. Lastly, the panel was validated in two independent cohorts.

Findings: According to the constructed five-molecule panel, disease-specific survival (DSS) was significantly poorer in high-risk patients than in low-risk ones in development cohort (HR 2.15, 95%CI 1.51−3.05, $P<0.0001$; AUC 0.67). In two validation cohorts, similar significant differences between the two groups were also observed (HR 3.18 and 3.31, 95%CI 1.89−5.37 and 1.78−6.16, All $P<0.0001$; AUC 0.72 and 0.73). In multivariate analyses, this panel was the sole prognosticator that was significant in each cohort. Furthermore, its predictive power for long-term survival, higher than its individual constituents, could be largely enhanced by combination with traditional clinicopathological variables. Finally, adjuvant chemotherapy (ACT) correlated with better DSS only in high-risk patients, uni- and multi-variately, in all the cohorts.

Interpretation: The novel prognostic panel developed by a systematically network-based strategy presents strong ability in prediction of post-resectional survival of PDAC patients. Furthermore, panel-defined high-risk patients might benefit more from ACT.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) has very poor prognosis, in United States of America, it carries a 5-year survival rate of 7.2% [1]. The incidence of PDAC has significantly increased in both developed and developing countries [2]. Less than 20% of patients are eligible for initial surgery, and even in patients who undergo radical resection, long-term prognosis remains poor. Conventional adjuvant chemotherapy (ACT) has been shown to improve the survival of PDAC patients after radical resection. However, the best reported effective rate was only approximately 30% [3]. Intensified ACT, such as Folfirinox, has recently been shown to improve survival at the expense of a higher risk of adverse events [4]; however, considering its high incidence of side effects, in the real world clinical practice, Folfirinox has not been routinely used as ACT, therefore,

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Research in context

Evidence before this study

Pancreatic ductal adenocarcinoma (PDAC) is well known as one of malignancies with most gloomy prognosis. The performance of current clinicopathological variables and prognostic factors is limited. Although multi-molecule signatures have been developed as an attempt to predict prognosis in PDAC, the majority suffered from limited performance and small sample size. Furthermore, whether those had superiority to clinicopathological variables and the power to predict the response to adjuvant chemotherapy (ACT) remain unclear.

Added value of this study

We firstly designed a novel computational strategy that integrated prognostic evidence from omics and literature on the basis of bioinformatics prediction to systematically identify potential high-confidence PDAC-related prognostic factors. Then, we constructed and validated a multi-molecule panel in three independent cohorts. According to the constructed five-molecule panel, high-risk groups had poorer disease-specific survival (DSS) than the low-risk ones. In multivariate analyses, this panel was the sole significant prognosticator in each cohort. Furthermore, its predictive power for long-term survival, higher than individual constituents, could be largely enhanced by combination with clinicopathological variables. Finally, ACT correlated with better DSS only in high-risk patients, uni- and multivariately, in all the cohorts.

Implications of all the available evidence

Multi-molecule signatures could predict prognosis in PDAC. The novel prognostic panel presents strong ability in prediction of post-resectional survival of PDAC patients. Furthermore, panel-defined high-risk patients might benefit more from ACT.

personalized treatment is still needed. The performance of current clinicopathological variables and prognostic factors is limited [5,6]. Several multi-molecule signatures have yielded prognostic information beyond their individual constituents and clinicopathological parameters in various tumour types [7,8]. In PDAC, although multi-molecule signatures have been developed as an attempt to predict prognosis, the majority suffered from limited performance and small sample sizes. Furthermore, comparison in prognostic efficiency between those signatures and clinicopathological variables has not been performed, and the power to predict the response to ACT remain unclear [9–11]. Therefore, a new approach to systematically detect multi-molecule signatures with better predictive power is needed.

In this study, we proposed a novel bioinformatics-based computational strategy to systematically identify tumour high-confidence prognostic markers by integrating prognostic evidences from omics and literatures on the basis of bioinformatics prediction [12]. As a result, a multi-molecule panel was developed and validated independently, which exhibited superiority to clinicopathological variables to stratify the survival of the PDAC patients after resection. And it distinguished a subpopulation that had better response to ACT who might benefit from intensified ACT.

2. Materials and methods

2.1. Patients

In total, 605 patients with stage I–III PDAC after radical resection (tumour margin >1 mm), according to the 8th edition American Joint Committee on Cancer staging system [13], were retrospectively enrolled from Jun. 2003 to Oct. 2015. In this study, we only enrolled resectable PDAC patients without pre-surgical therapies, the borderline resectable patients who probably underwent vascular resection or replacement, were excluded, as it was extremely difficult to accurately determine the surgical margin for these patients. We did not mark all of margins with ink, and the surgical margins were determined by the pathologists. Tumour negative >1 mm was adopted to enrol patients, as positive tumour margin could substantially influence the survival which may confound the prognostic roles of the panel. Moreover, PDAC patients died of complications within 3 months, and ampullary carcinoma and distal cholangiocarcinoma, were also excluded. ACT based on gemcitabine was recommended for the patients during that time. The patients were followed up every 3 months at outpatient clinic or telephone interview and the serum carbohydrate antigen (CA) 19–9, and intravenous enhanced CT scan were prescribed to evaluate local recurrence and distant metastasis. Only the patients who underwent at least three cycles of ACT were enrolled for analysis. The development cohort consisted of 280 patients from Peking Union Medical College Hospital (PUMCH) (Beijing, China), following the exclusion of 40 patients with incomplete expression data for the candidate molecules (Appendix Fig. 1). The validation cohorts were recruited from Renji Hospital (RJH), School of Medicine, Shanghai Jiao Tong University, Shanghai, China, and The First Affiliated Hospital of Harbin Medical University (HMH) (Harbin, China), and included 120 and 100 patients, after excluding 49 and 16 ones, respectively (Appendix Fig. 1). The median follow-up durations were 19.5 (range, 2.0–129.0), 18.0 (range, 2.0–96.3), and 16.0 (range, 4.0–40.0) months for patients in the development (PUMCH) and the validation (RJH and HMH) cohorts, respectively. These patients resided in 27 out of the 32 provinces of mainland China (Appendix Fig. 2). ACT information was available for 425 patients (85.0%). Of these, 239 were treated with ACT (186 were not). The most common regimens included gemcitabine alone or in combination with oral 5-fluorouracil (3–8 cycles). The baseline clinicopathological characteristics and surgery-related items of the three cohorts were shown in Appendix Table 1 and 2. This study was approved by the Ethical Review Board of each hospital, and the written informed consents were acquired from all the patients.

2.2. Selection of PDAC-related prognostic candidates

PDAC-related prognostic candidates were selected using a novel bioinformatics-based computational strategy (Appendix Fig. 3), consisting of two main steps: (1) unbiased identification of PDAC-related genes through the integration of bioinformatics prediction and meta-analysis of publically available microarray datasets (GSE15471, GSE16515, GSE28735, GSE62452), and (2) inferring prognostic candidates from those PDAC-related genes. Specifically, the bioinformatics prediction was performed by CIPHER [12], a state-of-art tool for unbiasedly prioritising disease genes on a genome-wide scale, which has deduced several novel findings [14–16]. To further infer novel prognostic candidates, we focused on PDAC-related genes with the following prognostic evidence (Appendix Fig. 4): (1) previously implicated in the prognosis of other types of cancer except PDAC (literature-derived prognostic evidence), (2) associated with overall survival in publicly available PDAC-related datasets (omics-derived prognostic evidence, GSE57495, GSE62452 and TCGA), and (3) has

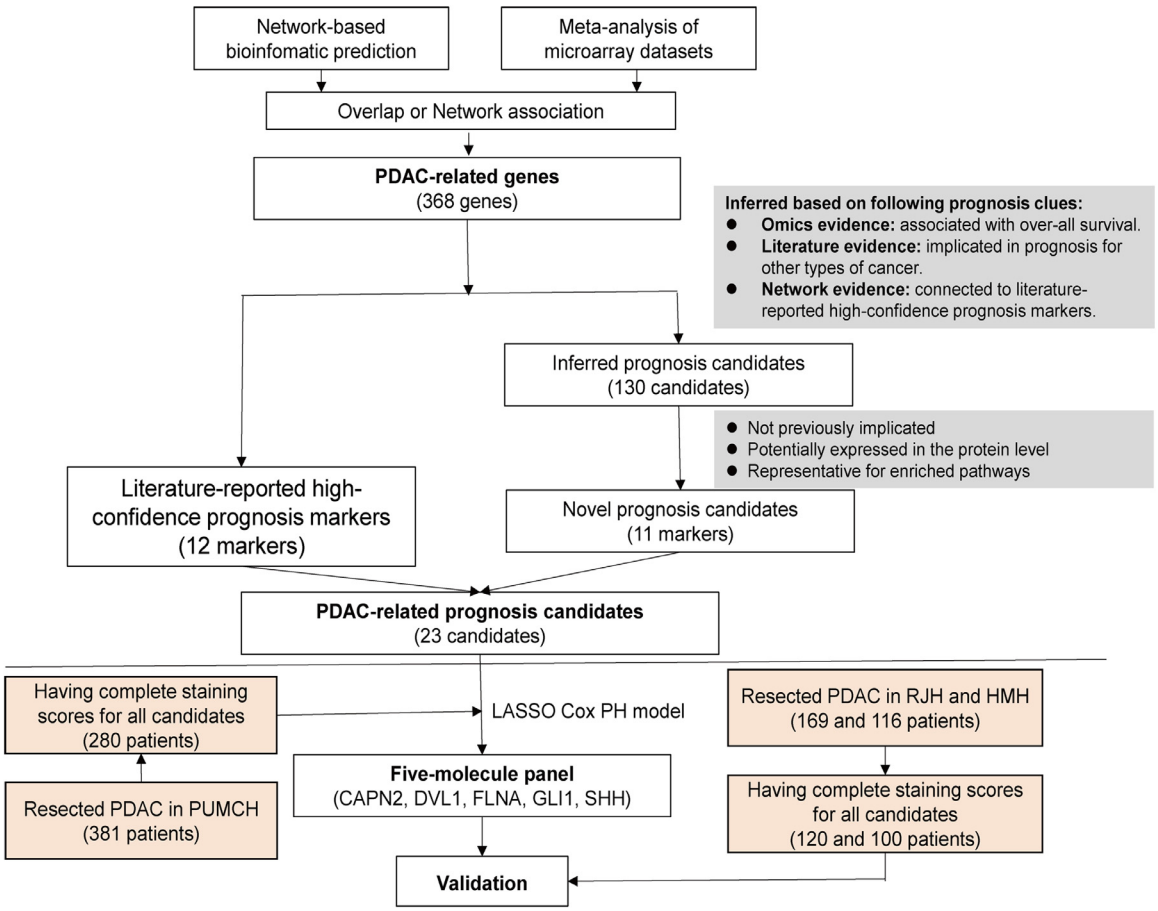

Fig. 1. The flow chart of this study.

exhibited strong network associations with high-confidence literature-reported prognostic markers in the literature (network-derived prognostic evidence). Taking inferred novel candidates and literature-supported markers together, PDAC-related prognostic candidates would finally be selected for detection by IHC in patients from the development cohort.

2.3. Tissue microarray construction, immunohistochemical staining and result evaluation

Tissue microarrays were constructed with tumour and non-tumour tissues. Primary antibodies (Appendix Table 3) and a two-step staining kit (EnVision™+; Dako, Glostrup, Denmark) were used for IHC. Relative details (including antibody specificity confirmation and intra-tumoural heterogeneity evaluation) are shown in appendix data (Appendix Fig. 5).

2.4. Construction of a multi-molecule prognostic panel

The least absolute shrinkage and selection operator (LASSO) Cox proportional hazards model is a popular method for feature selection when performing survival analysis in high-dimensional data. After evaluating immunoreactive scores for all PDAC-related prognostic candidates, LASSO Cox proportional hazards modelling was used to select the most informative candidates and to construct a multi-molecule panel on the basis of model coefficients for predicting disease-specific survival (DSS), which was defined as patient survival after calculating tumour-related deaths (from surgery to death), in PDAC patients in the development cohort. Sensitivity analyses, possible interactions and the proportional hazards assumption for the proportional hazards models were also performed. Relative details are shown in the appendix data (Appendix Fig. 6 and Appendix Table 4 and 5).

2.5. Statistical analyses

Clinicopathological variables were compared using the Chi-square test. Survival curves were analysed using the Kaplan-Meier method, log-rank and univariate tests. Multivariate Cox proportional hazards regression analysis was used to identify independent prognostic factors. The X-tile program [17], was used to determine the optimum cut-off point according to the minimum *P*-value defined by the Kaplan-Meier analysis and log-rank test [10]. The prognostic utility of variables was assessed using time-dependant receiver operating characteristic (ROC) curve analysis. We used 'penalised' and 'survival ROC' packages in R (version 3.3.1; http://cran.r-project.org) to perform the LASSO Cox proportional hazards regression and time-dependant ROC curve analyses, respectively. The assessment of the proportional hazards assumption, examined and met by scaled Schoenfeld residuals, for the proportional hazards models and sensitivity analysis for this model on a hazard ratio scale were also performed. Sensitivity analysis for residual confounding was performed using the R "obsSens" package. A *P*-value <0.05 was considered statistically significant.

3. Results

3.1. Construction of five-molecule prognostic panel

The overall flow chart of this study is shown in Fig. 1. We initially identified 368 PDAC-related genes by applying the bioinformatics-

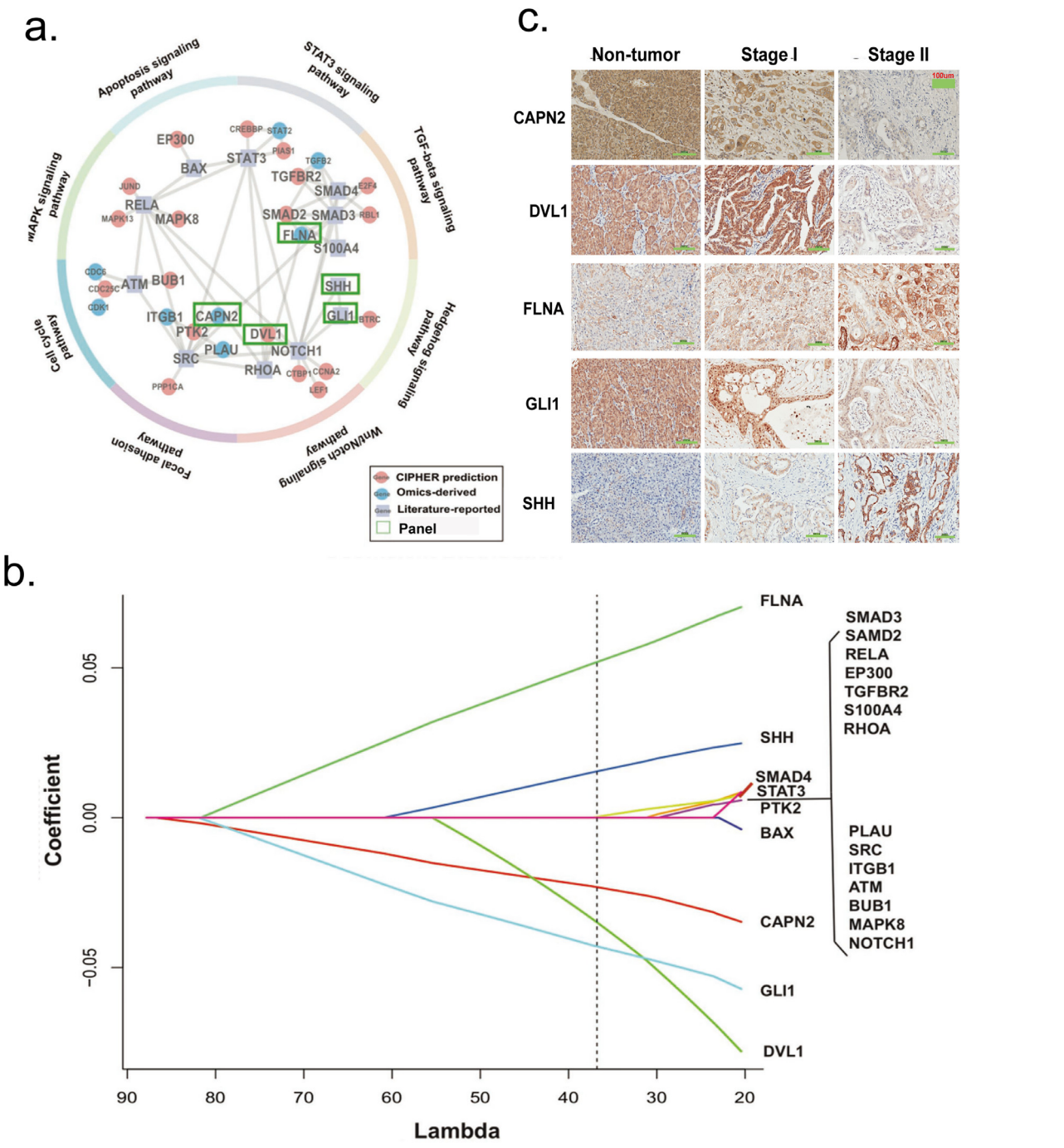

Fig. 2. Construction of the five-molecule prognostic panel. (a) Bioinformatics-based identification of PDAC-related prognostic candidates. The candidates labelled bold are those pathway-representative ones to be detected immunohistochemically in PUMCH cohort. (b) Construction of the five-molecule panel using the LASSO Cox PH model. (c) Immunohistochemical expression of the proteins included in the five-molecule panel in tumour and non-tumour tissues.

based unbiased integration strategy proposed in this study. Among them, 12 were reported high-confidence prognostic markers by manual literature searches and 11 were additionally inferred as PDAC-related novel prognostic candidates based on three types of prognostic evidence and their representation in involved pathways (Appendix Fig. 3). Taking together, 23 PDAC-related prognostic candidates, involved in several pivotal pathways related to PDAC, were identified (Fig. 2(a), Appendix Tables 6 and 7).

According to the score integrating positive ratio and staining intensity (Appendix fig. 7), a Cox proportional hazards regression model was used to assess the association between each of the 23 markers and DSS in the development cohort (Appendix fig. 8). The five most informative markers, CAPN2, DVL1, FLNA, GLI1, and SHH (Representative IHC images shown in Fig. 2(c)) were selected to build a multi-molecule prognostic panel using LASSO analysis (Fig. 2(b) and Appendix Fig. 9). Using the coefficients of the model, we subsequently derived a formula to calculate the risk score for assessing DSS for each patient (the risk score = −0.023*CAPN2 + −0.035 *DVL1 + 0.052*FLNA + −0.043*GLI1 + 0.015*SHH, forming a five-molecule panel). In the formula, coefficients above and below zero represented positive and negative associations between marker expression and DSS, respectively.

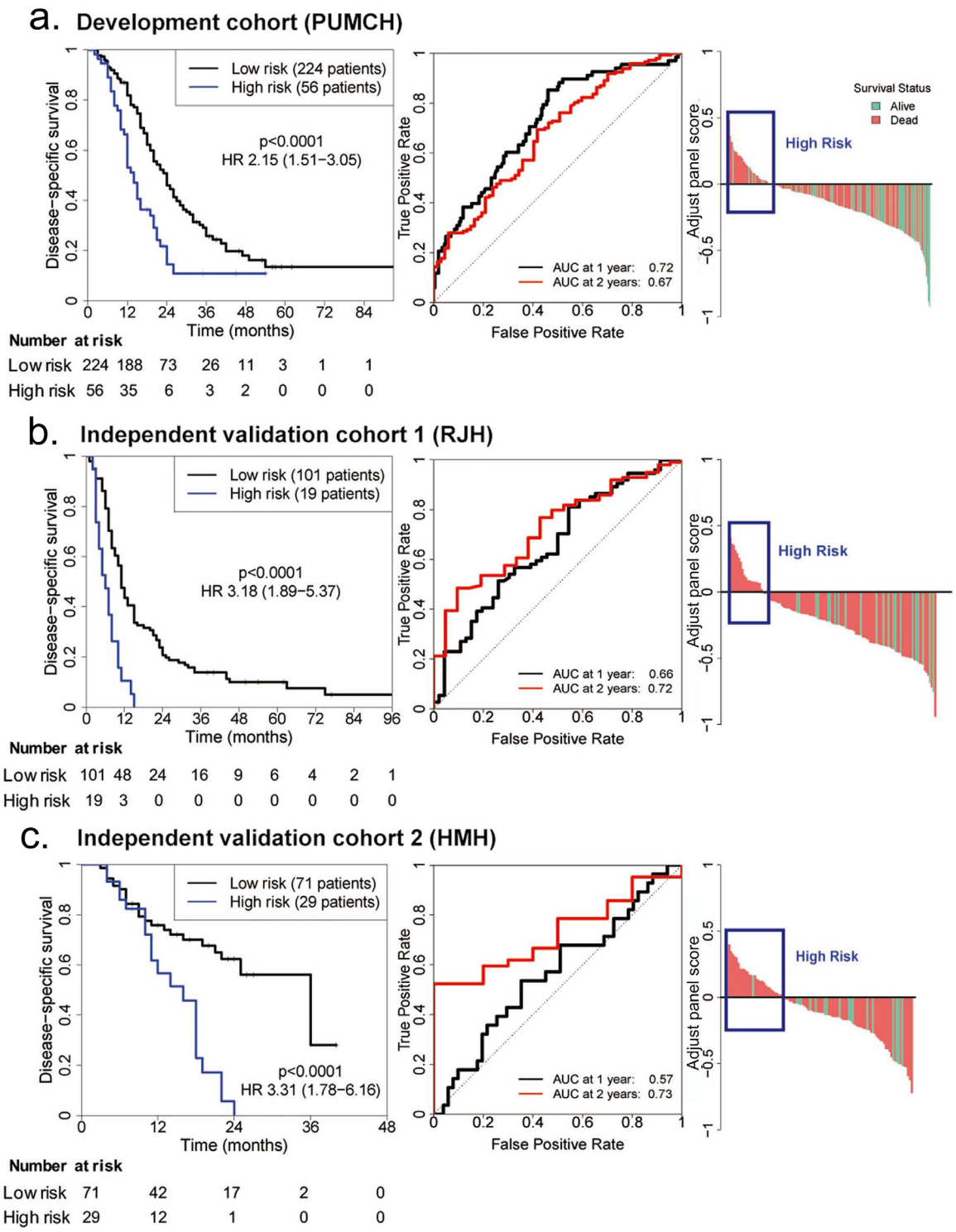

Fig. 3. Kaplan-Meier survival curves, time-dependant ROC curves and risk score by the five-molecule panel in development and validation cohorts of PDAC. (a) Survival curves of high- and low-risk groups (left panel); ROC curves for survival at 1 and 2 years (intermediate panel); survival status at 2 years after surgery in high- and low-risk groups (right panel) in the development cohort. (b) Survival curves of high- and low-risk groups (left panel); ROC curves for survival at 1 and 2 years (intermediate panel); survival status at 2 years after surgery in high- and low-risk groups (right panel) in the validation cohort 1. (c) Survival curves of high- and low-risk groups (left panel); ROC curves for survival at 1 and 2 years (intermediate panel); survival status at 2 years after surgery in high- and low-risk groups (right panel) in the validation cohort 2.

3.2. Prognostic value of five-molecule prognostic panel

Using risk scores for each patient based on their individual expression levels of the five markers and the optimal cut-off value determined by X-tile plots (Appendix Fig. 10), patients were classified into high-risk (risk score, ≥0.014) and low-risk (risk score, <0.014) groups. In the development cohort, high-risk patients had a significantly poorer DSS than low-risk ones (P<0.0001) (Fig. 3(a) left panel and Table 1). The AUCs for ROC curves at 1 and 2 years were 0.72 and 0.67, respectively (Fig. 3(a) intermediate panel). At 2 years post-surgery, a majority of patients in the high-risk group had died (2-year survival: 15.0% vs. 46.0% for the low-risk group; Fig. 3(a) right panel).

Table 1
Univariate and multivariate prognostic analyses of PDAC patients in development and validation cohorts.

Variables	Development Cohort (280 patients)				Validation Cohort 1 (120 patients)				Validation Cohort 2 (100 patients)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR	*P*	HR	*P*	HR	*P*	HR	*P*
Age (≥ 62 years* vs < 62 years)	0.93 (0.69–1.25)	0.63	1.21 (0.79–1.86)	0.39	1.2 (0.82–1.76)	0.34	1.18 (0.73–1.90)	0.5	1.79 (0.98–3.26)	0.055	1.82 (0.87–3.81)	0.11
Gender (Male vs Female)	1.12 (0.83–1.51)	0.47	0.95 (0.62–1.44)	0.8	1.06 (0.725–1.54)	0.77	0.98 (0.64–1.50)	0.93	1.26 (0.65–2.45)	0.5	1.39 (0.61–3.13)	0.43
Tumour site (Head vs Non-head)	1.05 (0.76–1.46)	0.77	0.95 (0.62–1.45)	0.8	0.687 (0.46–1.03)	0.073	0.74 (0.47–1.16)	0.19	0.85 (0.46–1.57)	0.6	1.45 (0.59–3.60)	0.42
T stage (T3 vs T1/2)	1.19 (0.88–1.61)	0.25	1.33 (0.85–2.08)	0.21	1.57 (1.05–2.33)	**0.031**	1.14 (0.72–1.82)	0.57	1.94 (1.01–3.73)	**0.044**	1.67 (0.63–4.49)	0.3
N stage (N1/N2 vs N0)	1.86 (1.35–2.56)	**0.00011**	2.38 (1.52–3.74)	**0.00017**	1.68 (1.14–2.46)	**0.0092**	1.54 (0.99–2.40)	0.058	0.88 (0.45–1.70)	0.7	0.83 (0.39–1.74)	0.62
CA 19–9 (≥ 37 vs < 37)	1.41 (0.92–2.15)	0.1	0.88 (0.50–1.55)	0.65	0.846 (0.481–1.49)	0.57	1.06 (0.57–1.94)	0.86	2.29 (0.70–7.43)	0.16	2.86 (0.85–9.60)	0.089
Histological Grade (G3 vs G1/G2)	1.45 (1.06–1.99)	**0.025**	1.35 (0.85–2.13)	0.2	2.2 (1.47–3.28)	**0.00019**	1.73 (1.06–2.81)	**0.027**	2.10 (1.05–4.20)	**0.033**	1.85 (0.81–4.25)	0.14
Adjuvant chemotherapy (No vs Yes)	1.32 (0.93–1.89)	0.12	1.48 (0.96–2.29)	0.076	1.87 (1.2–2.93)	**0.0089**	1.68 (0.97–2.90)	0.065	1.07 (0.585–1.97)	0.82	1.19 (0.57–2.49)	0.64
Five-molecule panel (High-risk vs low risk)	2.15 (1.51–3.05)	**<0.0001**	2.26 (1.34–3.81)	**0.0023**	3.18 (1.89–5.37)	**<0.0001**	2.48 (1.31–4.71)	**0.0054**	3.31 (1.78–6.16)	**<0.0001**	3.59 (1.71–7.54)	**0.00075**

* Median age of all patients.

When the same cut-off value was applied to the RJH validation cohort, DSS in high-risk group was also significantly worse than that in low-risk group ($P<0.0001$) (Fig. 3(b) left panel and Table 1). The AUCs for ROC curves at 1 and 2 years were 0.66 and 0.72, respectively (Fig. 3(b) intermediate panel). At 2 years post-surgery, none of patients in the high-risk group were alive (2-year survival: 0.0% vs. 21.0% for the low-risk group; Fig. 3(b) right panel). Similarly, low-risk group in the HMH validation cohort presented a significantly better DSS than high-risk group ($P<0.0001$) (Fig. 3(c) left panel and Table 1). The AUCs for ROC curves at 1 and 2 years were 0.56 and 0.68, respectively (Fig. 3(c) intermediate panel). At 2 years post-surgery, only one patient in the high-risk group was still alive (2-year survival: 6.0% vs. 62.0% for the low-risk group; Fig. 3(c) right panel).

In the development cohort, the five-molecule panel was identified as an independent prognostic factor of PDAC (hazard ratio [HR]: 2.26, 95% confidence interval [CI]: 1.34−3.81; $P = 0.0023$) (Table 1) in the multivariate analysis having adjusted for age, sex, tumour location, CA19-9 levels, tumour differentiation, T category, nodal involvement and adjuvant chemotherapy. Similar results were noted for RJH (HR: 2.48, 95% CI: 1.31−4.71; $P = 0.0054$) and HMH (HR: 3.59, 95% CI: 1.71−7.54; $P = 0.00075$) validation cohorts (Table 1), as well as, the entire cohort of 500 patients (HR: 2.03, 95% CI: 1.47−2.79; $P<0.0001$) (Appendix Table 8). Moreover, this five-molecule panel was the sole independent prognosticator in each of three cohorts, unlike clinicopathological characteristics.

The five-molecule panel was also effective at discriminating different DSS in most of subgroups stratified according to aforementioned clinicopathological parameters ($P<0.05$; Fig. 4 and Appendix fig. 11−13). The five-molecule panel exhibited stronger predictive power, proven by significantly larger AUC and higher HR, than its individual constituents (Fig. 5(a) and (b), Appendix Fig. 14), and the panel seemed to have advantages to some extent, in contrast to main clinicopathological factors, although that was not significantly better than N stage and histological grade (Appendix Fig. 15). These data suggested that this panel was better than its constituents in distinguishing long-term prognosis after radical resection in PDAC, while it was superior to major, not all, clinicopathological factors. Interestingly, we found that combination of the panel and all tested clinicopathological factors had remarkably higher prognostic efficiency than combined factors alone in all patients and development as well as validation cohorts (Fig. 5(c) and Appendix Fig. 16). Moreover, combination of individual clinicopathological variables and this panel show much better predictive power than individual clinicopathological factors alone (Fig. 5(d) and Appendix Fig. 16). Thus, we speculated that the five-molecule panel classifier could add significantly prognostic value to clinicopathological features.

Patients treated with post-operative ACT did not exhibit a better prognosis than patients without post-operative ACT in all three cohorts (the entire cohort, development cohort and combined validation cohorts). Among the high-risk factors, only the five-molecule panel was able to identify patients who could benefit most from post-operative ACT, different with grade, T and N categories. In the low-risk groups of all three cohorts, patient survival with and without post-operative ACT did not differ significantly, while patients without post-operative ACT had a significantly worse survival than those with ACT in the high-risk group (Fig. 6 and Appendix Fig. 17 and 18). Furthermore, ACT remained to be a significant factor in multivariate analyses adjusted for clinicopathological features in the high-risk patients (Appendix Table 9−11). And, we performed the interaction test between ACT and the panel, as well as clinicopathological variables, in the multivariate Cox proportional hazard model adjusted for grade, T and N status. We found a significant interaction between ACT and the panel in the entire cohort ($P_{(interaction)}$=0.01). This result was consistent with the Kaplan−Meier curves (Fig. 6). The significant interaction between ACT and the panel was also discovered in the development ($P_{(interaction)}$=0.04) and validation

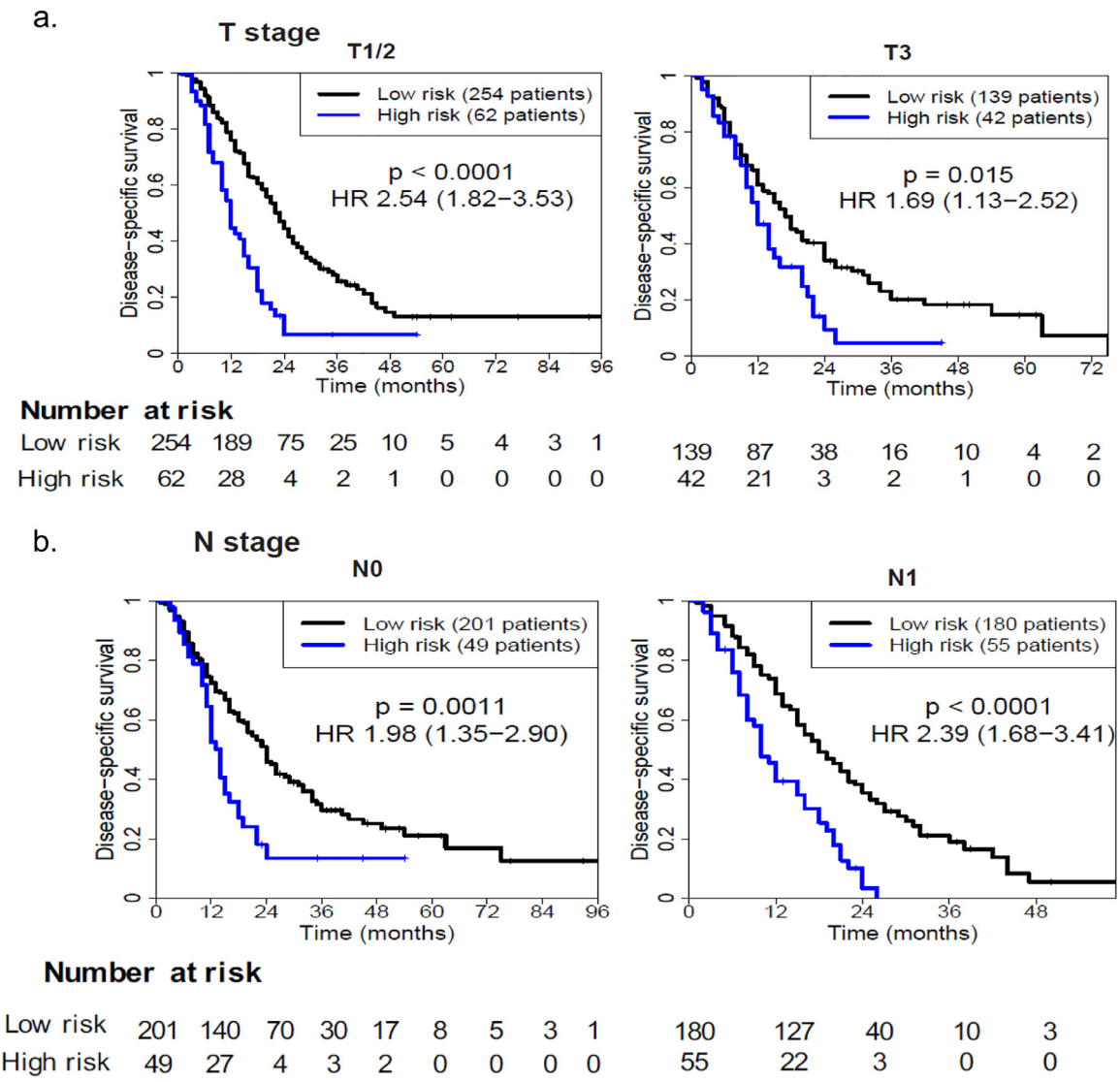

Fig. 4. Prognostic value of the five-molecule panel in some subgroups of PDAC for all the 500 patients. (a) The five-molecule panel for T1/2 or T3 patients. (b) The five-molecule panel for patients without or with nodal involvement. (c) The five-molecule panel for patients with well/moderately or poorly differentiated tumours. (d) The five-molecule panel for patients with or without adjuvant chemotherapy.

($P_{(interaction)}$=0.05) cohorts. Also, we found that neither grade ($P_{(interaction)}$=0.7), T ($P_{(interaction)}$=0.36), nor N categories ($P_{(interaction)}$=0.06), showed significant interactions with ACT. All the results that confirmed each other suggested that the five-molecule panel might be helpful for discriminating patients who could benefit more from ACT.

4. Discussion

Because of limited performance and inconsistent predictive values of clinicopathologic variables for survival [5,6], several molecular prognostic markers have been identified for PDAC [3,18]. In addition, signatures or scores that integrate multiple molecules to enhance the predictive power for prognosis have been reported in some cancer types [7,8]. In PDAC, some signatures or scores have also been reported. However, the majority were conducted using small-scale cohorts [9,19]. These signatures may be subject to low reproducibility. Also, messenger RNA (mRNA)-based prognostic markers might not be efficient at the protein level because of post-transcriptional modifications [20]. Furthermore, whether these signatures or scores are of greater prognostic significance than clinicopathological parameters remains unclear and their predictive roles for the response to ACT have rarely been elucidated. Herein, we proposed a novel network-based strategy for the identification of tumour-related prognostic markers, which integrated prognostic evidence from omics data and literatures according to network-based predictions and network modular associations.

We generated a five-molecule panel (CAPN2, DVL1, FLNA, GLI1 and SHH), which successfully stratified prognosis in all cohorts. Moreover, this panel was the only factor that was significant in the multivariate analysis for both development and validation cohorts following adjustment for general and tumour-related parameters. It was also found to be positively associated with survival in most subgroups stratified according to tested variables, thus carrying substantial prognostic value. These data also suggested that the panel had strong and reproducible prognostic value. In addition, this panel showed superiority to its individual constituents and some clinicopathological characteristics, suggesting the necessity of its construction. More importantly, it was shown that combination of the panel and both combined and individual clinicopathological factors could markedly enhance their predictive powers. This has overcome its

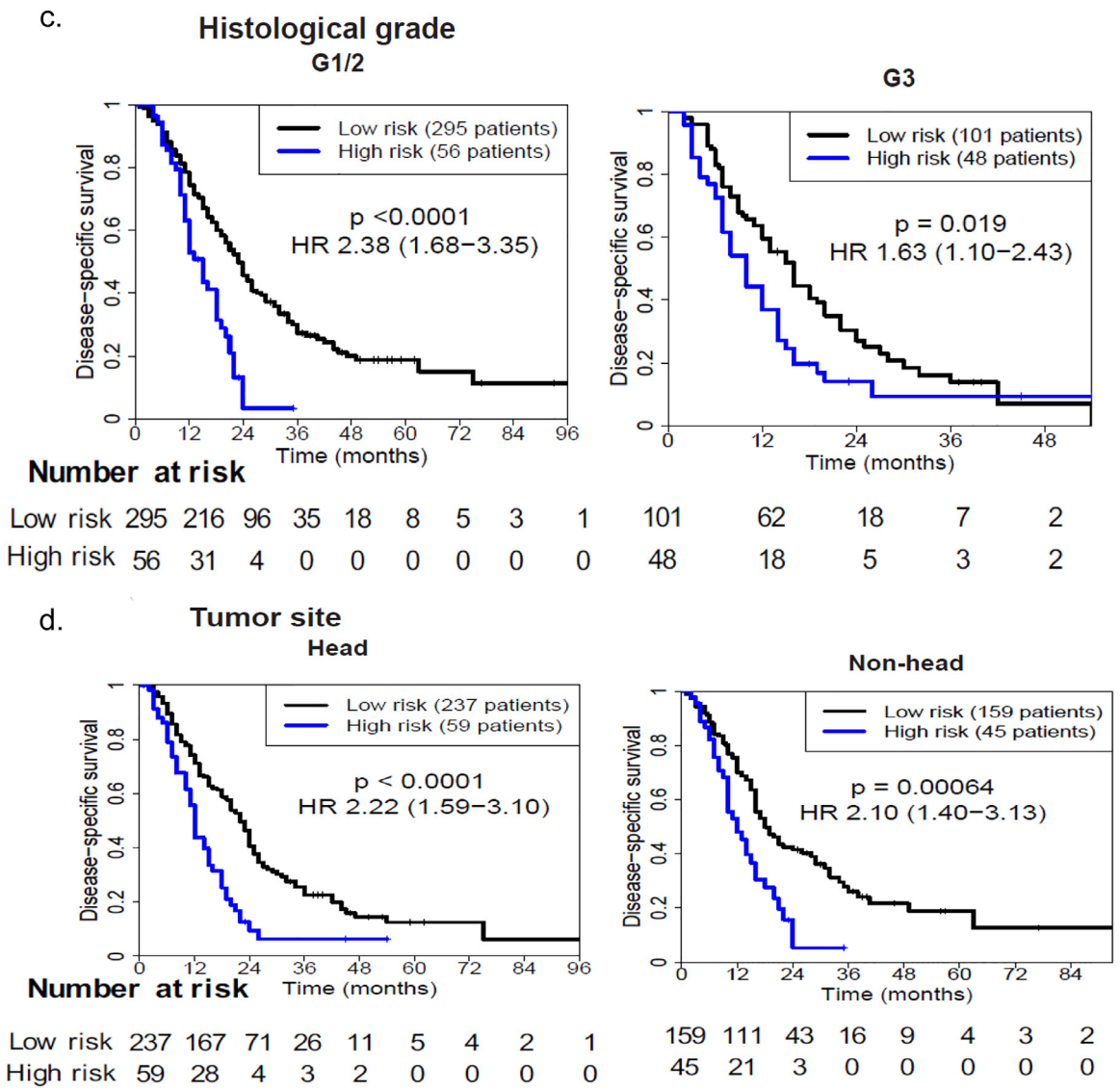

Fig. 4 Continued.

unsatisfactory efficiencies in contrast to some clinicopathological variables. Therefore, the panel, especially the combination, provided a potential as the supplemental prognostic tool for PDAC in the clinic. In particular, the multi-centre design of the study made our results which confirmed each other more solid and confident.

It is no doubt that ACT could substantially improve the survival of PDAC patients after radical resection in well-designed randomized controlled trials (RCTs) [21,22]. The recent RCT also found that as an intensified regimen, Folfirinox showed better efficacy for the resected patients, than gemcitabine alone [4]. However, in the real world clinical practice, it could be different, even if in US or some other economical well developed countries, only half of the patients underwent ACT, and less patients completed six cycles, therefore, in some retrospective studies, ACT did not show survival impact [23,24]. In this study, although ACT did not improved the survival in the whole cohort, however, in the high-risk patients, ACT significantly improved the survival, which indicated these patients were more sensitive to ACT. The high-risk patients had extremely poor survival, thus probably needing intensified ACT more urgently. Although the RCT has demonstrated Folfirinox regimen was better than gemcitabine, grade 3 and 4 side effects were up to 70% [4], which meant not each patient could tolerate or benefit from it. In the real world clinical practice, this panel may help to select patients for intensified ACT. To be frank, it did not mean the low risk patients should not be treated by intensified ACT, or, not all of the high-risk should be treated by intensified ACT, this panel only could be applied as a tool for personalized treatment. Since, this is only a retrospective study, there may be some selective bias which may confound the results and assumptions, further RCT or real world studies are needed to confirm its roles in clinical practice.

Although it was reported that SHH and GLI1 were oncogenic in PDAC [25], the latter predicted favourable survival in this study. Previous papers that showed GLI1-induced inhibition of PDAC progression through mechanisms independent of the Hedgehog pathway in vitro and in vivo provided mechanistic supports [26,27]. Furthermore, it was firstly evident for the prognostic significance of CAPN2, DVL1 and FLNA in PDAC, as preliminarily indicated and being consistent in part with data from other cancers [28,29]. It has been well known that these molecules belong to some pivotal signalling pathways, such as Hedgehog, focal adhesion, TGFβ and Wnt, which are involved in many important biological behaviours of inflammation and cancer [30−33].

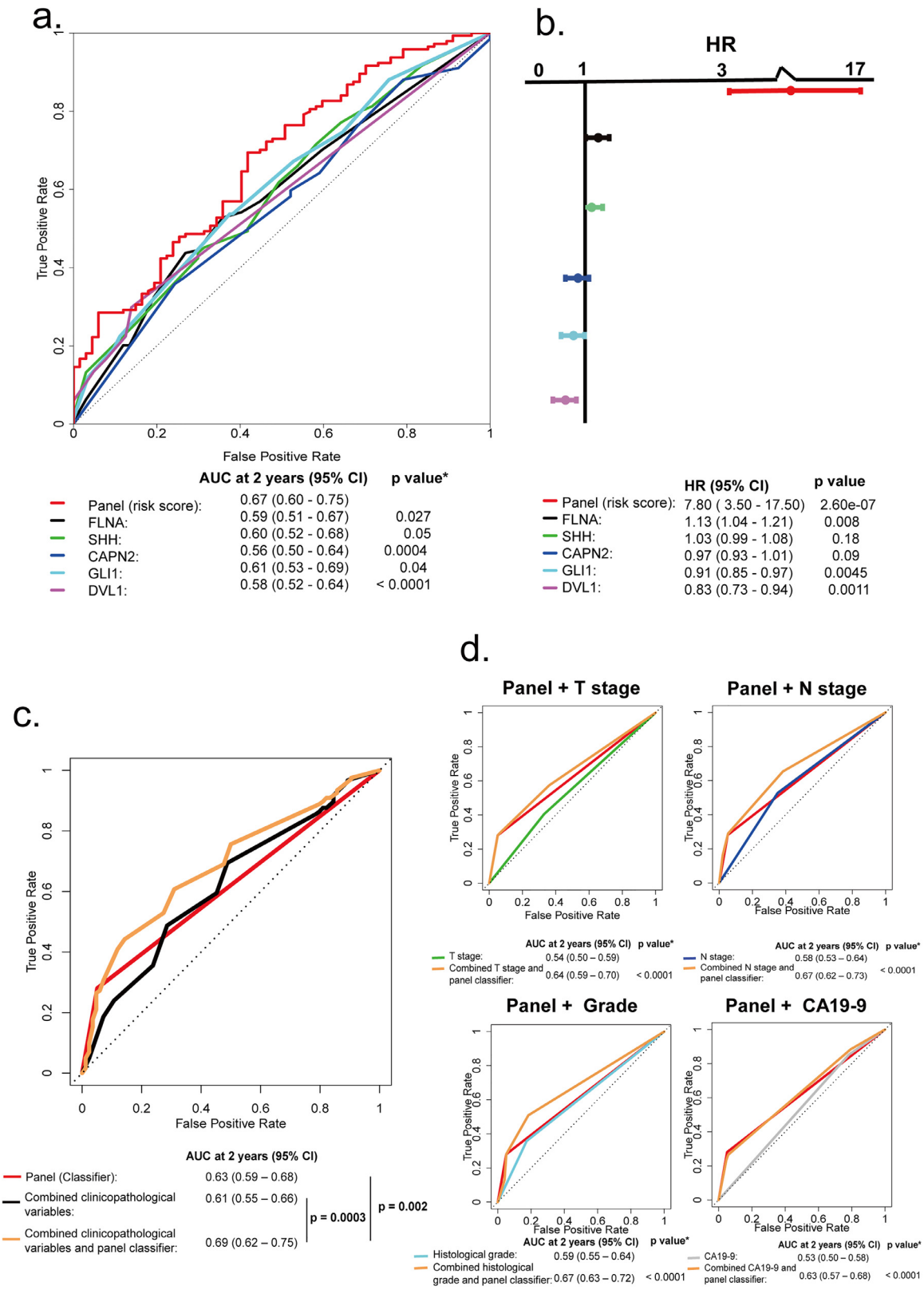

Fig. 5. Comparison of prognostic efficiencies between the five-molecule panel and its individual constituents, as well as clinicopathologic variables. (a) and (b) The 2 year-dependant ROC curves (a) and forest plot (b) of the five-molecule panel (risk score) and single markers in the development cohort. (c) and (d) The 2 year-dependant ROC curves of the five-molecule panel (classifier), and its combination with overall (c) or individual clinicopathological variables (d). *P* value with asterisk denotes the extent to which the predictive power of single component or clinicopathological variable, was lower than that of the five-molecule, and determined based on the bootstrap strategy using R package 'pROC'. HR, hazard ratio.

Therefore, the prognostic significance of these pathway-representative molecules in PDAC might be easily understood. However, why the combination of these molecules has significantly higher prognostic efficiency than the single ones in PDAC, as results from three independent cohorts, remains unclear. In the future, more studies for the related mechanisms might be quite needed.

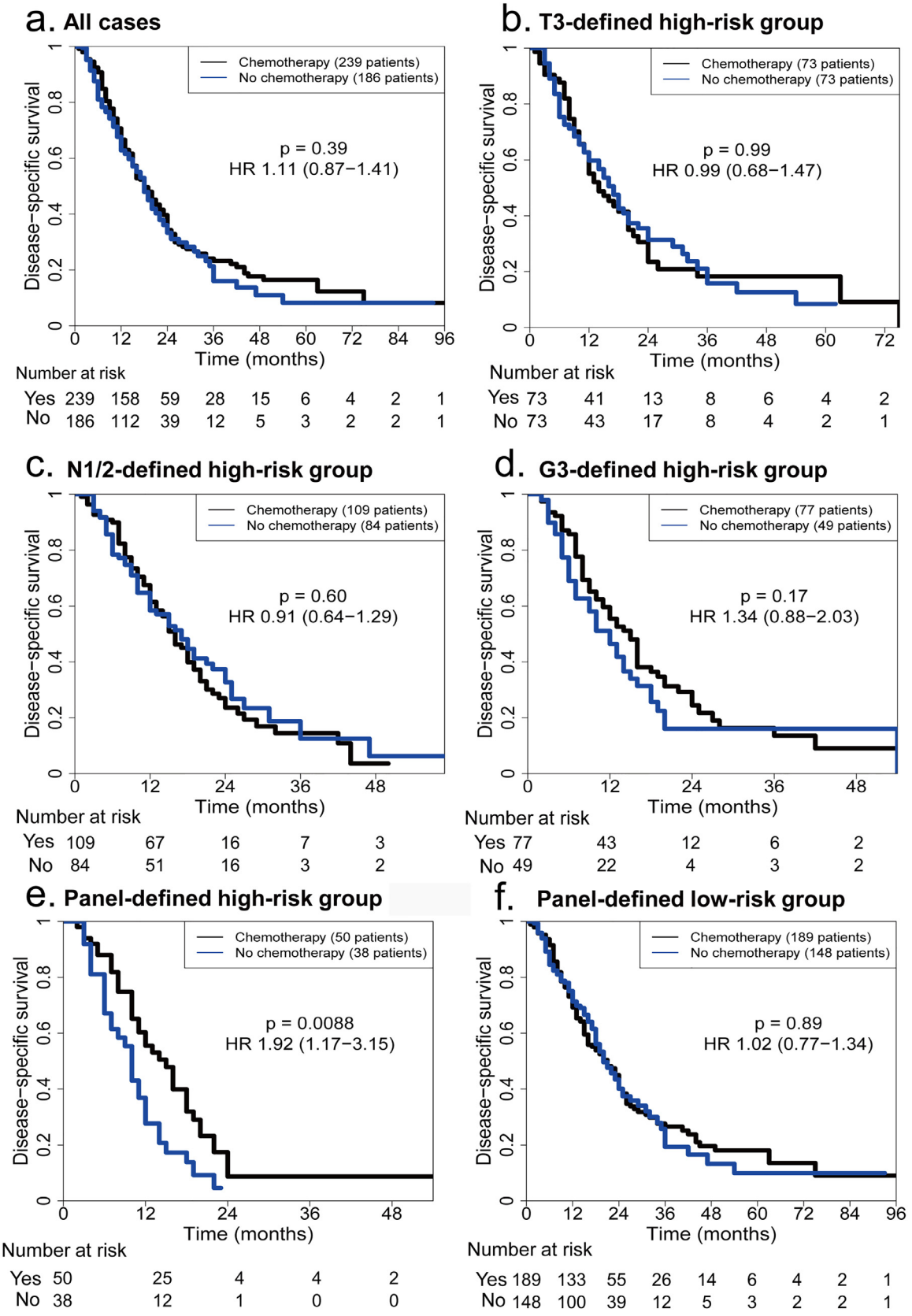

Fig. 6. The value of the five-molecule panel in distinguishing patients who benefit from adjuvant chemotherapy in PDAC. (a) All patients. (b) T3 patients. (c) N1/2 patients. (d) G3 patients. (e) High-risk patients. (f) Low-risk patients.

In conclusion, we developed a novel five-molecule panel using large-scale patient cohorts from three independent centres based on advanced bioinformatics strategy to predict the prognosis of PDAC. Moreover, panel-defined high-risk patients might benefit more from ACT. We believe that this panel might help clinicians to achieve more accurate prognosis prediction and treatment decision for patients with PDAC.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2020.102767.

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