Original research

Enhanced antitumour immunity following neoadjuvant chemoradiotherapy mediates a favourable prognosis in women with resected pancreatic cancer

Casper W F van Eijck ,[1,2] Dana A M Mustafa ,[3] Disha Vadgama,[3] Noel F C C de Miranda ,[4] Bas Groot Koerkamp,[1] Geertjan van Tienhoven,[5] Sjoerd H van der Burg,[6] Núria Malats ,[2] Casper H J van Eijck,[1,2] Dutch Pancreatic Cancer Group (DPCG)

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2023-330480).

For numbered affiliations see end of article.

Correspondence to
Prof. Dr. Casper H J van Eijck, Department of Surgery, Erasmus Medical Center, Rotterdam, South-Holland, Netherlands; c.vaneijck@erasmusmc.nl, Dr. Núria Malats, Genetic and Molecular Epidemiology Group, Spanish National Cancer Research Centre, and CIBERONC, Madrid, Spain; nmalats@cnio.es and Mr. Casper W F van Eijck, Department of Surgery, Erasmus Medical Center, Rotterdam, The Netherlands; c.w.f.vaneijck@erasmusmc.nl

NM and CHJvE are joint senior authors.

Received 12 June 2023
Accepted 1 August 2023

To cite: van Eijck CWF, Mustafa DAM, Vadgama D, *et al*. *Gut* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/gutjnl-2023-330480

ABSTRACT

Background This study investigates sex disparities in clinical outcomes and tumour immune profiles in patients with pancreatic ductal adenocarcinoma (PDAC) who underwent upfront resection or resection preceded by gemcitabine-based neoadjuvant chemoradiotherapy (nCRT).

Methods Patients originated from the PREOPANC randomised controlled trial. Upfront surgery was performed in 82 patients, and 66 received nCRT before resection. The impact of sex on overall survival (OS) was investigated using Cox proportional hazards models. The immunological landscape within the tumour microenvironment (TME) was mapped using transcriptomic and spatial proteomic profiling.

Results The 5-year OS rate differed between the sexes following resection preceded by nCRT, with 43% for women compared with 22% for men. In multivariate analysis, the female sex was a favourable independent prognostic factor for OS only in the nCRT group (HR 0.19; 95% CI 0.07 to 0.52). Multivariate heterogeneous treatment effects analysis revealed a significant interaction between sex and treatment, implying increased nCRT efficacy among women with resected PDAC. The TME of women contained fewer protumoural CD163+MRC1+M2 macrophages than that of men after nCRT, as indicated by transcriptomic and validated using spatial proteomic profiling.

Conclusion PDAC tumours of women are more sensitive to gemcitabine-based nCRT, resulting in longer OS after resection compared with men. This may be due to enhanced immunity impeding the infiltration of protumoral M2 macrophages into the TME. Our findings highlight the importance of considering sex disparities and mitigating immunosuppressive macrophage polarisation for personalised PDAC treatment.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer death, ranking third in the USA and Europe.[1 2] Diagnosis commonly occurs when the disease has already reached an advanced stage, limiting the possibility of curative resection to around 15% of cases. Even after successful resection, the 10-year overall survival (OS) rate remains less than 4%.[3] For years, the standard treatment to improve survival was upfront surgery followed by adjuvant chemotherapy.[4–9] However, not all patients who undergo surgery receive recommended adjuvant chemotherapy due to postoperative surgical complications, clinical deterioration, or early disease recurrence.[10–12] Neoadjuvant therapy has gained interest in treating patients with resectable and borderline resectable PDAC with the aim of improving survival and increasing resection rates, particularly in the latter group.[12 13] The Dutch PREOPANC trial was the first multicentre phase III randomised controlled trial (RCT) to demonstrate the benefits of neoadjuvant chemoradiotherapy (nCRT) in patients with (borderline) resectable PDAC. Patients who received gemcitabine-based nCRT prior to surgery had a 5-year OS rate of 20.5% vs. 6.5% in patients who underwent upfront resection.[14] Notably, stratified analyses based on resectability status revealed that the benefits of nCRT were predominantly observed in borderline resectable rather than resectable PDAC patients.

Although the use of neoadjuvant therapy in borderline resectable tumours is acknowledged, the question of whether patients with resectable tumours should receive neoadjuvant therapy remains unresolved.[12 13 15] Ongoing and recently completed randomised trials are crucial in answering this question and determining the optimal treatment approach in the neoadjuvant setting.[16 17] Furthermore, comprehending the impact of neoadjuvant therapy on the immunological, molecular and biological landscape of PDAC tumours is essential for facilitating tailored treatment approaches and improving therapeutic efficacy.[13]

The beneficial effects of nCRT may stem from restoring a potent antitumour immune response rather than cytotoxic effects alone.[18 19] Gemcitabine-based nCRT or its components have been shown to deplete various immune cells in the tumour microenvironment (TME) of PDAC associated with poor survival and the activation of immune cells with

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Gemcitabine-based neoadjuvant chemoradiotherapy (nCRT) can improve overall survival in patients with resected pancreatic ductal adenocarcinoma (PDAC).
⇒ Gemcitabine-based nCRT has immunomodulatory properties that aid in restoring a potent antitumour immune response, potentially resulting in increased sensitivity to nCRT in women with a generally more robust immune response than men.
⇒ Novel immune profiling technologies that preserve tissue context enable the investigation of the immunological landscape within distinct compartments of the tumour microenvironment.

WHAT THIS STUDY ADDS

⇒ The overall survival of women with resected PDAC who received gemcitabine-based nCRT was significantly longer than that of men receiving the same treatment.
⇒ Multivariate heterogeneous treatment effect analysis, allowed by the randomised controlled design of our study, showed an increased efficacy of gemcitabine-based nCRT in women with resected PDAC compared with men.
⇒ We unveiled that PDAC tumours of women exhibit a distinct transcriptomic response to gemcitabine-based nCRT, which enhances tumour immunity by, among other mechanisms, inhibiting M2 macrophage polarisation.
⇒ Genomic and spatial proteomic immune profiling validated that the infiltration of M2 macrophages into PDAC tumours is reduced in women compared to men following gemcitabine-based nCRT.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Women with (borderline) resectable PDAC benefit more from gemcitabine-based nCRT than men, emphasising the importance of integrating sex-specific considerations in therapeutic decision-making.
⇒ Our findings suggest that interventions aimed at modulating M2 macrophage polarisation hold promise to enhance the efficacy of gemcitabine-based nCRT and improve survival outcomes.

antitumour abilities.[20–23] Multiple studies have consistently reported better response rates and prolonged survival among women undergoing anticancer treatments, including nCRT.[24–29] Initially, these beneficial outcomes were ascribed to reduced exposure to risk factors or enhanced pharmacokinetic drug handling. However, accumulating evidence underscores the role of sex-dependent immunity. Women exhibit more robust innate and adaptive immune responses than men, potentially resulting in superior antitumour immunity following anticancer treatment.[27 30] Moreover, oestrogen signalling can modulate the TME, antigen presentation, immune checkpoint expression and infiltration of lymphocytes within the tumour.[31 32] Intriguingly, the PREOPANC intention-to-treat analysis revealed that gemcitabine-nCRT might be particularly effective in women compared with men with PDAC, although the statistical significance of the survival interaction models was not reached.

Given these findings, we hypothesised that gemcitabine-based nCRT triggers a more effective antitumour immune response in women than men, resulting in better survival outcomes for women with resected PDAC. To test this hypothesis, we conducted a comprehensive survival analysis that investigated the impact of sex on survival outcomes in patients with resected PDAC who received gemcitabine-based nCRT or underwent upfront surgery as part of an RCT. Furthermore, we mapped the sex-specific immune alterations in the different compartments of the TME by combining transcriptomic and spatial proteomic profiling. By elucidating immunological processes associated with the survival of patients with resected PDAC following gemcitabine-based nCRT, we provide insights for developing more effective and personalised treatment strategies.

METHODS

A schematic overview of the methodological steps can be found in figure 1.

Patient population and clinical procedure

This study included patients with resected PDAC originating from the multicentre phase III randomised controlled PREOPANC trial (EudraCT 2012-003181-40), of which the results have been published previously.[14] This study randomly assigned patients (1:1) to receive either gemcitabine-based nCRT or upfront surgery. The inclusion and exclusion criteria and treatment schedules can be found in the PREOPANC study protocol.[33] Notably, the current study only included patients who underwent surgical resection, had pathologically confirmed PDAC and completed nCRT if applicable.

Resection in the upfront surgery group occurred within 4 weeks after random assignment. Patients in the nCRT group underwent a staging laparoscopy before the treatment started, after which they received three cycles of gemcitabine (1000 mg/m^2) combined with hyperfractionated radiotherapy (36 Gy) in 15 fractions during the second cycle. Resection occurred 4–6 weeks after nCRT. Resection in both groups was performed only if no metastases or locally unresectable diseases were found. Following the consensus statement of the International Study Group on Pancreatic Surgery,[34] a pancreatoduodenectomy with locoregional lymph node dissection was performed for tumours in the pancreatic head, while tumours in the body or tail were resected using a pancreas body or tail resection with a splenectomy. Adjuvant gemcitabine (1000 mg/m^2) was administered within 12 weeks after surgery, with four cycles for the nCRT group and six cycles for the upfront surgery group.

The primary survival outcome in this study was OS, defined as the time between diagnosis (ie, histologically or cytologically confirmed PDAC) and death. Patients who were alive at the last follow-up were censored. This endpoint since diagnosis reflects the comprehensive impact of nCRT, diagnostic or staging procedures, surgical resection, and the natural history or other prognostic factors on patient survival.

Targeted multiplex gene expression profiling

Tumour tissue samples were obtained by surgical resection, after which they were formalin-fixed paraffin-embedded (FFPE). The PDAC surgical specimens of upfront resected and nCRT-treated patients were subjected to transcriptomic profiling using the NanoString nCounter module (NanoString Technologies, Seattle, Washington, USA). Details on RNA isolation and immune profiling protocols are available in online supplemental methods. The tissue RNA was analysed using the PanCancer Immune profiling panel (730 immune-related genes, 40 housekeeping genes) (online supplemental table S1A). Normalised and log2 transformed expression data were extracted from the NanoString nSolver software for downstream analysis.

Gene expression data analysis

Genes that were differentially expressed ($P<0.05$) between the sexes in any of the treatment groups were subjected to

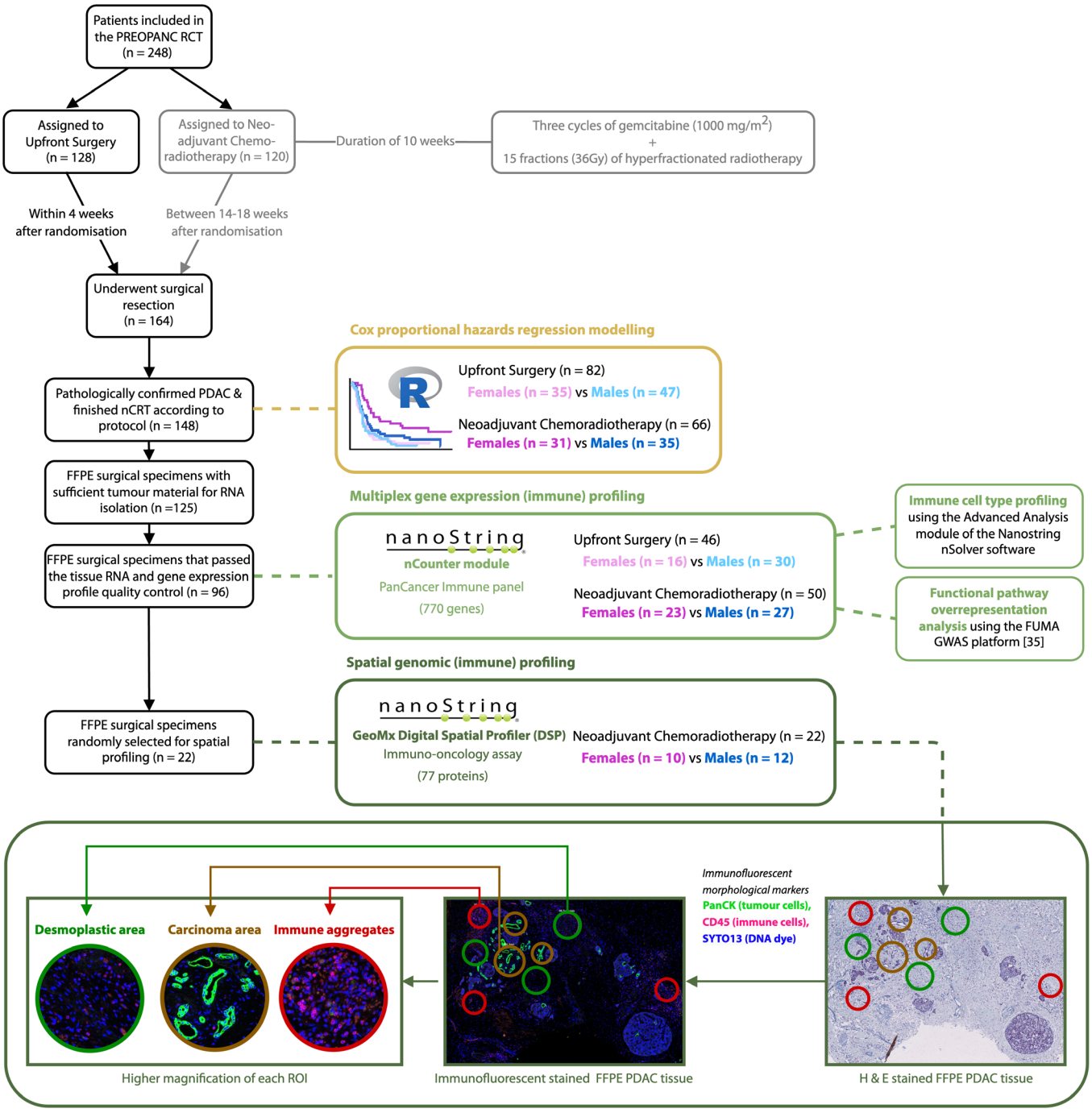

Figure 1 Schematic overview of the methodological steps The squircles illustrate the methodological steps of the study: (1) patient inclusion and clinical procedure, (2) Cox proportional hazards regression modelling, (3) gene expression profiling, and (4) digital spatial profiling (DSP). CD45, cluster of differentiation 45; FFPE, formalin-fixed paraffin-embedded; FUMA GWAS, Functional Mapping and Annotation of the Genome-Wide Association Studies; nCRT, neoadjuvant chemoradiotherapy; ORA, over-representation analysis; PanCK, Pan-cytokeratin; PDAC, pancreatic ductal adenocarcinoma; RCT, randomised controlled trial; ROI, region of interest.

functional pathway over-representation analysis using the Functional Mapping and Annotation of the Genome-Wide Association Studies platform.[35] The over-representation analysis was stratified by treatment, and the canonical pathways C2 collection (BioCarta, KEGG, PID and Reactome) and C5 collection (ontology gene sets) of the Human Molecular Signature Database were analysed.

We developed a genetic signature based on two criteria to map the transcriptomic response to nCRT, specifically in PDAC tumours of females. First, genes that showed significant differential expression between the sexes in the nCRT group ($P<0.05$) were included. Second, genes that exhibited enhanced expression within a similar sex compared with the upfront surgery group were excluded. Notably, the second criterion was applied irrespective of the statistical significance of the differential expression between the sexes in the upfront surgery group, which enhanced the robustness of the genetic signature.

The tumour immune cell infiltration was quantified using the Advanced Analysis module of the nSolver NanoString software. This module assigns cell type scores to each sample using marker genes from the PanCancer panel, specifically tailored to represent cell types in PDAC.[36] Marker genes were accepted to define an immune cell type if the pairwise similarity between all genes representing the cell type was sufficient ($R^2 \geq 0.6$) (online supplemental table S2).

GeoMx digital spatial profiling

FFPE tissue samples from PDAC patients who received nCRT were subjected to multiregional protein immune profiling using the NanoString GeoMx Digital Spatial Profiler (DSP).[37] The selection of samples was based on their availability. The GeoMx immuno-oncology protein panel (78 targets, including 2 housekeeping and 3 negative control targets) was used to map the sex-specific immunological changes across the different TME compartments (online supplemental table S1B). The selection of regions of interest (ROIs) was guided by morphological markers for tumour cells (Pan-cytokeratin; PanCK) and immune cells (CD45) accompanied by a DNA dye (SYTO13) to confirm that the selected ROIs contained nuclei. To account for intratumoural heterogeneity, three ROI replicas for each histological area were selected for each patient by a pathologist. Histological areas included carcinoma (PanCK-rich), desmoplasia (PanCK-absent) and immune aggregates (CD45-rich). Details on the generation and processing of the DSP data are available in online supplemental methods. Normalised data were exported from the GeoMx DSP Analysis Suite for downstream analysis.

Data exploration using dimension reduction

Dimension reduction of the gene expression data and spatial proteomic data was performed using t-Distributed Stochastic Neighbour Embedding (t-SNE) optimised with hyperparameters ('perplexity'=20, 'max_iter'=5000 and 'theta'=0). This technique visualised the high-dimensional datasets in a two-dimensional space but preserved the local structure of the data points, allowing us to investigate if the composition of the TME drove any clustering. The in-between-group comparison (women vs. men) could mask such clustering if the TME of some men behaved like that of women or vice versa.

Statistical analyses

Downstream statistical analyses and visualisations for all datasets were performed in R Statistical Software (V.4.1.2), and details are available in online supplemental methods. P values were adjusted (P.adj) for multiple hypothesis testing by calculating the false discovery rate using the Benjamini-Hochberg correction. P.adj<0.05 was considered statistically significant, except for pathways found differentially altered if P.adj<0.01. P values are indicated as follows: *P.adj<0.05, **P.adj<0.01, ***P.adj<0.001.

RESULTS

Patient characteristics

Among the 248 (borderline) resectable PDAC patients who participated in the phase III PREOPANC RCT between April 2013 and July 2017, 164 underwent surgical resection. For this study, we included 148 patients after excluding 12 patients without pathologically confirmed PDAC and 4 patients who did not complete the full course of gemcitabine-based nCRT. Further details on the clinical characteristics of the additional 16 excluded patients can be found in online supplemental table S3. Upfront surgery was performed in 82 patients (35 females and 47 males), and nCRT before surgery was administered in 66 patients (31 females and 35 males) (figure 1). There were no significant sex-based differences in preoperative clinical characteristics or postoperative pathological and surgical outcomes within the treatment groups (tables 1 and 2).

Women with resected PDAC exhibit prolonged OS compared with men following surgical resection preceded by gemcitabine-based CRT

After a median follow-up of 73 months, 32 (91%) females and 44 (94%) males in the upfront surgery group, and 20 (65%) females and 29 (83%) males in the nCRT group had died. Sex disparities in survival outcomes were evident among patients who received nCRT (figure 2A). Females had a median OS of 48 months (95% CI 6 to 108) with a 5-year OS rate of 43% (95% CI 29% to 65%), whereas males had a median OS of 18 months (95% CI 1 to 96) with a 5-year OS rate of 22% (95% CI 12% to 42%). In contrast, these survival outcomes were comparable between the sexes in the upfront surgery (figure 2A). Females and males in this group had a median OS of 17 months (95% CI 0 to 88) and 18 months (95% CI 1 to 71), with 5-year OS rates of 11.4% (95% CI 4.54% to 28.7%) and 6.38% (95% CI 2.14% to 19.1%), respectively.

Univariate Cox regression analyses stratified by treatment showed that the female sex was associated with prolonged OS in the nCRT group (HR 0.44; 95% CI 0.25 to 0.79; P.adj=0.017) but not in the upfront surgery group (HR 1.00; 95% CI 0.63 to 1.59; P.adj=0.99) (figure 2A). Univariate analysis in the entire cohort identified four potential confounders, including the number of adjuvant gemcitabine cycles, nodal status (N), resection classification (R) and tumour stage (T) (online supplemental table S4A). After correcting for these potential confounders, multivariate Cox regression analyses stratified by treatment revealed that the female sex remained a favourable prognostic factor for prolonged OS in the nCRT group (HR 0.44; 95% CI 0.24 to 0.81; P.adj=0.034), but not in the upfront surgery group (HR 1.22; 95% CI 0.76 to 1.96; P.adj=0.51) (figure 2B).

Importantly, the influence of sex on the effect of nCRT followed by resection versus resection alone on OS was evaluated using heterogeneous treatment effect analyses. Unstratified Cox regression interaction models showed a significant interaction between sex and treatment in univariate analysis (figure 3A). After adjusting for the potential confounders, multivariate analysis revealed an increasing benefit of nCRT in women with resected PDAC (HR 0.35; 95% CI 0.19 to 0.66; P=0.010) (figure 3B). Additionally, survival analyses for progression-free survival yielded comparable results (online supplemental figure S1, online supplemental tables S4B, S4D).

Transcriptomic alterations in PDAC tumours of women in response to gemcitabine-based CRT promote anticancer (immunological) properties

Among the 148 PDAC patients included in our study, the tumour material of 125 surgical specimens was suitable for RNA isolation. Following quality control of tissue RNA and raw gene expression profiles, the surgical specimens of 46 upfront surgery patients (16 females and 30 males) and 50 gemcitabine-based nCRT-treated patients (23 females and 27 males) were included in the transcriptomic immune profiling analyses (figure 1).

Preoperative clinicopathological characteristics and postoperative outcomes of the patient subset included in the transcriptomic analyses are provided in online supplemental table S5. Univariate (figure 4A) and multivariate (figure 4B) Cox

Table 1 Preoperative clinical characteristics of the 148 included patients with resected PDAC

Treatment group	Upfront surgery			nCRT			All patients	
Sex	Female (n=35)	Male (n=47)	P value	Female (n=31)	Male (n=35)	P value	Female (n=66)	Male (n=82)
Age at diagnosis, years								
Median (min, max)	67 (49, 80)	67 (40, 78)	0.15	65 (42, 80)	65 (52, 78)	0.47	66 (42, 80)	67 (40, 78)
BMI, kg/m^2								
Median (min, max)	25 (18, 43)	25 (18, 31)	0.57	25 (19, 44)	25 (20, 32)	0.19	25 (18, 44)	25 (18, 32)
Diabetes mellitus, n (%)								
No	21 (60)	34 (72)	0.38	25 (81)	27 (77)	0.77	46 (70)	61 (74)
Yes	14 (40)	13 (28)		6 (19)	8 (23)		20 (30)	21 (26)
Hypertension, n (%)								
No	26 (74)	35 (75)	>0.99	23 (74)	24 (69)	0.79	49 (74)	59 (72)
Yes	9 (26)	12 (26)		8 (26)	11 (31)		17 (26)	23 (28)
History of cardiovascular disease, n (%)								
No	26 (74)	34 (72)	>0.99	22 (71)	21 (60)	0.44	48 (73)	55 (67)
Yes	9 (26)	13 (28)		9 (29)	14 (40)		18 (27)	27 (33)
History of cancer, n (%)								
No	30 (86)	44 (94)	0.43	25 (81)	32 (91)	0.29	55 (83)	76 (93)
Yes	5 (14)	3 (6)		6 (19)	3 (9)		11 (17)	6 (7)
History of pancreatitis, n (%)								
No	33 (94)	46 (98)	0.81	27 (87)	32 (91)	0.70	60 (91)	78 (95)
Yes	2 (6)	1 (2)		4 (13)	3 (9)		6 (9)	4 (5)
Resectability, n (%)								
Borderline resectable	18 (51)	17 (36)	0.18	14 (45)	13 (37)	0.62	32 (49)	30 (37)
Resectable	17 (49)	30 (64)		17 (55)	22 (63)		34 (51)	52 (63)
CA19-9 preoperative, U/mL								
Median (min, max)	465 (48, 6000)	243 (1, 12 000)	0.63	176 (7, 4040)	207 (2, 4110)	0.69	141 (7, 6000)	222 (1, 12 000)
Missing, n (%)	8 (23)	7 (15)		5 (16)	2 (6)		13 (20)	9 (11)
Involvement of the SMA preoperative, n (%)								
Absent	33 (94)	44 (94)	> 0.99	26 (84)	33 (94)	0.24	59 (89)	77 (94)
Present	2 (6)	3 (6)		5 (16)	2 (6)		7 (11)	5 (6)
Tumour diameter preoperative before nCRT, mm								
Median (min, max)	30 (15, 55)	30 (16, 60)	0.31	29 (13, 50)	32 (15, 64)	0.28	30 (13, 55)	30 (15, 64)
Missing, n (%)	0 (0)	2 (4)		1 (3)	1 (3)		1 (2)	3 (4)
Tumour diameter preoperative after nCRT, mm								
Median (min, max)	30 (4, 55)	30 (16, 60)	0.29	27 (13, 50)	28 (14, 62)	0.62	30 (4, 55)	30 (14, 62)
Missing, n (%)	0 (0)	2 (4)		1 (3)	1 (3)		1 (2)	3 (4)
Regional suspicious lymph nodes preoperative, n (%)								
Absent	27 (77)	35 (75)	0.99	24 (77)	23 (66)	0.42	51 (77)	58 (71)
Present	8 (23)	12 (26)		7 (23)	12 (34)		15 (23)	24 (29)
Tumour location preoperative, n (%)								
Corpus/tail	5 (14)	5 (11)	0.87	6 (19)	6 (17)	>0.99	11 (17)	11 (13)
Head	30 (86)	42 (89)		25 (81)	29 (83)		55 (83)	71 (87)
WHO performance status preoperative, n (%)								
WHO 0	14 (40)	13 (28)	0.34	19 (61)	20 (57)	0.80	33 (50)	33 (40)
WHO 1	20 (57)	32 (68)		11 (35)	14 (40)		31 (47)	46 (56)
Missing	1 (3)	2 (4)		1 (3)	1 (3)		2 (3)	3 (4)
Response to nCRT (RECIST 1.1), n (%)								
Partial response	0 (0)	0 (0)	–	2 (6)	4 (11)	0.89	3 (5)	5 (6)
Stable disease	0 (0)	0 (0)		20 (65)	23 (66)		22 (33)	27 (23)
Progressive disease	0 (0)	0 (0)		2 (6)	3 (9)		2 (3)	3 (4)
Missing	35 (100)	47 (100)		7 (23)	5 (14)		39 (59)	47 (57)
Institute, n (%)								
Academical hospital	26 (74)	33 (70)	0.88	23 (74)	26 (74)	>0.99	49 (74)	59 (72)
General hospital	9 (26)	14 (30)		8 (26)	9 (26)		17 (26)	23 (28)

BMI, body mass index; nCRT, neoadjuvant chemoradiotherapy; PDAC, pancreatic ductal adenocarcinoma; RECIST, response evaluation criteria in solid tumours; SMA, superior mesenteric artery.

Table 2 Postoperative clinical, surgical and pathological outcomes of the 148 included patients with resected PDAC

Treatment group	Upfront surgery			Neoadjuvant chemoradiotherapy			All patients	
Sex	Female (n=35)	Male (n=47)	P value	Female (n=31)	Male (n=35)	P value	Female (n=66)	Male (n=82)
Nodal status (N) postoperative, n (%)								
N0	9 (26)	6 (13)	0.23	21 (68)	23 (66)	>0.99	30 (46)	29 (35)
N1	26 (74)	41 (87)		10 (32)	12 (3)		36 (55)	53 (65)
Perineural invasion postoperative, n (%)								
Absent	8 (23)	6 (13)	0.38	13 (42)	19 (54)	0.45	21 (32)	25 (30)
Present	26 (74)	39 (83)		16 (52)	14 (40)		42 (64)	53 (65)
Missing	1 (3)	2 (4)		2 (6)	2 (6)		3 (4)	4 (5)
Resection classification (R) postoperative, n (%)								
R0	15 (43)	20 (43)	>0.99	22 (71)	26 (74)	0.79	37 (56)	46 (56)
R1	20 (57)	27 (57)		9 (29)	9 (26)		29 (44)	36 (44)
SAE reported (any grade), n (%)								
No	17 (49)	29 (62)	0.38	12 (39)	13 (37)	>0.99	29 (44)	42 (51)
Yes	18 (51)	18 (38)		19 (61)	22 (63)		37 (56)	40 (49)
Tumour grade, postoperative, n (%)								
Moderately differentiated	17 (49)	26 (55)	0.96	13 (42)	15 (43)	0.78	30 (45)	41 (50)
Poorly differentiated	7 (20)	11 (23)		11 (35)	8 (23)		18 (27)	19 (23)
Well differentiated	5 (14)	4 (9)		2 (7)	4 (11)		7 (11)	8 (10)
Missing	6 (17)	6 (13)		5 (16)	8 (23)		11 (17)	14 (17)
Tumour stage (T) postoperative, n (%)								
T1/T2	1 (3)	1 (2)	0.10	11 (36)	6 (17)	>0.99	12 (18)	7 (9)
T3/T4	34 (97)	46 (98)		20 (65)	29 (83)		54 (82)	75 (92)
Type of resection, n (%)								
Pancreas body and tail resection	1 (3)	2 (4)	0.39	4 (13)	4 (11)	0.85	5 (8)	6 (7)
Pancreatoduodenectomy	31 (89)	44 (94)		26 (84)	31 (89)		57 (86)	75 (92)
Total pancreatectomy	3 (9)	1 (2)		1 (3)	0 (0)		4 (6)	1 (1)
Vascular invasion postoperative, n (%)								
Absent	15 (43)	14 (30)	0.48	19 (61)	20 (57)	0.8	34 (52)	34 (41)
Present	20 (57)	29 (62)		11 (36)	14 (40)		31 (47)	43 (53)
Missing	0 (0)	4 (8)		1 (3)	1 (3)		1 (1)	5 (6)
Adjuvant gemcitabine completed, n (%)								
No	25 (71)	30 (64)	0.63	20 (65)	25 (71)	0.60	45 (68)	55 (67)
Yes	10 (29)	17 (36)		11 (36)	10 (29)		21 (32)	27 (33)
Adjuvant gemcitabine cycles								
Median (min, max)	4 (0, 6)	4 (0, 6)	0.63	3 (0, 4)	2 (0, 4)	0.12	3 (0, 6)	3 (0, 6)
OS since diagnosis, months								
Median (min, max)	17 (0, 88)	18 (1, 71)	0.81	48 (6, 108)	18 (1, 96)	**0.002**	29 (0, 108)	18 (1, 96)
PFS since diagnosis, months								
Median (min, max)	12 (0, 85)	12 (1, 71)	0.85	24 (60, 108)	13 (1, 96)	**0.007**	16 (0, 108)	12 (1, 96)
Survival status, n (%)								
Alive	2 (6)	1 (2)	0.66	10 (32)	3 (9)	**0.037**	12 (18)	4 (5)
Alive with disease	1 (3)	2 (4)		1 (3)	3 (9)		2 (3)	5 (6)
Diseased	32 (91)	44 (94)		20 (65)	29 (83)		52 (79)	73 (90)
Disease status, n (%)								
No progression	2 (6)	1 (2)	0.79	9 (29)	3 (9)	0.053	11 (17)	4 (5)
Progression	33 (94)	46 (98)		22 (71)	32 (92)		55 (83)	78 (95)

Bolded P values indicate statistical significance (P < 0.05).
OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; SAE, Serious Adverse Event.

proportional hazards for this patient subset demonstrated results similar to those of the survival analyses in the total cohort of 148 PDAC patients, as did the heterogeneous treatment effects analysis (online supplemental table S6A).

The gene expression data were explored using t-SNE dimensionality reduction analyses. This exploration showed no apparent segregation of patients by sex in the entire cohort (figure 4C) or when stratified by treatment (figure 4D). Nonetheless, several genes were differentially expressed between the sexes (P.adj<0.05), with 18 genes in the upfront surgery group (8 upregulated in females and 10 in males) and 7 genes in the nCRT group (2 upregulated in females and 5 in males) (figure 5A,B and online supplemental table S7).

The differentially expressed genes were subjected to stratified functional pathway over-representation analysis to elucidate sex-specific differences in immune and biological processes across both treatment groups. Following nCRT treatment,

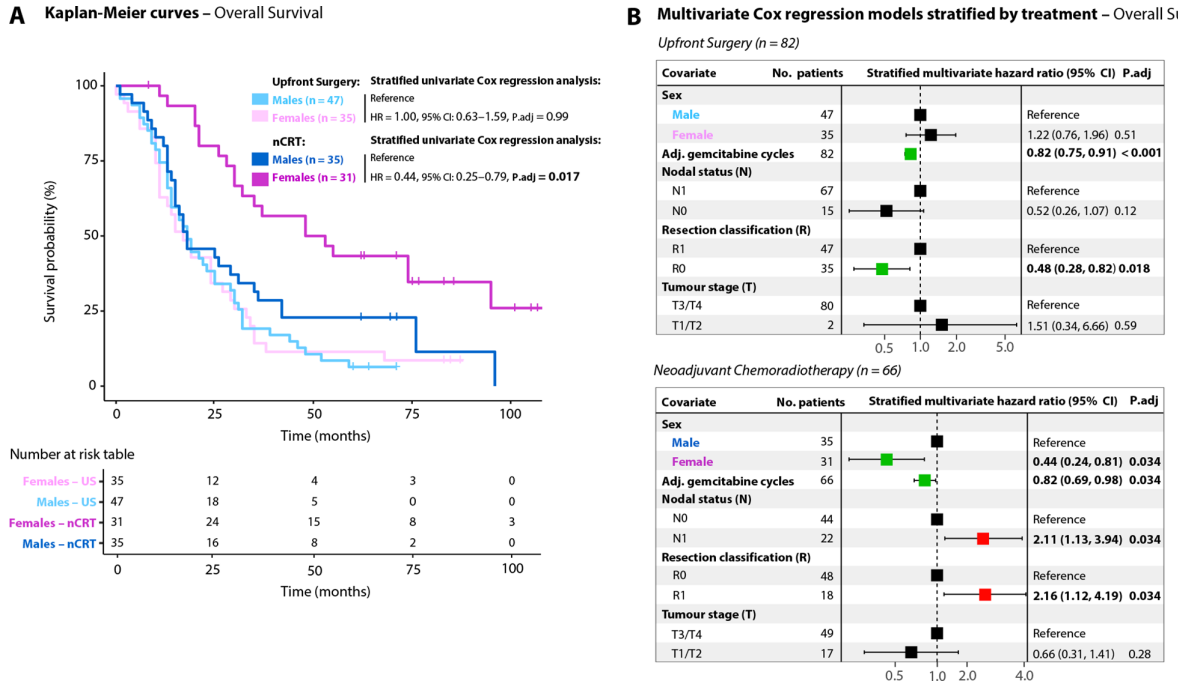

Figure 2 Survival analysis stratified by treatment in patients with resected PDAC who received nCRT or upfront surgery (A) Kaplan-Meier curves and univariate Cox regression models, stratified by treatment, illustrating the significantly prolonged OS in women with resected PDAC who received nCRT compared with men. The x-axis displays the survival time (months) and the y-axis displays the survival probability (%). Cross-symbols denote censored patients. The number-at-risk table provides information on the number of patients at risk of death at each specific time point. (B) Forest plots of the stratified multivariate Cox proportional hazards models illustrate that the female sex is a favourable independent prognostic factor for OS in the nCRT group but not in the upfront surgery group. nCRT, neoadjuvant chemoradiotherapy; OS, overall survival; P.adj, P value adjusted; PDAC, pancreatic ductal adenocarcinoma; US, upfront surgery.

multiple immune activation pathways with gene sets of fewer than 100 were found to be enhanced in female PDAC patients (P.adj<0.01) (online supplemental table S8). The pathways showing the most profound enhancement (ie, pathways with a proportion of overlapping genes of over 3%) were those related to chemokine receptor binding and activity, specifically CXCR3 and lymphocyte chemotaxis (figure 5C). Notably, no pathways were significantly enhanced in the US group or in males of the nCRT group (online supplemental table S8).

We developed a signature to further map the distinct transcriptomic alterations of PDAC tumours of females in response to gemcitabine-based nCRT. Genes that were differentially expressed between the sexes in the nCRT group were included, and genes with enhanced expression within the same sex compared with the upfront surgery group were excluded. Following gemcitabine-based nCRT, PDAC tumours of females exhibited elevated expression of *CXCL10* and *CXCL11* and diminished expression of *CCL2* and *IL34* (P.adj<0.05), associated with the enhancement of antitumoural (immunological) properties (figure 5D).

Gemcitabine-based CRT reduces the infiltration of protumoural M2 macrophages in PDAC tumours of women

Gene expression-based immune profiling revealed that both the total infiltration of immune cells (*PTRPC*+, ie, *CD45*+cells) and the infiltration of immune cell subpopulations were not significantly different between the sexes in both treatment groups (online supplemental figure S2). Interestingly, following nCRT treatment, the abundance of protumourous M2 macrophages (*CD163*+ and *MRC1*+ cells) in the TME of female PDAC patients was lower (P.adj=0.016) than in the TME of males (figure 5E). In women who received nCRT, the number of M2 Macrophages in the TME negatively correlated with OS (Pearson's correlation=−0.6, P=0.003) (figure 5F). However, in the US group or in men who received nCRT, no correlation was observed between the number of M2 macrophages and OS.

To confirm these observations at the protein level, spatial protein immune profiling was performed on 22 surgical specimens from nCRT-treated PDAC patients (10 females and 12 males) (figure 1). Preoperative clinicopathological characteristics and postoperative outcomes of this patient subset are provided in online supplemental table S9. There was no bias in the selection of the ROI used for immune profiling, evident from the fact that data exploration using t-SNE showed no clear segregation of ROIs based on each patient (figure 6A) or based on median OS groups (online supplemental figure S3A). The t-SNE analysis based on sex did not reveal any apparent segregation of ROIs (figure 6A), nor when stratified by the histological area (online supplemental figure S3B). Concordant with these t-SNE results, the expression of immune checkpoints and infiltration of immune cells were comparable between the sexes within the different histopathological areas (online supplemental figures S4, S5). As expected, the t-SNE analysis based on the histological area demonstrated clear segregation of ROIs (figure 6A), irrespective of sex stratification (online supplemental figure S3C).

To account for interpatient variations in immune cell infiltration, we evaluated the marker expression of immune cell subdivisions relative to their respective immune cell compartment. This revealed a sex difference in the proportion of protumourous M2 macrophages (CD163+) within the total macrophage compartment (CD68+). The M2 to total macrophage ratio (CD163+/CD68+) after nCRT was lower in carcinoma areas and immune aggregates of females compared with males (P.

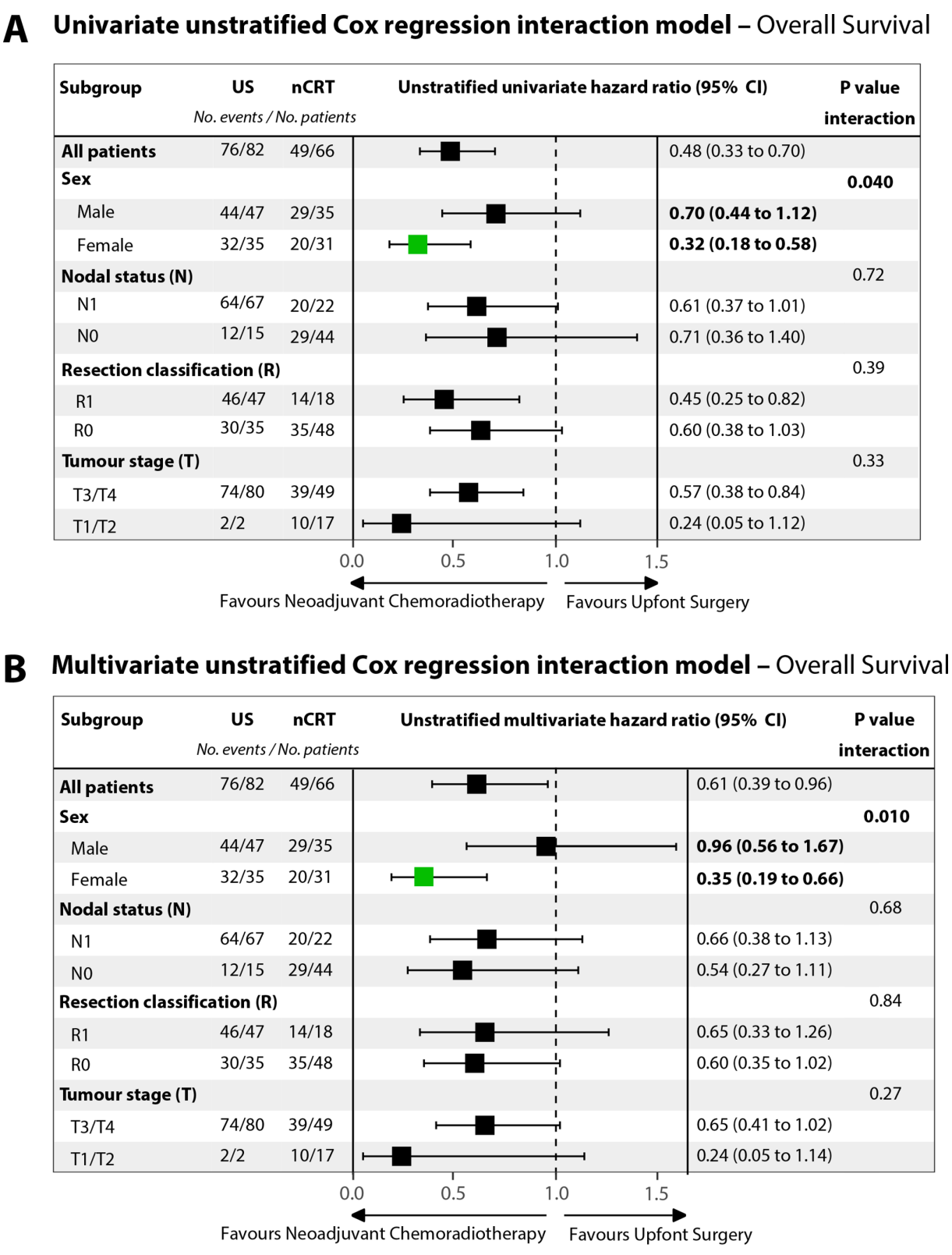

Figure 3 Unstratified heterogeneous treatment effect survival analysis in patients with resected PDAC who received nCRT or upfront surgery forest plots of the univariate (A) and multivariate (B) unstratified Cox proportional hazards interaction models illustrate the impact of nCRT followed by resection versus resection on OS across various subgroups. A statistically significant interaction between the sex and treatment was observed in both univariate and multivariate analysis, with an increased benefit of nCRT in women with resected PDAC. nCRT, neoadjuvant chemoradiotherapy; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; US, upfront surgery.

adj=0.035 and P.adj=0.011, respectively) and, although not statistically significant after correction for multiple testing, a similar trend was observed in areas of desmoplasia (P=0.016) (figure 6B). Importantly, in carcinoma areas of males (Pearson's correlation=–0.6, P=0.02) as well as desmoplasia areas (Pearson's correlation=–0.7, P=0.02) and immune aggregates

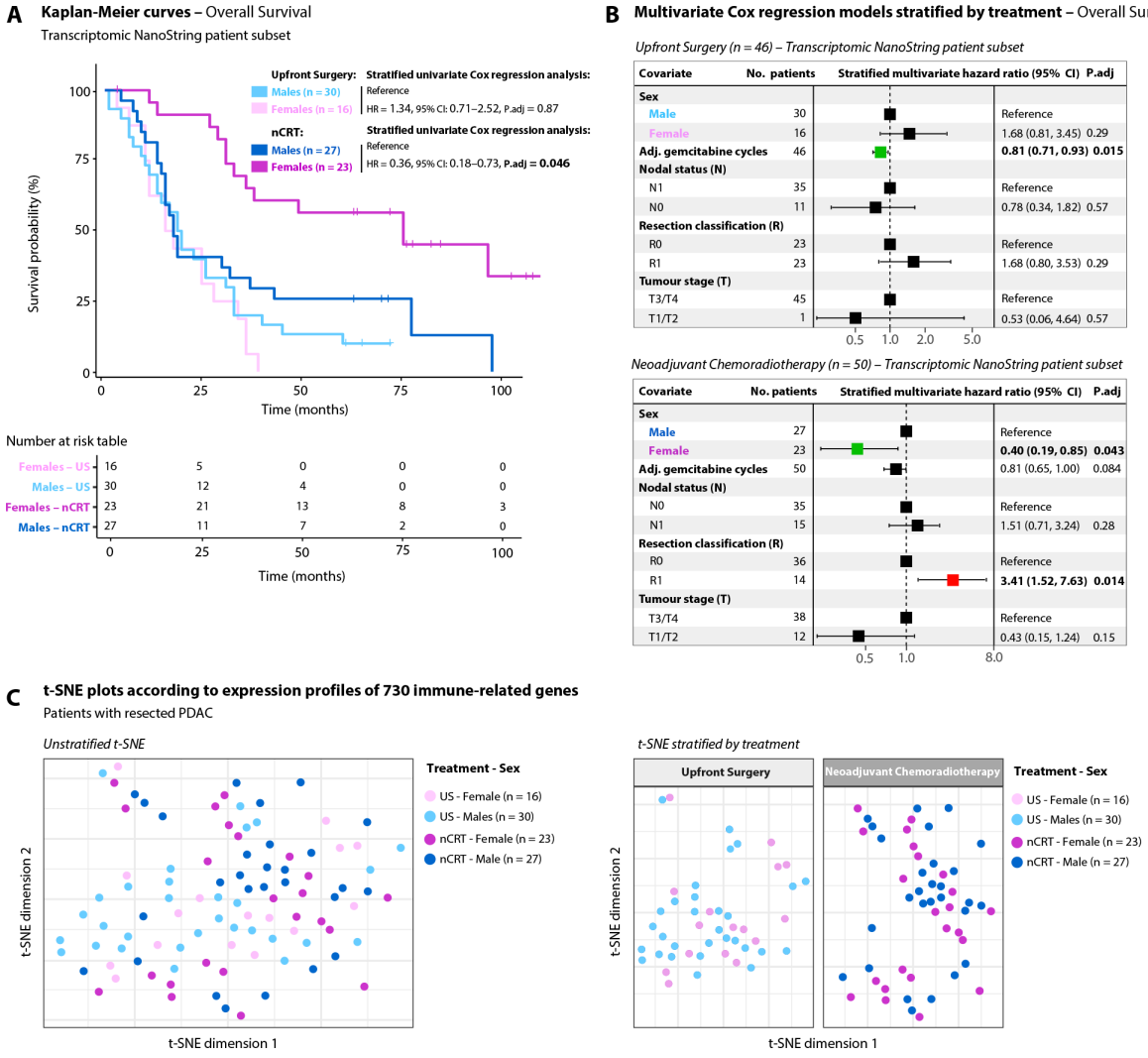

Figure 4 Survival and data exploration analysis in resected PDAC patients included in the transcriptomic Nanostring analysis (A) Kaplan-Meier curves and univariate Cox regression models, stratified by treatment, illustrating the preservation of the significantly prolonged OS in women with resected PDAC who received nCRT compared with men in this patient subset. The x-axis displays the survival time (months), and the y-axis displays the survival probability (%). Crosses denote censored patients. The number-at-risk table provides information on the number of patients at risk of death at each specific time point. (B) Forest plots of the stratified multivariate Cox proportional hazards models illustrate that the female sex remains a favourable independent prognostic factor for OS in the nCRT group but not in the upfront surgery group for this patient subset. (C, D) t-SNE biplots illustrating the expression of 730 immune-related genes reveal no apparent segregation of patients based on sex (C), even after stratifying by treatment (D). Each dot represents a patient, with coordinates depicting the first (x-axis) and second (y-axis) t-SNE dimensions. nCRT, neoadjuvant chemoradiotherapy; OS, overall survival; P.adj, P value adjusted for multiple testing using the Benjamini-Hochberg correction; PDAC, pancreatic ductal adenocarcinoma; t-SNE, t-Distributed stochastic neighbour embedding; US, upfront surgery.

(Pearson's correlation=–0.9, P<0.001) of females, the M2 to total macrophage ratio negatively correlated to OS (figure 6D).

Taken together, these data support the notion that gemcitabine-based nCRT modifies the PDAC TME to favour antitumour immunity, particularly in women. Notably, following nCRT, carcinoma areas of females exhibited elevated levels of the proapoptotic BAD protein compared with those in males (P.adj= 0.04), possibly due to increased immune activity (figure 6C).

DISCUSSION

This study found sex disparities in survival outcomes among patients with resected PDAC who received gemcitabine-based nCRT. While patients who underwent upfront surgery showed no sex-related differences in survival, women who received gemcitabine-based nCRT showed better median OS and 5-year OS rates than men. Multivariate Cox regression analyses confirmed that the female sex was a favourable prognostic factor for OS. Moreover, analysis of heterogeneous treatment effects, allowed by our data's randomised controlled design, demonstrated increased gemcitabine-based nCRT efficacy in women with resected PDAC. These findings collectively provide compelling evidence that differences in gemcitabine-based nCRT sensitivity predominantly drive the survival disparities between the sexes. Consistent with our results, several studies have reported improved survival outcomes in women with various cancers, including PDAC treated with (neoadjuvant) chemotherapy[24–27] or nCRT.[28 29]

Our comprehensive immune profiling analysis revealed reduced immune suppression as a potential mechanistic basis for the increased efficacy of gemcitabine-based nCRT in PDAC

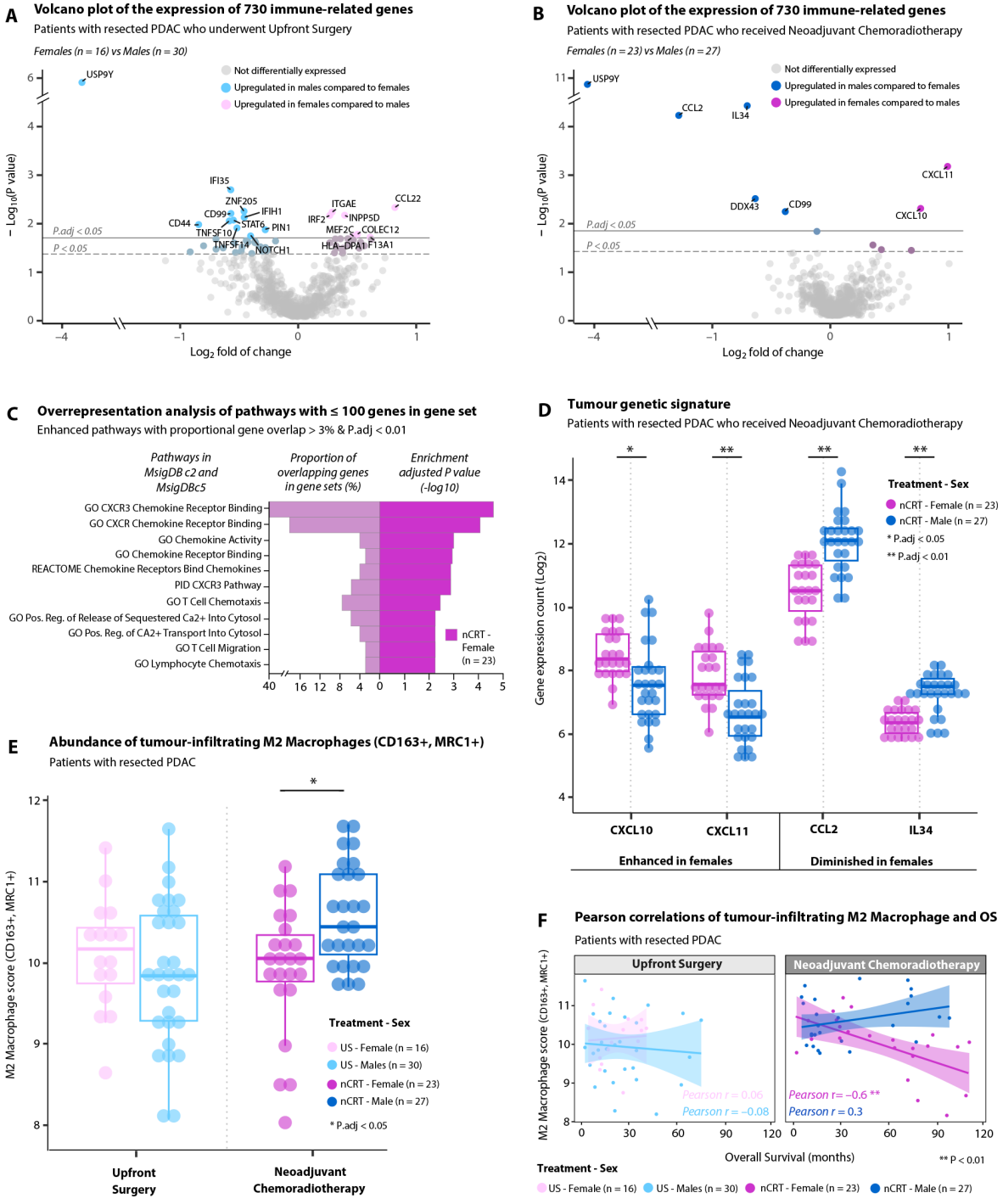

Figure 5 Tumourous transcriptomic NanoString analysis in patients with resected PDAC who received nCRT or upfront surgery (A, B). Volcano plots illustrate the differential gene expression profiles between the sexes in the upfront surgery (A) and nCRT group (B). The x-axis displays the log2 fold of change, while the y-axis displays the –log10 P value. Each dot corresponds to a gene, with genes on the right (positive) upregulated in females compared with male PDAC patients and genes on the left (negative) upregulated in males compared with female PDAC patients. (C) Barplot illustrating the results of the over-representation analysis for pathways characterised by a gene set size of ≤100. Only pathways exhibiting a proportional overlap of ≥3% (displayed on the left side of the x-axis) and a P.adj<0.01 (displayed on the right side of the x-axis) are presented. Each bar represents a specific pathway, and the analysis revealed that the pathways showing significant enhancement were exclusively observed in females (ie, diminished in males) following the administration of gemcitabine-based nCRT. (D) Boxplots illustrating the gene alterations in response to nCRT reveal a signature with antitumourous properties in female PDAC patients. The x-axis displays enhanced (left) and diminished (right) genes in females, and the y-axis displays the log2 gene expression count. (E) Boxplots illustrating the abundance of protumourous M2 macrophages, quantified by *CD163* and *MRC1* expression, reveal significantly lower infiltration in the TME of female PDAC patients than males. The y-axis displays the M2 macrophage score. (F) Scatterplots illustrating Pearson's correlations between the M2 macrophage score (y-axis) and overall survival (OS) in months (x-axis), stratified by treatment groups and sex. A significant association in females who received nCRT can be observed, where a higher number of M2 macrophages in the TME was negatively correlated with OS. In D–F, each dot represents a patient. CD163, cluster of differentiation 163; MRC1, Mannose Receptor C-Type 1; nCRT, neoadjuvant chemoradiotherapy; PDAC, pancreatic ductal adenocarcinoma; US, upfront surgery.

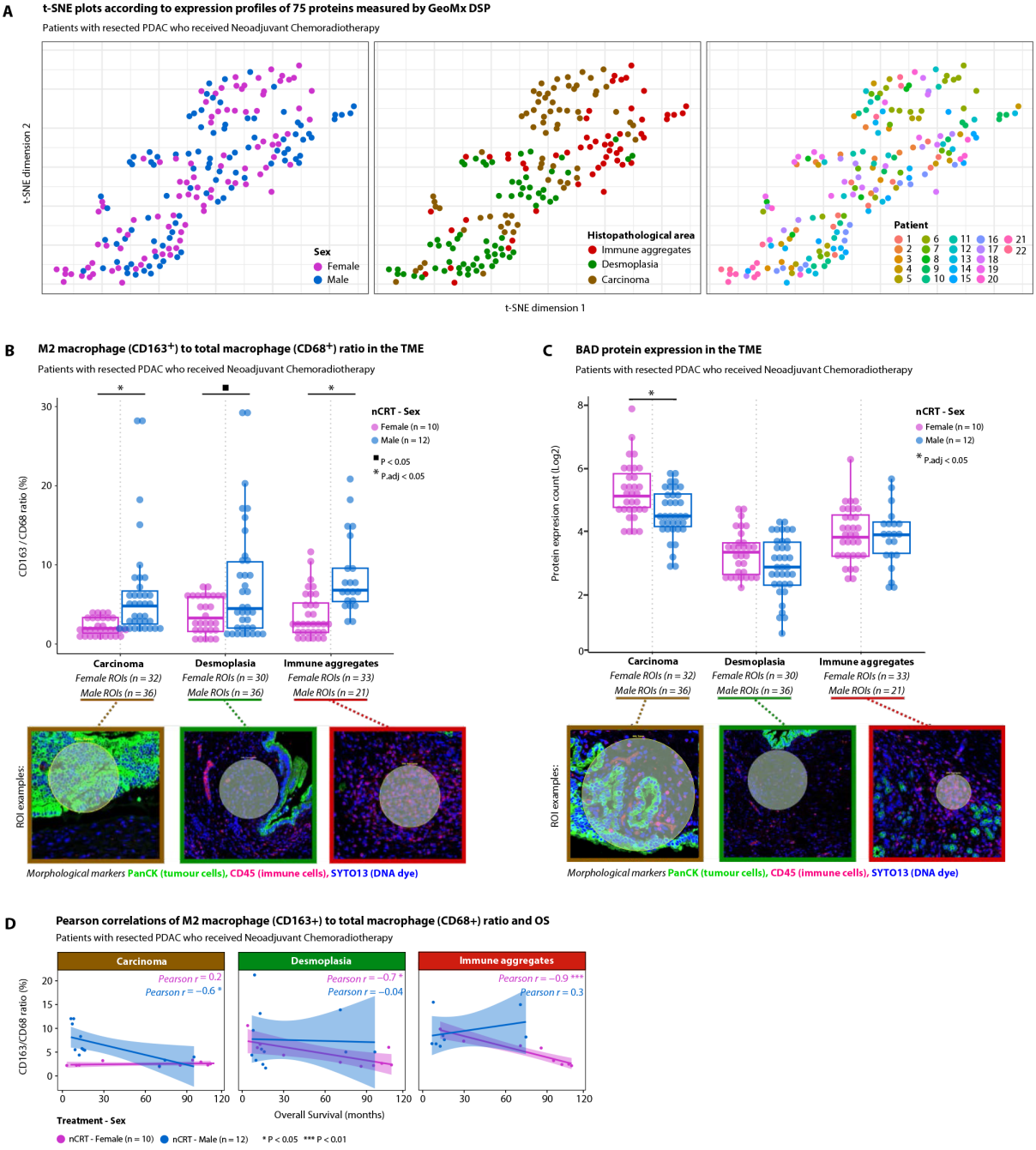

Figure 6 Protein-based GeoMx digital spatial profiling in patients with resected PDAC who received nCRT (A) t-SNE biplot illustrating the expression of 75 proteins reveals no apparent segregation of ROIs by sex or patient, while ROIs clearly segregate by their histological areas. Each dot represents an ROI, with coordinates depicting the first (x-axis) and second t-SNE dimensions (y-axis). (B) Boxplots illustrate the proportion of protumourous M2 macrophages, quantified by CD163 protein expression, within the total macrophage compartment, quantified by CD68 protein expression. Various compartments (x-axis) of the PDAC TME of females show significantly higher CD163 to CD68 (ie, M2 to total macrophage) ratios than males. (C) Boxplots illustrating the BAD protein expression in the PDAC TME reveal significantly higher expression in carcinoma areas of females than in males. The x-axis displays the different TME compartments, and the y-axis displays the Log2 protein expression count. (D) Scatterplots illustrating Pearson's correlations between the CD163 to CD68 ratios (y-axis) and overall survival (OS) in months (x-axis), stratified by sex. A significant association in carcinoma areas of males as well as desmoplasia areas and immune aggregates of females can be observed, where the M2 to total macrophage ratio negatively correlates to OS. In B–D, each dot represents a patient's ROI and immunofluorescent microscopic images below the boxplots in B and C exemplify different TME compartments (ie, histopathological areas) stained with morphological markers for tumour cells (PanCK), immune cells (CD45) and DNA (SYTO13). BAD, BCL2 associated agonist of cell death; CD, cluster of differentiation; nCRT, neoadjuvant chemoradiotherapy; P.adj, P value adjusted for multiple testing using the Benjamini-Hochberg correction; PanCK, Pan-cytokeratin; PDAC, pancreatic ductal adenocarcinoma; ROI, region of interest; TME, tumour microenvironment; t-SNE, t-distributed stochastic neighbour embedding.

tumours of females. Transcriptomic immune profiling unveiled a significant reduction in protumoural M2 macrophages (*CD163*+ and *MRC1*+ cells) within the TME of females with PDAC after gemcitabine-based nCRT compared with males. This finding was validated at the protein level using DSP, allowing us to pinpoint the specific TME compartments where the alterations occurred. Remarkably, all investigated TME compartments of females, including carcinoma areas, desmoplastic areas and immune aggregates, exhibited a significantly lower proportion of infiltrating CD163+M2 macrophages within the total macrophage population compared with these compartments in males. By assessing the M2 to total macrophage ratio, we effectively accounted for interpatient variability in immune infiltration. Moreover, our findings also revealed a negative correlation between the number of M2 macrophages within the TME and OS after gemcitabine-based nCRT. This was predominantly observed in women but also partially in men receiving nCRT.

Our analysis unveiled elevated levels of the proapoptotic BAD protein (BCL-2-associated death promoter) within carcinoma areas of female PDAC patients compared with their male counterparts. This finding is consistent with the observed enhanced efficacy of gemcitabine-based nCRT in female patients, as BAD, acting as an initiator of the intrinsic apoptotic pathway, can induce (cancer) cell death.[38] Furthermore, this pathway is known to be activated by cellular stresses, including treatment with gemcitabine chemotherapy and radiotherapy,[39 40] but also improved immune reactions may trigger this pathway.[41]

Gemcitabine-based nCRT has been shown to deplete immune cells associated with poor survival in cancer, including intratumoural M2 macrophages, myeloid-derived suppressor cells, T regulatory cells (Treg),[20 21] cancer-associated fibroblasts,[42] and immunosuppressive CD19+CD20+B cells.[22] In addition, gemcitabine-based nCRT releases damage-associated molecular pattern molecules, which activate T cells and dendritic cells, and enhance the cytolytic response of CD8+T cells and natural killer cells.[23 43] Notably, a previous study investigating the interplay between nCRT (gemcitabine plus S-1 with 30 Gy radiation therapy), sex, and antitumour immunity in PDAC patients also reported a potential association between improved survival in women and the reduced presence of tumour-associated macrophages in their TME. In that study, a significant reduction in CD204+ macrophages and a trend for reduced CD163+ macrophages were found among women.[28] However, a heterogeneous treatment effects analysis was lacking in this study due to its non-randomised controlled design. In addition, the statistical analyses needed to account for multiple testing and the relatively small sample size of 58 patients raises concerns about potentially overfitting their multivariate analyses that included nine variables. Nonetheless, the fact that two studies found that nCRT reduces CD163+ macrophages has important clinical implications, as these macrophages are known to influence clinical outcomes negatively. Furthermore, they hamper the response to immunotherapy and require specific targeting as they are non-responsive to colony-stimulating factor 1 receptor targeted strategies.[44]

In parallel with the observed differences in immune cell presence, we revealed a tumour transcriptomic response unique to women receiving gemcitabine-based nCRT. This genetic signature consisted of two upregulated (*CXCL10* and *CXCL11*) and two downregulated genes (*CCL2* and *IL34*) in PDAC tumours of females. CXCL10 and CXCL11 are proinflammatory cytokines that promote antitumour immunity[45] and were found to be associated with the prevention of M2 macrophage polarisation in PDAC[46 47] and glioblastoma.[48] In addition, elevated levels of these cytokines were associated with an improved (immunological) response to nCRT in rectal cancer[49] and breast cancer[50] and positively correlated to prolonged survival in PDAC patients treated with chemotherapy.[51] In contrast, elevated serum levels of the cytokine CCL2 were found to be associated with poor PDAC survival,[52] and both *CCL2* and *IL34* promote M2 macrophage polarisation.[53–56] Furthermore, the CCR2-CCL2 axis promotes the recruitment of monocytes to infiltrate tumours, subsequently undergoing further differentiation into M2 macrophages.[57] These alterations in immune modifying and recruitment factors and the decreased presence of M2 macrophages, as shown by transcriptomic and proteomic immune profiling, further reinforce that restoring antitumour immunity is a critical determinant of nCRT efficacy.[18 19] Considering this concept in conjunction with the generally more robust immune response observed in women,[27 30] it is not surprising that we observed improved survival outcomes in women with PDAC following gemcitabine-based nCRT. Collectively, our data strongly point towards the existence of an impaired M2 macrophage recruitment phenomena in women with PDAC following gemcitabine-based nCRT. Expanding on these findings, future studies should examine the intricate and multifaceted mechanisms that govern M2 macrophage infiltration. Specifically, exploring the diverse modifying factors involved and characterising the spectrum of macrophages transitioning between the M1 and M2 states would be valuable.

Despite surpassing the limitations of previous reports, our study had some limitations. First, this study only includes patients with resected PDAC that generally have superior survival outcomes, as surgical specimens were necessary to achieve our objective of elucidating sex-specific immune alterations in the TME. Although no intention-to-treat analysis could be performed, the insights obtained still provide valuable guidance for developing more effective and personalised treatment strategies for PDAC. Second, patients in our study received gemcitabine-based nCRT. However, the optimal treatment approach in the neoadjuvant setting for borderline resectable and resectable PDAC remains a topic of ongoing debate. A consensus has yet to be reached regarding the optimal treatment approach in (borderline) resectable PDAC tumours.[12 13] Encouragingly, ongoing and recently completed randomised trials, such as the NorPACT-1 (NCT02919787),[16] PREOPANC-2 (EudraCT2017-002036-17/NL7094),[17] and PREOPANC-3 (NCT04927780) may provide definite answers. It would be of interest to investigate potential sex disparities within this study, aiming to determine whether sex differences are observed explicitly following gemcitabine-based nCRT or if the disparities extend to other treatment approaches as well. Third, patients with rapid progression were ineligible for surgical resection in the nCRT group, whereas those with rapid progression in the upfront surgery group underwent resection. This discrepancy could introduce a bias favouring improved survival outcomes in the nCRT group compared with the upfront surgery group. However, the primary objective of the current study was to investigate sex disparities within the treatment groups rather than directly comparing the two treatment arms. Moreover, we anticipated that this bias would equally affect both sexes, mitigating its impact on the observed sex-related differences. Fourthly, a subset of 96 out of 148 PDAC tumours in this study were subjected to (immune) gene expression profiling due to limited sample availability and the quality of tissue RNA. However, Cox proportional hazards models confirmed that the female sex remained a favourable prognostic factor for prolonged OS in this subset of patients. Furthermore, the results obtained through gene expression profiling were validated using spatial protein immune profiling.

Lastly, we did not investigate potential differences in molecular subtypes, such as classical and basal-like PDAC tumours, between the sexes within our treatment groups. This decision was deliberate, driven by the current knowledge gaps regarding the utility of molecular subtypes in the neoadjuvant setting. Moreover, increasing evidence supports the lack of association between molecular subtypes and survival or treatment response following gemcitabine-based chemotherapy.[58 59] Encouragingly, an ongoing clinical trial (NCT04683315) is currently evaluating the feasibility and clinical significance of molecular subtyping through RNA expression profiling of endoscopic ultrasound aspiration samples to aid in therapy selection for patients undergoing neoadjuvant chemotherapy.

In conclusion, this study provides compelling evidence of sex-based disparities in survival outcomes among patients with resected PDAC who received gemcitabine-based nCRT. We underscore the pivotal role of enhanced antitumour immunity as the mechanistic basis of the heightened sensitivity to gemcitabine-based nCRT in PDAC tumours of women. Our findings warrant tailored treatment approaches in PDAC, encompassing considerations of sex disparities and the modulation of M2 macrophage polarisation to optimise therapeutic outcomes.

Author affiliations
[1]Department of Surgery, Erasmus Medical Center, Rotterdam, The Netherlands
[2]Genetic and Molecular Epidemiology Group, Spanish National Cancer Research Centre, and CIBERONC, Madrid, Spain
[3]Department of Pathology, Tumour-Immuno Pathology Laboratory, Erasmus Medical Center, Rotterdam, The Netherlands
[4]Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands
[5]Department of Radiation Oncology, Amsterdam UMC Locatie AMC, Amsterdam, The Netherlands
[6]Department of Medical Oncology, Oncode Institute, Leiden University Medical Center, Leiden, The Netherlands

Acknowledgements The authors would like to express their gratitude to Dr María Casanova-Acebes for her invaluable contributions to interpreting and translating the immune profiling analysis result. Additionally, the authors are grateful to Anne Moreno Oya for her exceptional assistance in conducting the comprehensive survival analyses.

Collaborators Dutch Pancreatic Cancer Group (DPCG)

Contributors CWFvE: conceptualisation, formal analysis, investigation, methodology, validation, visualisation and writing (original draft). DAMM: formal analysis, investigation, methodology, resources, supervision, and validation. DV: investigation, resources, and validation. NFCCdM: methodology and formal analysis. SHvdB: conceptualisation. BGK and GvT: investigation, project administration. NM: conceptualisation, formal analysis, methodology, supervision and writing (original draft). CHJvE: conceptualisation, formal analysis, funding acquisition, methodology, project administration, supervision and writing (original draft). All authors performed writing (review and editing) and agreed on its content. Guarantors: NM and CHJvE.

Funding This work was financially supported by the Survival with Pancreatic Cancer Foundation (www.supportcasper.nl) (grant number OVIT17-06).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and the participating patients in this study originated from the phase III PREOPANC RCT (EudraCT 2012-003181-40) performed in 16 high-volume pancreatic surgery centres from the Dutch Pancreatic Cancer Group (DPCG). This trial was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committees of Erasmus MC (MEC-2012-249; 11 December 2012). Written informed consent was obtained from all patients. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The datasets used during the current study are available from the corresponding authors upon reasonable request.

ORCID iDs
Casper W F van Eijck http://orcid.org/0000-0002-1461-6725
Dana A M Mustafa http://orcid.org/0000-0003-0644-078X
Noel F C C de Miranda http://orcid.org/0000-0001-6122-1024
Núria Malats http://orcid.org/0000-0003-2538-3784

REFERENCES

1 Sung H, Ferlay J, Siegel RL, *et al*. Global cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71:209–49.
2 Dalmartello M, La Vecchia C, Bertuccio P, *et al*. European cancer mortality predictions for the year 2022 with focus on ovarian cancer. *Ann Oncol* 2022;33:330–9.
3 Paniccia A, Hosokawa P, Henderson W, *et al*. Characteristics of 10-year survivors of pancreatic ductal adenocarcinoma. *JAMA Surg* 2015;150:701–10.
4 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;389:1011–24.
5 Conroy T, Hammel P, Hebbar M, *et al*. FOLFIRINOX or gemcitabine as adjuvant therapy for pancreatic cancer. *N Engl J Med* 2018;379:2395–406.
6 Conroy T, Castan F, Lopez A, *et al*. Five-year outcomes of FOLFIRINOX vs gemcitabine as adjuvant therapy for pancreatic cancer: a randomized clinical trial. *JAMA Oncol* 2022;8:1571–8.
7 Evans DB, Hess KR, Pisters PWT. ESPAC-1 trial of adjuvant therapy for resectable adenocarcinoma of the pancreas. *Ann Surg* 2002;236:694;
8 Oettle H, Neuhaus P, Hochhaus A, *et al*. Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: the CONKO-001 randomized trial. *JAMA* 2013;310:1473–81.
9 Uesaka K, Boku N, Fukutomi A, *et al*. Adjuvant chemotherapy of S-1 versus gemcitabine for resected pancreatic cancer: a phase 3. *Lancet* 2016;388:248–57.
10 Bakens MJ, van der Geest LG, van Putten M, *et al*. The use of adjuvant chemotherapy for pancreatic cancer varies widely between hospitals: a nationwide population-based analysis. *Cancer Med* 2016;5:2825–31.
11 Mayo SC, Gilson MM, Herman JM, *et al*. Management of patients with pancreatic adenocarcinoma: national trends in patient selection, operative management, and use of adjuvant therapy. *J Am Coll Surg* 2012;214:33–45.
12 van Dam JL, Janssen QP, Besselink MG, *et al*. Neoadjuvant therapy or upfront surgery for resectable and borderline resectable pancreatic cancer: a meta-analysis of randomised controlled trials. *Eur J Cancer* 2022;160:140.
13 Springfeld C, Ferrone CR, Katz MHG, *et al*. Neoadjuvant therapy for pancreatic cancer. *Nat Rev Clin Oncol* 2023;20:318–37.
14 Versteijne E, van Dam JL, Suker M, *et al*. Neoadjuvant Chemoradiotherapy versus Upfront surgery for resectable and borderline resectable pancreatic cancer: long-term results of the dutch randomized PREOPANC trial. *J Clin Oncol* 2022;40:1220–30.
15 Ghaneh P, Palmer D, Cicconi S, *et al*. Immediate surgery compared with short-course neoadjuvant gemcitabine plus capecitabine, FOLFIRINOX, or chemoradiotherapy in patients with borderline resectable pancreatic cancer (ESPAC5): a four-arm. *Lancet Gastroenterol Hepatol* 2023;8:157–68.
16 Labori KJ, Lassen K, Hoem D, *et al*. Neoadjuvant chemotherapy versus surgery first for resectable pancreatic cancer (Norwegian Pancreatic cancer trial - 1 (Norpact-1)) - study protocol for a national multicentre randomized controlled trial. *BMC Surg* 2017;17:94.
17 Janssen QP, van Dam JL, Bonsing BA, *et al*. Total neoadjuvant FOLFIRINOX versus neoadjuvant gemcitabine-based chemoradiotherapy and adjuvant gemcitabine for resectable and borderline resectable pancreatic cancer (PREOPANC-2 trial): study protocol for a nationwide multicenter randomized controlled trial. *BMC Cancer* 2021;21:300.
18 Shi M, Chen Y, Ji D. The implications from the interplay of neoadjuvant chemoradiotherapy and the immune microenvironment in rectal cancer. *Future Oncol* 2022;18:3229–44.

19 Zhang H, Ye L, Yu X, *et al*. Neoadjuvant therapy alters the immune microenvironment in pancreatic cancer. *Front Immunol* 2022;13:956984.
20 Michelakos T, Cai L, Villani V, *et al*. Tumor microenvironment immune response in pancreatic ductal adenocarcinoma patients treated with neoadjuvant therapy. *J Natl Cancer Inst* 2021;113:182–91.
21 Ren L, Mota Reyes C, Friess H, *et al*. Neoadjuvant therapy in pancreatic cancer: what is the true oncological benefit *Langenbecks Arch Surg* 2020;405:879–87.
22 Farren MR, Sayegh L, Ware MB, *et al*. Immunologic alterations in the pancreatic cancer microenvironment of patients treated with neoadjuvant chemotherapy and radiotherapy. *JCI Insight* 2020;5:e130362.
23 Murakami T, Homma Y, Matsuyama R, *et al*. Neoadjuvant chemoradiotherapy of pancreatic cancer induces a favorable immunogenic tumor microenvironment associated with increased major histocompatibility complex class I-related chain A/B expression. *J Surg Oncol* 2017;116:416–26.
24 Athauda A, Nankivell M, Langley RE, *et al*. Impact of sex and age on chemotherapy efficacy, toxicity and survival in localised oesophagogastric cancer: a pooled analysis of 3265 individual patient data from four large randomised trials (OE02, OE05, MAGIC and ST03). *Eur J Cancer* 2020;137:45–56.
25 Lopes-Ramos CM, Quackenbush J, DeMeo DL. Genome-wide sex and gender differences in cancer. *Front Oncol* 2020;10:597788.
26 Yang Y, He C. Construction and validation of a nomogram model to predict the overall survival rate of esophageal cancer patients receiving neoadjuvant chemotherapy: a population-based study. *Front Surg* 2022;9:1066092.
27 Rakshith HT, Lohita S, Rebello AP, *et al*. Sex differences in drug effects and/or toxicity in oncology. *Curr Res Pharmacol Drug Discov* 2023;4:100152.
28 Matsuki H, Hiroshima Y, Miyake K, *et al*. Reduction of gender-associated M2-like tumor-associated macrophages in the tumor microenvironment of patients with pancreatic cancer after neoadjuvant chemoradiotherapy. *J Hepatobiliary Pancreat Sci* 2021;28:174–82.
29 Wang J, Ye C, Zhang C, *et al*. Sex differences in cancer-specific survival for locally advanced esophageal cancer after neoadjuvant chemoradiotherapy: a population-based analysis. *Front Surg* 2022;9:989204.
30 Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016;16:626–38.
31 Polanczyk MJ, Hopke C, Vandenbark AA, *et al*. Treg suppressive activity involves estrogen-dependent expression of Programmed Death-1 (PD-1). *Int Immunol* 2007;19:337–43.
32 Rothenberger NJ, Somasundaram A, Stabile LP. The role of the estrogen pathway in the tumor microenvironment. *Int J Mol Sci* 2018;19:611.
33 Versteijne E, van Eijck CHJ, Punt CJA, *et al*. Preoperative radiochemotherapy versus immediate surgery for resectable and borderline resectable pancreatic cancer (PREOPANC trial): study protocol for a multicentre randomized controlled trial. *Trials* 2016;17:127.
34 Tol J, Gouma DJ, Bassi C, *et al*. Definition of a standard Lymphadenectomy in surgery for pancreatic ductal adenocarcinoma: a consensus statement by the International Study Group on Pancreatic surgery (ISGPS). *Surgery* 2014;156:591–600.
35 Watanabe K, Taskesen E, van Bochoven A, *et al*. Functional mapping and annotation of genetic associations with FUMA. *Nat Commun* 2017;8:1826.
36 de Koning W, Latifi D, Li Y, *et al*. Identification, validation, and utilization of immune cells in pancreatic ductal adenocarcinoma based on marker genes. *Front Immunol* 2021;12:649061.
37 Hernandez S, Lazcano R, Serrano A, *et al*. Challenges and opportunities for Immunoprofiling using a spatial high-Plex technology: the nanostring Geomx((R)) digital spatial profiler. *Front Oncol* 2022;12:890410.
38 Legrand AJ, Konstantinou M, Goode EF, *et al*. The diversification of cell death and immunity: memento Mori. *Mol Cell* 2019;76:232–42.
39 de Sousa Cavalcante L, Monteiro G. Gemcitabine: metabolism and molecular mechanisms of action, sensitivity and chemoresistance in pancreatic cancer. *Eur J Pharmacol* 2014;741:8–16.
40 Jan R, Chaudhry G-E-S. Understanding apoptosis and apoptotic pathways targeted cancer therapeutics. *Adv Pharm Bull* 2019;9:205–18.
41 Campani D, Esposito I, Boggi U, *et al*. Bcl-2 expression in pancreas development and pancreatic cancer progression. *J Pathol* 2001;194:444–50.
42 Miyashita T, Tajima H, Makino I, *et al*. Neoadjuvant chemotherapy with gemcitabine plus NAB-paclitaxel reduces the number of cancer-associated fibroblasts through depletion of pancreatic stroma. *Anticancer Res* 2018;38:337–43.
43 Fang H, Ang B, Xu X, *et al*. TLR4 is essential for dendritic cell activation and anti-tumor T-cell response enhancement by damps released from chemically stressed cancer cells. *Cell Mol Immunol* 2014;11:150–9.
44 van Elsas MJ, Labrie C, Etzerodt A, *et al*. Invasive margin tissue-resident macrophages of high CD163 expression impede responses to T cell-based immunotherapy. *J Immunother Cancer* 2023;11:e006433.
45 Tokunaga R, Zhang W, Naseem M, *et al*. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - A target for novel cancer therapy. *Cancer Treat Rev* 2018;63:40–7.
46 Huang H, Zhou W, Chen R, *et al*. CXCL10 is a tumor microenvironment and immune infiltration related prognostic biomarker in pancreatic adenocarcinoma. *Front Mol Biosci* 2021;8:611508.
47 Pandey V, Fleming-Martinez A, Bastea L, *et al*. CXCL10/CXCR3 signaling contributes to an inflammatory microenvironment and its blockade enhances progression of murine pancreatic precancerous lesions. *Elife* 2021;10:e60646.
48 Wang G, Zhang Z, Zhong K, *et al*. CXCL11-armed oncolytic adenoviruses enhance CAR-T cell therapeutic efficacy and reprogram tumor microenvironment in glioblastoma. *Mol Ther* 2023;31:134–53.
49 Li C, Wang Z, Liu F, *et al*. CXCL10 mRNA expression predicts response to neoadjuvant chemoradiotherapy in rectal cancer patients. *Tumor Biol* 2014;35:9683–91.
50 Fang D, Li Y, Li Y, *et al*. Identification of immune-related biomarkers for predicting neoadjuvant chemotherapy sensitivity in her2 negative breast cancer via bioinformatics analysis. *Gland Surg* 2022;11:1026–36.
51 Qian L, Yu S, Yin C, *et al*. Plasma IFN-Γ-inducible Chemokines CXCL9 and CXCL10 correlate with survival and chemotherapeutic efficacy in advanced pancreatic ductal adenocarcinoma. *Pancreatology* 2019;19:340–5.
52 Feng L, Qi Q, Wang P, *et al*. Serum level of CCL2 predicts outcome of patients with pancreatic cancer. *Acta Gastroenterol Belg* 2020;83:295–9.
53 Ségaliny AI, Mohamadi A, Dizier B, *et al*. Interleukin-34 promotes tumor progression and metastatic process in osteosarcoma through induction of angiogenesis and macrophage recruitment. *Int J Cancer* 2015;137:73–85.
54 Kalbasi A, Komar C, Tooker GM, *et al*. Tumor-derived CCL2 mediates resistance to radiotherapy in pancreatic ductal adenocarcinoma. *Clinical Cancer Research* 2017;23:137–48.
55 Lankadasari MB, Mukhopadhyay P, Mohammed S, *et al*. Taming pancreatic cancer: combat with a double edged sword. *Mol Cancer* 2019;18:48.
56 Yang S, Liu Q, Liao Q. Tumor-associated macrophages in pancreatic ductal adenocarcinoma: origin, polarization, function, and reprogramming. *Front Cell Dev Biol* 2020;8:607209.
57 Franklin RA, Liao W, Sarkar A, *et al*. The cellular and molecular origin of tumor-associated macrophages. *Science* 2014;344:921–5.
58 Zhou X, Kurilov R, Neoptolemos JP, *et al*. Abstract A010: persister cell phenotypes contribute to poor patient outcomes after neoadjuvant chemotherapy in PDAC. *Cancer Res* 2022;82(22_Supplement):A010.
59 Martinelli P, Carrillo-de Santa Pau E, Cox T, *et al*. Gata6 regulates EMT and tumour dissemination, and is a marker of response to adjuvant chemotherapy in pancreatic cancer. *Gut* 2017;66:1665–76.