European Journal of Cancer 196 (2024) 113440

Contents lists available at ScienceDirect

European Journal of Cancer

journal homepage: www.ejcancer.com

Original research

FOLFIRINOX chemotherapy modulates the peripheral immune landscape in pancreatic cancer: Implications for combination therapies and early response prediction

Casper W.F. van Eijck [a,b], Gaby Strijk [a], Eveline E. Vietsch [a,c], Fleur van der Sijde [a], Maaike Verheij [a], Dana A.M. Mustafa [d], Madelief Vink [c,e], Joachim G.J.V. Aerts [c,e], Casper H. J. van Eijck [a,b], Marcella Willemsen [c,e,*]

[a] Department of Surgery, Erasmus University Medical Centre, Rotterdam, the Netherlands
[b] Genetic and Molecular Epidemiology Group, Spanish National Cancer Research Centre, Madrid, Spain
[c] Erasmus MC Cancer Institute, Erasmus University Medical Centre, Rotterdam, the Netherlands
[d] Department of Pathology, Erasmus University Medical Centre, Rotterdam, the Netherlands
[e] Department of Pulmonary Medicine, Erasmus University Medical Centre, Rotterdam, the Netherlands

ARTICLE INFO

Keywords:
Blood proteins
Biomarkers
FOLFIRINOX
Immunomodulation
Pancreatic neoplasms
Combined modality therapy

ABSTRACT

Background: FOLFIRINOX chemotherapy has improved outcomes for pancreatic cancer patients, but poor long-term survival outcomes and high toxicity remain challenges. This study investigates the impact of FOLFIRINOX on plasma proteins and peripheral immune cells to guide immune-based combination therapies and, ideally, to identify a potential biomarker to predict early disease progression during FOLFIRINOX.
Methods: Blood samples were collected from 86 pancreatic cancer patients before and two weeks after the first FOLFIRINOX cycle and subjected to comprehensive immune cell and proteome profiling. Principal Component Analysis and Linear Mixed Effect Regression models were used for data analysis. FOLFIRINOX efficacy was radiologically evaluated after the fourth cycle.
Results: One cycle of FOLFIRINOX diminished tumour-cell-related pathways and enhanced pathways related to immune activation, illustrated by an increase in pro-inflammatory IL–18, IL–15, and TNFRSF4. Similarly, FOLFIRINOX promoted the activation of CD4 + and CD8 + T cells, the proliferation of NK(T), and the activation of antigen-presenting cells. Furthermore, high pre-treatment levels of VEGFA and PRDX3 and an elevation in

Abbreviations: ANGPT1, Angiopoietin 1; ALAT, Alanine Aminotransferase; ASAT, Aspartate Aminotransferase; BACH1, Transcription regulator protein BTB domain and CNC homolog 1; BMI, Body Mass Index; BH, Benjamini-Hochberg; CA19–9, Cancer Antigen 19–9; CD, Cluster of Differentiation; cDC, Conventional Dendritic Cells; CEA, Carcinoembryonic Antigen; CRP, C-Reactive Protein; CI, Confidence Interval; CT, Computed Tomography; CTLA–4, Cytotoxic T Lymphocyte Associated Protein 4; EPCAM, Epithelial Cell Adhesion Molecule; FACS, Fluorescence-Activated Sorting; FDR, False-discovery rate; FOC, Fold Of Change; FOLFIRINOX, Chemotherapeutic regimen of 5-fluorouracil, folinic acid,irinotecan, and oxaliplatin; FUMA GWAS, Functional Mapping and Annotation of the Genome-Wide Association Studies; GAL, Galanin; G-CSF, Granulocyte-Colony Stimulating Factor; GFR, Glomerular Filtration Rate; ICOS, Inducible T cell Stimulator; IL, Interleukin; ITGB6, Integrin αvβ6; LAG–3, Lymphocyte Activating Gene 3; LAPC, Locally Advanced Pancreatic Cancer; Lipegfilgrastim, glycoPEGylated human N-methionyl granulocyte-colony stimulating factor; LMER, Linear Mixed Effects Regression Models; MSigDB, The Human Molecular Signature Database; MYO9B, Myosin IXB; NK cells, Natural Killer cells; NKT cells, Natural Killer T cells; NLR, Neutrophil-To-Lymphocyte ratio; NPX, Normalized Protein Expression; OS, Overall Survival; PBMC, Peripheral Blood Mononuclear Cell; PCA, Principle Component Analysis; PD, Progressive Disease; PDAC, Pancreatic Ductal Adenocarcinoma; pDC, plasmacytoid Dendritic Cell; PDGFB, Platelet Derived Growth Factor subunit B; PD–1, Programmed cell Death 1; PD-L1, Programmed cell Death Ligand 1; PEA, Proximity Extension Assays; PFS, Progression-Free Survival; PR, Partial Response; PRDX3, Peroxiredoxin 3; R2, Correlation Coefficient; P.adj, P values adjusted for multiple comparisons with the Benjamin-Hochberg correction; RECIST, Response Evaluation Criteria In Solid Tumours; RCT, Randomized Controlled Trial; SII, Systemic Immune-Infiltration Index; SIT1, Signalling Threshold Regulating Transmembrane Adaptor 1; TCF–1, T cell Factor 1; Tcm cells, Central memory T cells; Tem, Effector memory T cells; Temra, Terminally differentiated effector memory T cells; TIM–3, T cell Immunoglobulin and Mucin-Domain Containing 3; TME, Tumour Microenvironment; Tn, Naive T cells; TNF, Tumour Necrosis Factor; TNFRSF4, Tumour Necrosis Factor Receptor Superfamily 4; VEGFA, Vascular Endothelial Growth Factor A.

* Correspondence to: Department of Pulmonary Medicine, Erasmus MC Cancer Institute, Dr. Molenwaterplein 40, P.O. Box 2040, 3015GD Rotterdam, the Netherlands.

E-mail address: m.willemsen@erasmusmc.nl (M. Willemsen).

https://doi.org/10.1016/j.ejca.2023.113440
Received 13 October 2023; Accepted 1 November 2023
Available online 17 November 2023

FCRL3 levels after one cycle predicted early progression under FOLFIRINOX. Finally, patients with progressive disease exhibited high levels of inhibitory markers on B cells and CD8 + T cells, while responding patients exhibited high levels of activation markers on CD4 + and CD8 + T cell subsets.
Conclusion: FOLFIRINOX has immunomodulatory effects, providing a foundation for clinical trials exploring immune-based combination therapies that harness the immune system to treat pancreatic cancer. In addition, several plasma proteins hold potential as circulating predictive biomarkers for early prediction of FOLFIRINOX response in patients with pancreatic cancer.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive and lethal malignancy with an alarming five-year overall survival (OS) of around 9% across all stages of the disease [1]. The sole potential curative treatment option is surgical resection. However, only 10–20% of tumours are resectable at diagnosis because most patients present with advanced disease [2–4]. Despite the limited success of novel therapies in recent years, the multi-drug chemotherapeutic regimens of 5-fluorouracil, folinic acid, irinotecan, and oxaliplatin (FOLFIRINOX) and gemcitabine/nab-paclitaxel (Gem/Nab) have emerged as a cornerstone for treating all stages of PDAC.

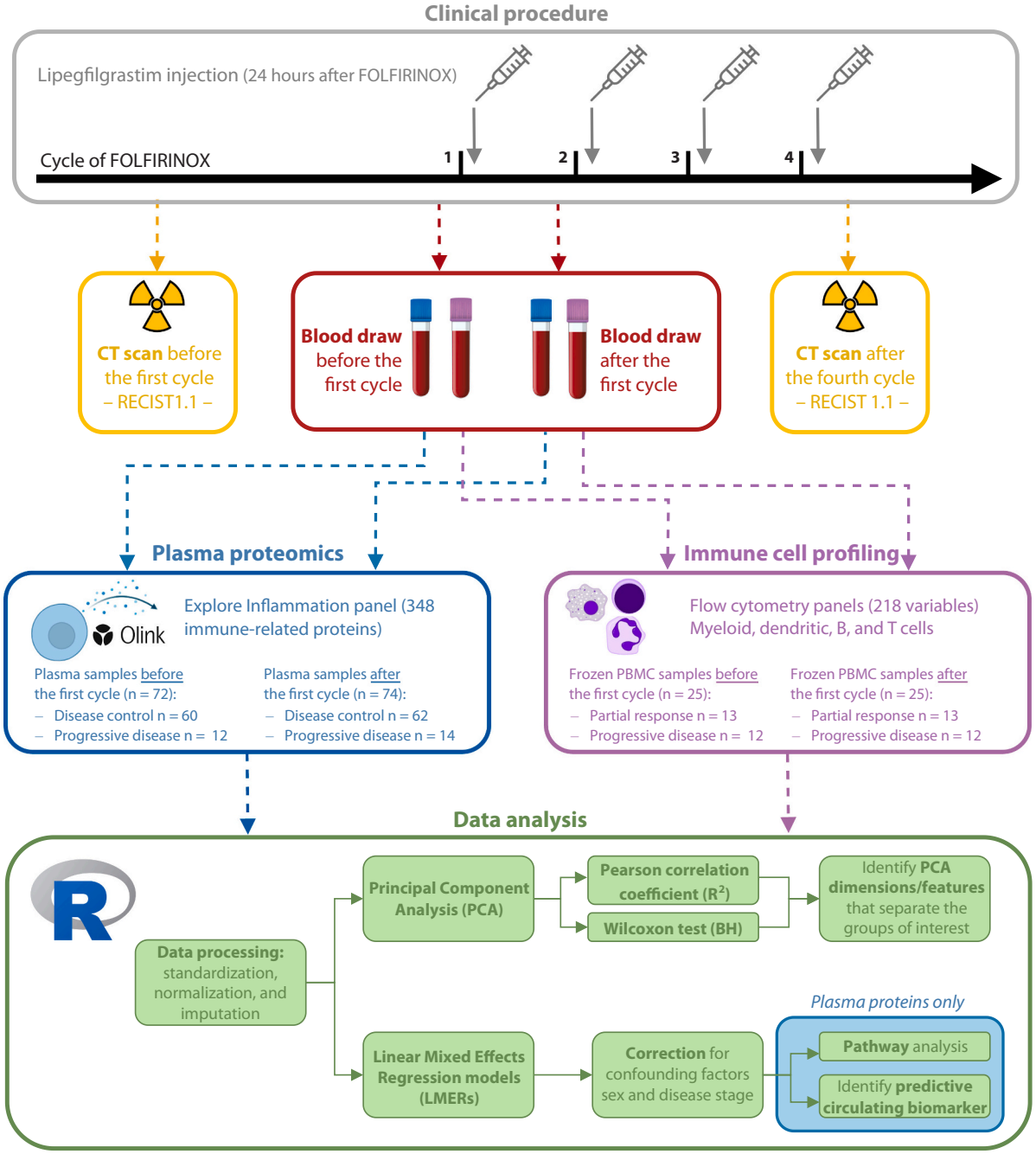


Fig. 1. Schematic methodological overview of the clinical procedure and data analyses.

Compared to gemcitabine monotherapy, both chemotherapeutic regimens have demonstrated increased but limited efficacy in all stages of PDAC [5–7]. Patients are also increasingly treated with neoadjuvant FOLFIRINOX or Gem/Nab since previous studies have shown that this improves survival, although results from large randomised controlled trials (RCTs) are lacking [8–12]. A systemic review and patient-level meta-analysis including 315 patients with locally advanced pancreatic cancer (LAPC) reported improved median OS after first-line FOLFIRINOX (24 months vs. 6–13 months) [13], and a recent study demonstrated that patients receiving neoadjuvant FOLFIRINOX had a better pathologic response than neoadjuvant Gem/Nab [14].

Despite these promising results, survival outcomes remain far from optimal, highlighting the need for multimodal therapies to improve survival outcomes. One possible approach is combining chemotherapy with immunotherapy. First, chemotherapeutic efficacy is often influenced by the immune cell composition in the tumour microenvironment (TME) [15,16]. Second, chemotherapeutic agents may restore the immune cell-mediated anti-tumour response in addition to their direct cytotoxic effects [15,17], which may help to overcome the immunosuppressive TME in PDAC that has hindered the success of immunotherapy compared to other cancer types [18,19]. Undoubtedly, FOLFIRINOX modulates the peripheral [20,21] and intra-tumoral immune landscape in PDAC [22–24]. The administration of multiple (at least three cycles with a mean of 6.5 cycles) of neoadjuvant FOLFIRINOX has even been associated with the expansion of effector T cells and concomitant reduction in regulatory T cells (Tregs) [21]. Furthermore, oxaliplatin, a component of FOLFIRINOX, induced immunogenic cancer cell death and enhanced the adaptive and systemic immune response in other cancer types [25,26]. Leveraging these favourable immunological properties by combining FOLFIRINOX with immunotherapy could shift the balance from a pro-tumoral to an anti-tumoral TME, ultimately improving treatment outcomes. A recent paper by Rojas *et al.* (2023) demonstrates that personalised mRNA neoantigen vaccination in combination with anti-PD-L1 and FOLFIRINOX in patients with resectable PDAC led to neoantigen-specific T cells and that high abundance correlated with delayed recurrence [27], highlighting the potential of combining chemotherapy with immunotherapy.

Another challenge is predicting response to FOLFIRINOX because toxicity occurs in 60–70% of patients [5,13,28,29]. Currently, treatment response is only evaluated after four cycles through computed tomography (CT) imaging, and Carbohydrate Antigen 19–9 (CA19–9) showed not to be predictive of FOLFIRINOX response early on [20] but only after multiple cycles [30]. Furthermore, approximately 20% of the population cannot synthesise CA19–9, limiting its clinical utility [31]. This belated response evaluation leads to ineffective but toxic treatment that reduces a patient's quality of life and delays alternative therapies that are potentially more effective. Identifying patients benefiting from FOLFIRINOX treatment as early as possible is essential to address this issue. Liquid biopsies are promising in this regard, and we have previously demonstrated the potential of circulating biomarkers [20,32]. In addition, liquid biopsies have several advantages, including no need for invasive tumour biopsies or resections, and they can be easily repeatedly measured [33]. Furthermore, they probably better represent tumour heterogeneity of the primary tumour and metastatic site [34].

This study aimed to investigate if the immunological effects of one cycle of FOLFIRINOX could guide the development of more effective immuno-based combinational therapies and to identify a potential early easy-to-use circulating biomarker predictive of early progression under FOLFIRINOX chemotherapy. To address these issues, we analysed the immune cell and proteome repertoire in baseline and early on-treatment samples.

2. Methods

2.1. Patient population

Patients with histologically confirmed PDAC treated with FOLFIRINOX in a tertiary referral centre (Erasmus University Medical Centre) were enrolled in this study. The inclusion criteria included adults aged 18 years or older who had not undergone prior chemotherapy. (Borderline) resectable patients originated from the randomised clinical PREOPANC–2 trial (Dutch trial register NL7094), and patients with advanced disease (LAPC or metastatic disease) originated from the prospective iKnowIT cohort (Dutch trial register NL7522). These studies were approved by the medical ethical committee of Erasmus University Medical Centre (MEC−2018–004, MEC−2018–087).

2.2. Clinical procedure

Patients were treated with first-line FOLFIRINOX chemotherapy between February 2015 and October 2019. Twenty-four hours after every FOLFIRINOX cycle, patients were prophylactically treated with the long-acting granulocyte-colony stimulating factor (G-CSF) lipegfilgrastim (Lonquex; Teva Ltd, Petach Tikva, Israel), to reduce FOLFIRINOX-induced neutropenia [35]. Peripheral venous blood samples were collected from each patient at two time points: at baseline (immediately before the first cycle) and after the first FOLFIRINOX cycle (just before the second cycle, approximately two weeks later) (Fig. 1). The collected samples were stored in 10 mL plasma EDTA tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and underwent density gradient centrifugation for 10 min at room temperature to isolate plasma and peripheral blood mononuclear cells (PBMCs). The samples were then cryopreserved in aliquots at −80 °C (plasma) or in 10% DMSO in liquid nitrogen until further analysis.

The radiological response to FOLFIRINOX was evaluated by comparing CT scans made at baseline and after the fourth cycle, following the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria. Patients who showed stable disease, partial response (PR), or complete response were defined as having "disease control" (DC), while patients showing disease progression were defined as having "progressive disease" (PD).

2.3. Plasma proteome profiling

Proteomic analysis was performed on cryopreserved peripheral plasma samples. Plasma concentrations of 368 immune-related proteins were quantified using the Olink Explore Inflammation panel (Supplementary Table S1), following the manufacturer's protocol. Olink Proteomics (Uppsala, Sweden) uses the Proximity extension assay (PEA) technology [36], which detects nucleotide-labelled antibody probe pairs to individualise proteins by a real-time PCR as previously described [37].

Protein data was normalised, standardised, and assessed for patient-level and protein-level quality using the standard pipeline of Olink Proteomics [38]. Final protein quantities were presented as normalised protein expression (NPX) values. Patient samples that deviated less than 0.3 NPX from the median of their internal control passed the quality assessment. Only proteins detected in more than 75% of patient samples and with less than 25% of values below the reported limit of detection were statistically analysed. When values were below the detection limit, actual NPX values were used to impute best-guess values. Possible sample outliers were identified using principal component analyses (PCAs) and excluded if necessary.

2.4. Immune cell profiling

To enhance the validity of our immune proteomic analysis, we performed flow cytometry staining on PBMCs. We selectively utilised

PBMCs from patients who exhibited either partial response or progressive disease. By targeting patients at opposite ends of the disease spectrum, we aimed to optimise the detectability of immune-related alterations, ultimately enhancing the reliability and validity of our research findings. Four panels were designed to characterise T cells (co-stimulatory and co-inhibitory), B cells, and myeloid or dendritic cells (Supplementary Tables S2, S3, and Supplementary Fig. S1). Cells were surface stained at 4 °C for 30 min, followed by incubation with Fixable Viability Dye (eBioscience, ThermoFisher, Waltham, MA, USA) for 15 min. Subsequently, the cells were fixed and permeabilised using the FoxP3 Transcription Factor Staining Buffer set (eBioscience) and stained intracellularly for 60 min. The acquisition was performed on a FACSymphony A5 (BD Biosciences) using BD FACSDiva Software (BD Biosciences).

2.5. Data visualisation and statistical analyses

Data visualisation and statistical analysis were performed with R Statistical Software (v.4.1.2) using the EnhancedVolcano (v1.11.3), 'ggplot2' (v3.4.2), 'OlinkAnalyze' (v3.3.1), 'Rtsne' (v0.16), and 'survival' (3.5–5) packages. All analyses were stratified based on two groups: the timing of blood sample collection (baseline vs. after one cycle) and response to FOLFIRINOX treatment. P values were considered statistically significant if adjusted for multiple comparisons with the Benjamini-Hochberg correction (P.adj) and below 0.05. Statistical significance was indicated as follows: *P.adj < 0.05, **P.adj < 0.01, ***P.adj < 0.001, and ****P.adj < 0.0001. Clinical characteristics were statistically analysed using Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables and were considered statistically significant if the uncorrected P value was below 0.05. The effect of protein levels on OS and progression-free survival (PFS) was predicted using multivariate Cox proportional hazards models, which corrected potential confounders as identified by univariate analysis (the disease stage and the total number of FOLFIRINOX cycles).

2.6. Principle component analyses

PCA was performed for the flow cytometry and the proteome datasets. To facilitate PCA, missing data were imputed and assessed for accuracy, after which the values were $\log_{10}$ (flow cytometry percentages) or $\log_2$ (NPX) transformed. PCA dimensions capable of separating between subgroups were identified by calculating Pearson R^2 and statistical evaluation of the PCA dimension coordinates. Wilcoxon tests with Benjamini-Hochberg correction were used for statistical comparison of the coordinates. The top ten flow cytometry variables or plasma proteins with the highest contribution to each PCA dimension were considered to drive the dimension.

2.7. Linear Mixed Effects Regression models and pathway overrepresentation analysis

To correct for the confounding factors of sex and disease stage, Linear Mixed Effects Regression models (LMERs) were fitted to the datasets. In addition, plasma proteins that were differentially expressed (P.adj < 0.05) between baseline and after the first FOLFIRINOX cycle samples were subjected to functional pathway overrepresentation analysis using the Functional Mapping and Annotation of the Genome-Wide Association Studies (FUMA GWAS) platform [39]. The overrepresentation analysis analysed the canonical pathway collections C2 (BioCarta, KEGG, PID, and Reactome) and C5 (ontology gene sets) of the Human Molecular Signature Database (MSigDB). The FUMA GWAS platform automatically analysed the results, and pathways were found to be differentially altered if P.adj < 0.01.

Table 1
Clinical characteristics of the PDAC patient cohort.

Characteristics	Disease control ($n = 65$)	Progressive disease ($n = 21$)	P value	Overall ($n = 86$)
Age, median years (range)[a]	63 (46–83)	60 (47–78)	*0.13*	62 (46–83)
BMI, median kg/m2 (range)	25 (16–36)	25 (19–36)	*0.62*	25 (16–36)
Diabetes mellitus, *n (%)*				
No	50 (77)	17 (81)	*1.0*	67 (78)
Yes	15 (23)	4 (19)		19 (22)
Disease stage, *n (%)*				
(Borderline) resectable disease	30 (46)	7 (33)	*0.56*	37 (43)
LAPC	21 (32)	9 (43)		30 (35)
Metastatic disease	14 (22)	5 (24)		19 (22)
Family history for PDAC, *n (%)*				
No	59 (91)	18 (86)	*0.68*	77 (90)
Yes	6 (9)	3 (14)		9 (10)
History of malignancy, *n (%)*				
No	55 (85)	18 (86)	*1.0*	73 (85)
Yes	10 (15)	3 (14)		13 (15)
History of pancreatitis, *n (%)*				
No	63 (97)	20 (95)	*1.0*	83 (96)
Yes	2 (3)	1 (5)		3 (4)
Sex, *n (%)*				
Female	28 (43)	10 (48)	*0.80*	38 (44)
Male	37 (57)	11 (52)		48 (56)
Alcohol use, *n (%)*				
Current/former	40 (62)	14 (67)	*0.89*	54 (63)
Never	25 (38)	7 (33)		32 (37)
Smoking status, *n (%)*				
Current/former	35 (54)	15 (71)	*0.36*	50 (58)
Never	30 (46)	6 (29)		36 (42)
Baseline clinical parameters, median (range)				
ALAT (U/L)	28 (9 – 1240)	27 (14 – 636)	*0.42*	28 (9 – 1240)
ASAT (U/L)	26 (10 – 1100)	26 (15 – 285)	*0.83*	26 (10 – 1100)
Albumin (g/L)	40 (23–48)	40 (28–49)	*0.68*	40 (23–49)
Bilirubin (μmol/L)	10 (3–45)	15 (4–83)	*0.24*	13 (3–83)
CA19–9 (U/mL)	286 (0 – 26500)	388 (4 – 83300)	*0.10*	294 (0 – 83300)
CEA (ng/L)	4 (1 – 119)	4 (1 – 226)	*0.35*	4 (1 – 226)
Creatinine (μmol/L)	69 (33 – 149)	61 (47 – 1103)	*0.20*	69 (33 – 149)
CRP (mg/L)	6 (0 – 91)	6 (1–88)	*0.21*	6 (0 – 91)
GFR (mL/min)	89 (39 – 124)	90 (55 – 126)	*0.15*	90 (39 – 126)
Leukocytes (x10^9/L)	8 (3–14)	8 (5–15)	*0.27*	8 (3 – 145
NLR[b]	4 (1–20)	3 (1–7)	*0.88*	3 (1–20)
SII[b]	839 (264 – 7250)	1010 (201 – 2380)	*0.52*	869 (201 – 7250)
FOLFIRINOX cycles, median (range)	8 (4–12)	4 (4–8)	< *0.001*	8 (4–12)
Survival status, *n (%)*				
Alive	35 (54)	2 (10)	< *0.001*	37 (43)
Diseased	30 (46)	19 (90)		49 (57)
OS, median months (range)[c]	24 (6–54)	5 (1–30)	< *0.001*	17 (1–54)
Progression status, *n (%)*				
No progression	35 (54)	0 (0)	< *0.001*	35 (35)
Progression	30 (46)	21 (100)		51 (65)
PFS, median months (range)[c]	23 (4–52)	3 (1–20)	< *0.001*	15 (1–52)
Type of profiling, *n (%)*				
Immune cells	3 (5)	7 (33)	< *0.001*	10 (12)
Serum proteins	52 (80)	9 (43)		61 (71)

(*continued on next page*)

Table 1 (*continued*)

Characteristics	Disease control ($n = 65$)	Progressive disease ($n = 21$)	P value	Overall ($n = 86$)
Serum proteins and Immune cells	10 (15)	5 (24)		15 (17)
RECIST1.1 after four cycles, *n (%)*[d]				
Partial response (PR)	18 (28)	0 (0)	< *0.001*	18 (21)
Stable disease (SD)	47 (72)	0 (0)		47 (55)
Progressive disease (PD)	0 (0)	21 (100)		21 (24)

[a] Age was calculated from the date of birth until the date of initiation of FOLFIRINOX.

[b] NLR and SII values were missing for a total of 9 patients.

[c] Overall and progression-free survival outcomes were calculated from the date of initiation of FOLFIRINOX until the date of death or disease progression.

[d] Detailed description of the RECIST1.1 definitions, in which patients with disease control are divided into those with stable disease and progressive disease.

3. Results

3.1. Patient and sample characteristics

A total of 202 peripheral blood samples from 101 PDAC patients were collected at baseline and after the first cycle of FOLFIRINOX chemotherapy (on treatment sample). After quality control, immune cell profiling using flow cytometry was performed on 50 samples, and plasma protein profiling using Olink Proteomics was performed on 146 samples. These samples correspond to 86 PDAC patients whose clinical characteristics are summarised in Table 1. This seemingly incoherent number of patients was due to a partial overlap between the two profiling methods ($n = 10$) and the selective exclusion of a patient's baseline or on-treatment sample during the quality control. The clinical characteristics stratified by profiling type can be found in Supplementary Table S4.

3.2. FOLFIRINOX treatment is associated with systemic immune activation

To investigate the immunological effects of FOLFIRINOX, we conducted plasma protein and immune cell validation profiling of

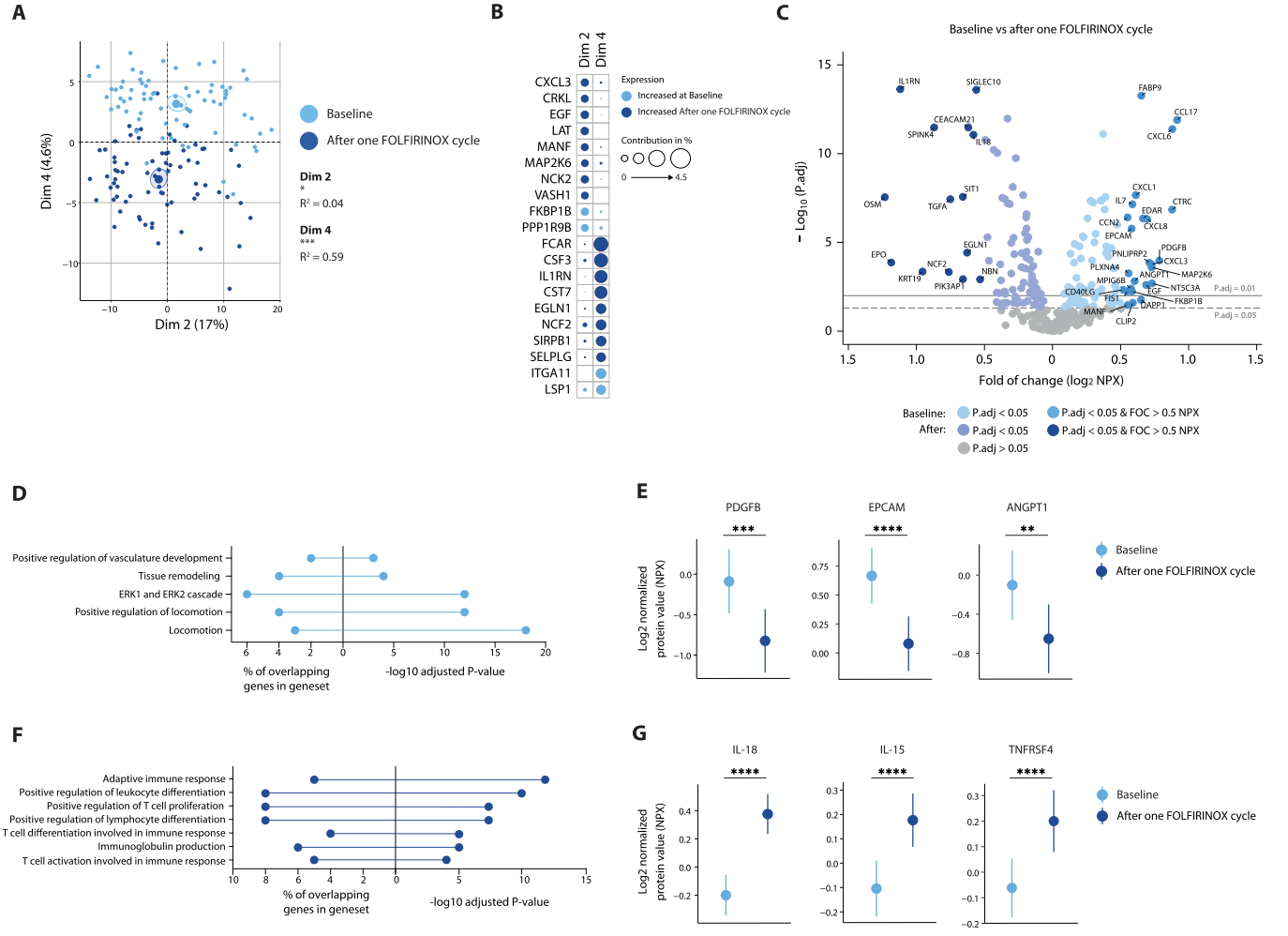


Fig. 2. Analyses of 368 plasma proteins in baseline and on-treatment blood samples. A PCA biplot that shows time-point-based clustering based on blood sample similarity in dimensions 2 (x-axis) and 4 (y-axis). The percentage indicates the variance that is explained by the dimension. B Balloon plot that highlights the ten most contributing proteins to dimensions 2 and 4. The size of the circle indicates the total contribution percentage. C Volcano plot that depicts the FOC (log2 NPX) of all proteins between the two blood sample time points. D-F Visual representations of the pathway overrepresentation analysis using significantly overexpressed proteins in baseline (D) and on-treatment (F) samples. The y-axis displays the pathway names, the x-axis displays on the left the percentage of overlapping genes included in these pathways, and the -log10 adjusted P values on the right. E-G Point-range plots that depict the expression (log2 NPX, 95% CI) of three highlighted proteins in baseline (E) and on-treatment (G) samples. *P.adj < 0.05, **P.adj < 0.01, ***P.adj < 0.001, and ****P.adj < 0.0001.

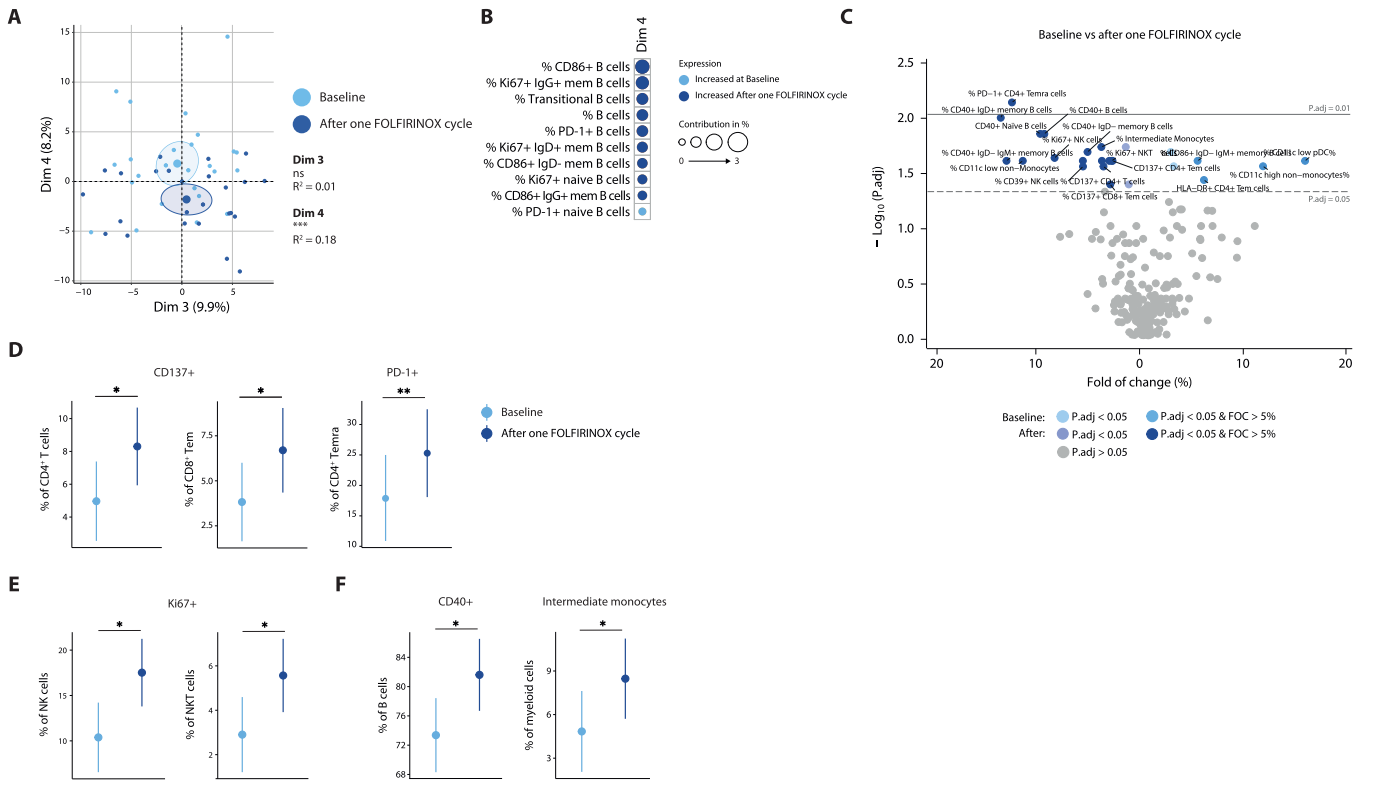


Fig. 3. Analyses of 218 flow cytometry variables in baseline and on-treatment blood samples. A PCA biplot that shows time-point-based clustering based on sample similarity in dimension 4 (y-axis) but not in dimension 3 (x-axis). The percentage indicates the variance that is explained by the dimension. B Balloon plot that highlights the ten most contributing variables to dimension 4. The size of the circle indicates the total contribution percentage. C Volcano plot that depicts the relative FOC (%) of all variables between the two blood sample time points. D-F Point-range plots that depict the value (mean %, 95% CI) of several highlighted variables in baseline and on-treatment samples. *P.adj < 0.05, **P.adj < 0.01, ***P.adj < 0.001, and ****P.adj < 0.0001.

peripheral blood samples. Dimension reduction was performed for both datasets using PCA, and to identify variables that were confidently associated with treatment-induced changes, we fitted LMERs that corrected for the covariates of disease stage and sex.

Unsupervised PCA of proteome data (368 proteins) showed clustering of baseline and on-treatment samples based on the second (R^2 = 0.04, P.adj < 0.05) and fourth (R^2 = 0.59, P.adj < 0.001) dimension (Figs 2A,2B). LMERs revealed 89 enriched plasma proteins at baseline (P.adj < 0.01) against 103 on-treatment (P.adj < 0.01) (Fig. 2C). Pathway overrepresentation analysis (Supplementary Table S5) of these plasma proteins revealed baseline enrichment of tumour-cell-related pathways such as positive regulation of vasculature development, tissue remodelling, and locomotion (Fig. 2D), illustrated by high expression of PDGFB, EPCAM, and ANGPT1 (Fig. 2E). Following one cycle of FOLFIRINOX, pathways of the adaptive immune response, activation, proliferation, differentiation of T cells, and immunoglobulin production were highly enriched (Fig. 2F). Subsequent LMER analyses revealed interleukin (IL)−18, IL−15, and TNFRSF4 as significant regulators of these pathways (Fig. 2G).

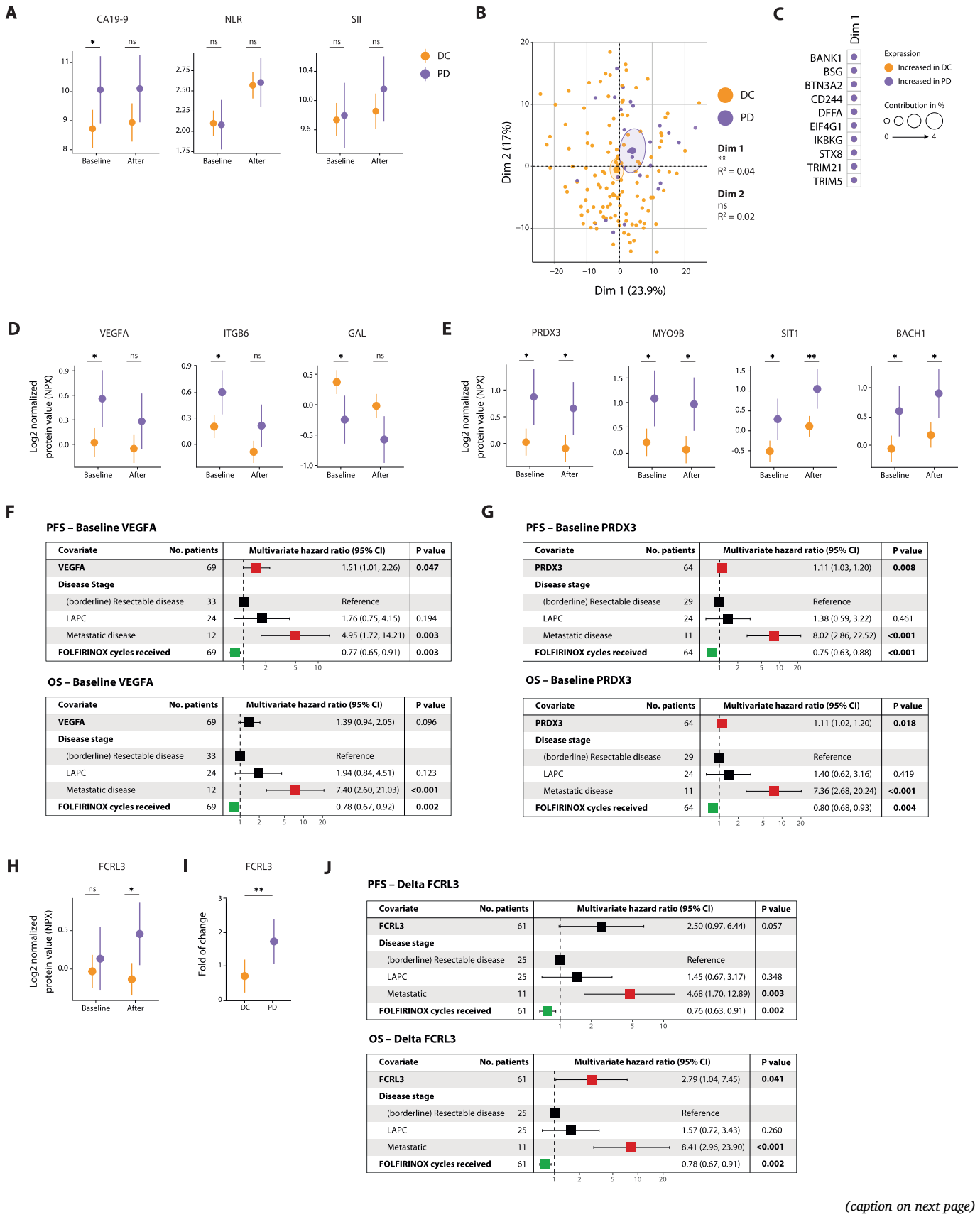
Similarly, unsupervised PCA of flow cytometry data (218 variables) showed clustering of baseline and on-treatment samples based on the fourth dimension (R^2 = 0.18, P.adj < 0.001) (Fig. 3A). This dimension was primarily driven by the increased proliferation and activation of B cell subsets following FOLFIRINOX treatment (Fig. 3B). LMERs revealed six enhanced variables at baseline (P.adj < 0.05) against 19 enhanced variables on-treatment (P.adj < 0.05) (Fig. 3C). Specifically, we observed increased expression of markers in T cell activation (i.e., CD137 and PD–1) upon treatment (Fig. 3D). Moreover, the frequency of proliferating natural killer (NK) and NKT cells increased upon treatment, indicating systemic lymphocyte activation (Fig. 3E). Finally, we observed activation signals of antigen-presenting cells upon treatment, as illustrated by increased CD40 expression on B cells and a higher abundance of specialised antigen-presenting intermediate monocytes (Fig. 3F).

3.3. *Plasma proteins have the potential to predict early disease progression before starting or on FOLFIRINOX treatment*

To elucidate patterns of initial (immune) resistance to FOLFIRINOX, we attempted to differentiate between patients with DC from PD by analysing distinct immune cell and plasma protein profiles using unsupervised PCA clustering. We also accounted for the potential covariates of disease stage and sex by fitting LMERs.

To date, CA19–9 is commonly used to predict therapy response early on treatment. However, as already indicated, CA19–9 can only be predictive after multiple cycles, which is in correspondence with our cohort (Fig. 4A). Furthermore, neutrophil-lymphocyte ratio (NLR) and systemic immune-inflammation index (SII) were not predictive of early treatment response (Fig. 4A). Therefore, there is a need to identify an early biomarker predictive for FOLFIRINOX response.

Our proteome dataset had an overrepresentation of samples from DC patients compared to PD patients ($n = 62$ vs. $n = 14$). Nevertheless, unsupervised PCA revealed clustering of samples corresponding to DC and PD patients based on the first dimension (R^2 = 0.04, P < 0.01) (Figs. 4B, 4C). LMERs revealed seven plasma proteins to be differentially expressed between DC and PD patients at baseline (P.adj < 0.01). Pre-treatment levels of VEGFA, ITGB6, PRDX3, MYO9B, SIT1, and BACH1 were elevated in PD patients, while pre-treatment levels of GAL were elevated in DC patients (Figs. 4D, 4E). Notably, four of these proteins remained differentially expressed in on-treatment samples (PRDX3, MYO9B, SIT1, BACH1). Multivariate survival analyses revealed FOLFIRINOX-independent baseline VEGFA and PRDX3 levels to predict



PFS – Baseline VEGFA

Covariate	No. patients	Multivariate hazard ratio (95% CI)	P value
VEGFA	69	1.51 (1.01, 2.26)	**0.047**
Disease Stage			
(borderline) Resectable disease	33	Reference	
LAPC	24	1.76 (0.75, 4.15)	0.194
Metastatic disease	12	4.95 (1.72, 14.21)	**0.003**
FOLFIRINOX cycles received	69	0.77 (0.65, 0.91)	**0.003**

OS – Baseline VEGFA

Covariate	No. patients	Multivariate hazard ratio (95% CI)	P value
VEGFA	69	1.39 (0.94, 2.05)	0.096
Disease stage			
(borderline) Resectable disease	33	Reference	
LAPC	24	1.94 (0.84, 4.51)	0.123
Metastatic disease	12	7.40 (2.60, 21.03)	**<0.001**
FOLFIRINOX cycles received	69	0.78 (0.67, 0.92)	**0.002**

PFS – Baseline PRDX3

Covariate	No. patients	Multivariate hazard ratio (95% CI)	P value
PRDX3	64	1.11 (1.03, 1.20)	**0.008**
Disease stage			
(borderline) Resectable disease	29	Reference	
LAPC	24	1.38 (0.59, 3.22)	0.461
Metastatic disease	11	8.02 (2.86, 22.52)	**<0.001**
FOLFIRINOX cycles received	64	0.75 (0.63, 0.88)	**<0.001**

OS – Baseline PRDX3

Covariate	No. patients	Multivariate hazard ratio (95% CI)	P value
PRDX3	64	1.11 (1.02, 1.20)	**0.018**
Disease stage			
(borderline) Resectable disease	29	Reference	
LAPC	24	1.40 (0.62, 3.16)	0.419
Metastatic disease	11	7.36 (2.68, 20.24)	**<0.001**
FOLFIRINOX cycles received	64	0.80 (0.68, 0.93)	**0.004**

PFS – Delta FCRL3

Covariate	No. patients	Multivariate hazard ratio (95% CI)	P value
FCRL3	61	2.50 (0.97, 6.44)	0.057
Disease stage			
(borderline) Resectable disease	25	Reference	
LAPC	25	1.45 (0.67, 3.17)	0.348
Metastatic	11	4.68 (1.70, 12.89)	**0.003**
FOLFIRINOX cycles received	61	0.76 (0.63, 0.91)	**0.002**

OS – Delta FCRL3

Covariate	No. patients	Multivariate hazard ratio (95% CI)	P value
FCRL3	61	2.79 (1.04, 7.45)	**0.041**
Disease stage			
(borderline) Resectable disease	25	Reference	
LAPC	25	1.57 (0.72, 3.43)	0.260
Metastatic	11	8.41 (2.96, 23.90)	**<0.001**
FOLFIRINOX cycles received	61	0.78 (0.67, 0.91)	**0.002**

(caption on next page)

Fig. 4. Analyses of 368 plasma proteins in baseline and on-treatment blood samples stratified by the radiological response after four FOLFIRINOX cycles. A Point-range plots that depict the expression (log2 NPX, 95% CI) of CA19–9, NLR, and SII at baseline and on-treatment stratified by treatment. B PCA biplot that shows radiological response-based clustering based on sample similarity in dimension 1 (x-axis) but not in dimension 2 (y-axis). The percentage indicates the variance that is explained by the dimension. C Balloon plot that highlights the ten most contributing proteins to dimension 1. The size of the circle indicates the total contribution percentage. D-E Point-range plots that depict the overexpression (log2 NPX, 95% CI) of several proteins in baseline only (D) and baseline as well as on-treatment (E) samples stratified by treatment. F-G Forest plots of the multivariate Cox regression hazards models that predict OS and PFS, corrected by disease stage and the total number of FOLFIRINOX cycles, using continuous values (log2 NPX) for VEGFA (F) and PRDX3 (G). The baseline values of VEGFA and PRDX3 are independent negative predictors for OS and PFS. H-I Point-range plots that depict the expression (log2 NPX, 95% CI) of FCRL3 (H) and the absolute FOC between the two blood sample time points (I) stratified by the response. (J) Forest plots of the multivariate Cox regression hazards models that predict OS and PFS, corrected by disease stage and the total number of FOLFIRINOX cycles, using continuous delta values (log2 NPX) for FCRL3. The delta values of FCRL3 are independent negative predictors for OS and PFS. *P.adj < 0.05, **P.adj < 0.01, ***P.adj < 0.001, and ****P.adj < 0.0001.

early disease progression (Figs. 4F, 4G). High pre-treatment VEGFA correlated with worse PFS (Fig. 4F). Similarly, elevated PRDX3 levels indicated treatment unresponsiveness associated with PFS and OS (Fig. 4G). Furthermore, an increase in FCRL3 during FOLFIRINOX treatment was specific for patients with PD, with a fold change in plasma levels of 1.74 vs 0.826 for PR (Figs. 4H, 4I). Finally, delta FCRL3 correlated borderline significant to PFS and significant to OS (Fig. 4J). These analyses demonstrate the potential of plasma proteins to predict early disease progression before the start of treatment or early on-treatment.

3.4. Patients with disease control exhibit increased levels of T cell activation markers, while patients with disease progression show high levels of PD-L1 + B cells

Following proteome analyses, we aimed to validate these findings with immune cell profiling to identify a signature indicative of the response to FOLFIRINOX treatment. To enhance the robustness of our results, we restricted our patient selection to those with PD or PR, representing the two extreme ends of the disease spectrum. Unsupervised PCA of flow cytometry data showed clustering of samples corresponding to PR and PD patients based on the first ($R^2 = 0.13$, $P < 0.01$) and second ($R^2 = 0.63$, P.adj < 0.001) dimension (Figs. 5A, 5B). Patients with PD were enriched for inhibitory markers by B cells and CD4 + and CD8 + T cells, compared to PR patients (Fig. 5B). Specifically, PD-L1 expression was increased on various B cell subsets. In contrast, T cells in PD patients seemed less functional, based on the expression of CD39 and LAG–3. Conversely, activation markers on CD4 + and CD8 + T cells were highly abundant in PR patients.

LMERs revealed 45 variables at baseline and 54 variables on-treatment to be differentially expressed between PR and PD patients (P.adj < 0.05). After accounting for potential covariates, we could still delineate high expression of inhibitory PD-L1 on B cell(s) (subsets) and conventional dendritic cells (cDC) in PD patients (Fig. 5C), illustrating the robustness of our PCA. Likewise, activation markers were increased on lymphocytes in PR patients, characterised by high ICOS, PD–1, and TCF–1 expression (Fig. 5D). However, inhibitory TIM–3 and CTLA–4 were also expressed at significantly higher levels on T cells in PR patients (Fig. 5E). Only LAG–3 was specifically enriched on naïve CD8 + T cells, as well as TOX on CD8 + central memory T (Tcm) cells in PD patients (Fig. 5F). Finally, PD–1 expression of CD4 + Tcm cells was found to increase in PD patients upon one cycle of FOLFIRINOX, which was significantly opposite in PR patients (Figs. 5G, 5H), which may suggest that PD–1 + CD4 + T cells restrain clonal expansion in PD patients. Altogether, these data indicate that high levels of B and T cell inhibitory markers might be partially related to FOLFIRINOX response. In contrast, increased levels of activation markers seem to be most specific for PR patients.

4. Discussion

This prospective multicentre study investigated the pre- and on-treatment immune cell and proteome profiles of 86 PDAC patients. Our findings reveal that only one cycle of FOLFIRINOX chemotherapy promotes several facets of the anti-tumour response, namely increased activation of T cells and antigen-presenting cells, which could be leveraged by adding immunotherapy. Moreover, we identified seven plasma proteins with the potential to predict progression under FOLFIRINOX before starting treatment and could serve as valuable targets for combination therapy.

Combining FOLFIRINOX with immunotherapy is gaining interest and leading to several studies investigating the immunological effects of FOLFIRINOX alone. We further investigated this immune-modulating effect after only one cycle of FOLFIRINOX. Comprehensive immune profiling demonstrated that one cycle of FOLFIRINOX elicits systemic immune activation and induces the proliferation of T, B, and NK(T) cells. Indeed, FOLFIRINOX or its components have demonstrated immunomodulatory properties in PDAC [20,22,25,26,40]. Consistent with our findings, previous research has shown that multiple (at least three cycles with and a mean of 6.5 cycles) of neoadjuvant FOLFIRINOX enhances anti-tumour immunity by decreasing the abundance of regulatory T cells (Tregs) and increasing the abundance of effector T cells in the peripheral blood of PDAC patients [21]. This demonstrates the robustness of this response, which can be detected early on-treatment.

Simultaneously modulating the populations of immune cells, FOLFIRINOX markedly increased the plasma levels of pro-inflammatory cytokines and regulators belonging to the TNF superfamily, including TNFSF4, IL−18, and IL−15. TNFSF4 (CD134 or OX40) is a secondary co-stimulatory immune checkpoint molecule vital in antigen-specific T cell expansion and survival [41]. Furthermore, the intra-tumoral expression of TNFSF4 on T cells is described as an independent prognostic factor for favourable prognosis [42,43]. Likewise, IL−18 can induce IFN-γ production by T cells. However, IL−18 receptor signalling is also described to regulate the exhaustion of tumour-reactive CD8 + T cells in a pre-clinical PDAC mouse model [44]. Finally, IL−15 regulates T and NK cell activation and proliferation without stimulating immune-suppressive Tregs [45]. In addition, IL−15 can induce NK cell-mediated cytotoxicity against pancreatic cell lines in vitro [46]. These early systemic effects on-treatment have not been previously reported but emphasise the potential of immune-based combinational therapies.

Elevated pre-treatment plasma levels of VEGFA, ITGB6, PRDX3, MYO9B, SIT1, and BACH1 and low pre-treatment plasma levels of GAL were significantly associated with PDAC progression after four cycles of FOLFIRINOX. These associations remained significant after one FOLFIRINOX cycle for PRDX3, MYO9B, SIT1, and BACH1. Most interestingly, elevated VEGFA and PRDX3 pre-treatment levels correlated with poor treatment response. Our results are consistent with previous research on the roles of these proteins in cancer treatment and pathogenesis. The transcription regulator protein BTB domain and CNC homolog 1 (BACH1) and VEGFA promote the metastatic behaviour of PDAC and positively correlate to a poor prognosis [47,48]. Likewise, myosin IXB (MYO9B), a protein regulating cell migration, promotes metastasis in several cancer types [49]. The integrin αvβ6 (ITGB6) has been shown to drive PDAC progression through pro-tumoral and TME-related mechanisms [50]. Peroxiredoxin 3 (PRDX3), a protein that protects cells from oxidative stress, has a well-established tumour-promoting role in various cancer types and induces chemotherapy resistance [51]. Although the function of the signalling threshold regulating

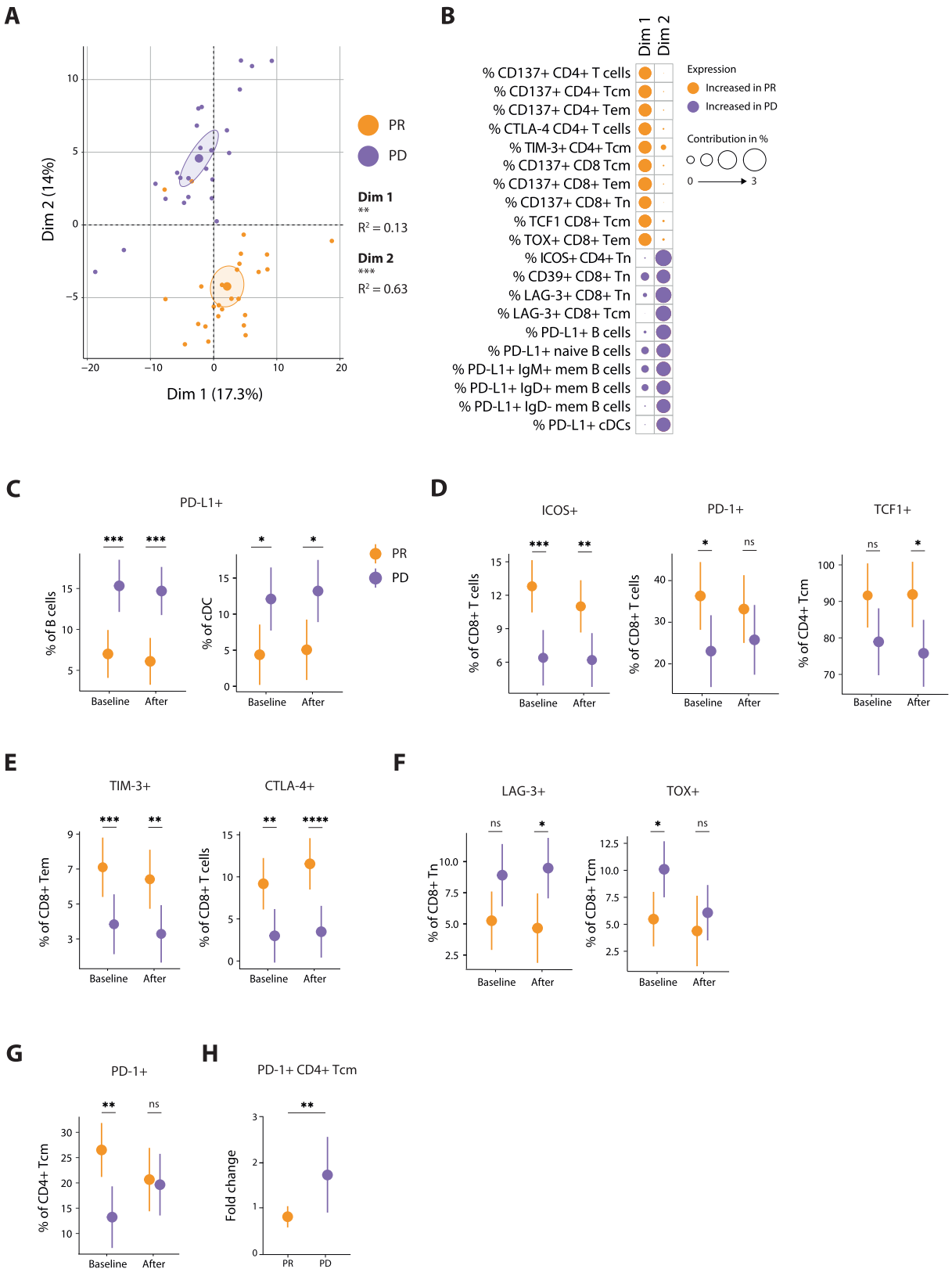


Fig. 5. Analyses of 218 flow cytometry variables in baseline and on-treatment blood samples stratified by the radiological response after four FOLFIRINOX cycles. A PCA biplot that shows radiological response-based clustering based on sample similarity in dimensions 1 (x-axis) and 2 (y-axis). The percentage indicates the variance that is explained by the dimension. B Balloon plot that highlights the ten most contributing proteins to dimensions 1 and 2. The size of the circle indicates the total contribution percentage. C Point-range plots that depict the value (mean %, 95% CI) of several variables in baseline and on-treatment samples stratified by the response. *P.adj < 0.05, **P.adj < 0.01, ***P.adj < 0.001, and ****P.adj < 0.0001.

transmembrane adaptor 1 (SIT1) in cancer is still unknown, it may regulate tumour immunity through its ability to inhibit T cell receptor-mediated signalling [52]. Finally, galanin (GAL) has a controversial role in the tumorigenesis of PDAC, but stimulating or inhibiting GAL may be a promising therapeutic approach [53]. In addition to VEGFA and PRDX3, FOLFIRINOX-induced FCRL3 significantly correlated with poor treatment response. FCRL3 is a receptor expressed by various lymphocytes and regulates the immune system. The elevated expression has been described to increase susceptibility to autoimmunity, and polymorphisms in the FCLR3 loci are associated with numerous autoimmune diseases [54,55]. However, little information on its role in cancer and therapy response is available.

In addition to elevated levels of tumour-promoting plasma proteins, patients with PD were enriched for inhibitory markers by B cells and CD8 + T cells, suggesting these cells are immune suppressive or less functional. Contrarily, activation markers on CD4 + and CD8 + T cells were highly abundant in PR patients, indicating that systemically activated T cells may contribute to the response to FOLFIRINOX, further highlighting the potential of combination immunotherapy approaches.

A previous study of our group failed to identify serum proteins using Luminex immunoassays that differed between DC and PD patients. However, we did observe elevated levels of IL−1RA in patients with DC, and IL−7, IL−18, and MIP−1β were correlated to OS [32]. Unsurprisingly, the findings of the present study are different from the previous study, which can be explained by the evaluation of plasma vs. serum analyses, the correction for disease stage, the number of cycles of FOLFIRINOX, and using models with continuous protein values instead of groups with arbitrary cut-off. More importantly, we now used a highly sensitive and comprehensive technique for proteomic profiling, resulting in the detection of 368 plasma proteins, as opposed to 11 cytokines.

We acknowledge that this study has several limitations. Firstly, the conclusions regarding the clinical applicability of our identified predictive protein are constrained by the uneven representation of DC and PD PDAC patients in our proteome analysis, and therefore, external validation is needed to accurately determine a cut-off protein value for predicting early progression under FOLFIRINOX. Secondly, full validation that complies with the REMARK guidelines [56] is still needed to determine if these proteins can serve as predictive biomarkers. Thirdly, our results were not validated in a cohort of patients receiving no G-CSF, or any other type of chemotherapy like Gemcitabine/Nab-Pa-CSF was injected 24 h after every FOLFIRINOX cycle, which may affect the immune cell and proteome profile by stimulating granulocyte production in the bone marrow [20]. However, combining G-CSF and FOLFIRINOX is standard practice in the Netherlands to reduce the risk of neutropenia, and G-CSF maladministration could have negative consequences [35]. Therefore, withholding G-CSF administration is unethical. In addition, G-CSF is suggested to have a minimal direct impact on lymphocytes, as the G-CSF receptor is only expressed by myeloid cells. Nonetheless, G-CSF administration may boost myeloid-derived suppressor cells that may have inhibitory effects on T cell proliferation and activation and may have impacted the expression of inhibitory or activating checkpoint molecules on T cells in this study. Fourth, we used blood samples after one cycle to explore the immunological effect of FOLFIRINOX. Although this time point is optimal for early response prediction, longitudinal on-treatment samples would have been preferable to investigate whether FOLFIRINOX's influence on systemic immunity remained consistent or intensified during response evaluation. Furthermore, such an approach would have provided enhanced insight into its predictive influence and the sustained viability of plasma protein biomarkers for long-term prognosis. Fifth, the effect of FOLFIRINOX on intra-tumoral immune cells could not be evaluated due to the lack of tumour tissue material. Establishing a correlation between our peripheral blood findings and changes within the tumour microenvironment is crucial to ensure that the chemotherapy does not hinder local immune cell activation. Finally, due to sample availability restrictions, not all patients underwent both immune cell and proteome profiling, limiting our findings' generalizability between both profiling methods.

5. Conclusion

This study provides evidence that a single cycle of FOLFIRINOX exerts immunological effects that bolster the immune cell-mediated antitumour response in PDAC patients. These findings provide a promising foundation for developing immune-based combination therapies that harness the beneficial immunological properties of FOLFIRINOX. Furthermore, we identified five protein-based liquid biomarkers that hold promise for early prediction of disease progression during FOLFIRINOX treatment. Future studies should validate and examine the utility of these biomarkers in the clinical setting.

Ethics approval and consent to participate

Participating patients in this study originated from the phase III PREOPANC–2 RCT (Dutch trial register NL7094) and the prospective cohort study iKnowIT (Dutch trial register NL7522). Both studies were conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committees of Erasmus MC; the PREOPANC–2 trial (MEC–2018–004, June 2018); the prospective cohort study iKnowIT (MEC–2018–087, February 2019). Written informed consent was obtained from all patients.

Author contributions

CWFvE and MW: conceptualisation, methodology, investigation, formal analysis, visualisation, writing – original draft, and writing – review & editing. GS, EEV, and FS: conceptualisation, methodology, and writing – review & editing. MVe, DM, and MVi: investigation. JGJVA and CHJvE: supervision, project administration, resources, writing – review & editing.

Funding

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

CRediT authorship contribution statement

Casper W.F. van Eijck and **Marcella Willemsen**: Conceptualisation, Methodology, Investigation, Formal analysis, Visualisation, Writing – original draft, and Writing – review & editing. **Gaby Strijk, Eveline E. Vietsch**, and **Fleur van der Sijde**: Conceptualisation, Methodology, and Writing – review & editing. **Maaike Verheij**, **Dana A. M. Mustafa**, and **Madelief Vink**: Investigation. **Joachim G.J.V. Aerts** and **Casper W.F. van Eijck**: Supervision, Project administration, Resources, Writing – review & editing.

Declaration of Competing Interest

J.G.J.V. Aerts reports personal fees and nonfinancial support from MSD, personal fees from BMS, Boehringer Ingelheim, Amphera, Eli-Lilly, Takeda, Bayer, Roche, Astra Zeneca outside the submitted work; in addition, J.G.J.V. Aerts holds ownership interest (including patients) in Amphera BV and is a consultant/advisory board member for Amphera. The other authors declare that they have no competing interests.

Data availability

The datasets used and analysed during the current study are available, with permission of the Erasmus Medical Centre Rotterdam, from the corresponding author on reasonable request.

Acknowledgements

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.113440.

References

[1] Rangarajan K, Pucher PH, Armstrong T, Bateman A, Hamady Z. Systemic neoadjuvant chemotherapy in modern pancreatic cancer treatment: a systematic review and meta-analysis. Ann R Coll Surg Engl 2019;101(7):453–62.
[2] Wolfgang CL, Herman JM, Laheru DA, Klein AP, Erdek MA, Fishman EK, et al. Recent progress in pancreatic cancer. CA Cancer J Clin 2013;63(5):318–48.
[3] Adamska A, Domenichini A, Falasca M. Pancreatic ductal adenocarcinoma: current and evolving therapies. Int J Mol Sci 2017;18(7).
[4] Fan JQ, Wang MF, Chen HL, Shang D, Das JK, Song J. Current advances and outlooks in immunotherapy for pancreatic ductal adenocarcinoma. Mol Cancer 2020;19(1):32.
[5] Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011;364(19):1817–25.
[6] Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med 2013;369(18):1691–703.
[7] Williet N, Saint A, Pointet AL, Tougeron D, Pernot S, Pozet A, et al. Folfirinox versus gemcitabine/nab-paclitaxel as first-line therapy in patients with metastatic pancreatic cancer: a comparative propensity score study. Ther Adv Gastroenterol 2019;12. 1756284819878660.
[8] Ielpo B, Caruso R, Duran H, Diaz E, Fabra I, Malave L, et al. A comparative study of neoadjuvant treatment with gemcitabine plus nab-paclitaxel versus surgery first for pancreatic adenocarcinoma. Surg Oncol 2017;26(4):402–10.
[9] Janssen QP, Buettner S, Suker M, Beumer BR, Addeo P, Bachellier P, et al. Neoadjuvant FOLFIRINOX in patients with borderline resectable pancreatic cancer: a systematic review and patient-level meta-analysis. J Natl Cancer Inst 2019;111(8):782–94.
[10] Murphy JE, Wo JY, Ryan DP, Jiang W, Yeap BY, Drapek LC, et al. Total neoadjuvant therapy with FOLFIRINOX followed by individualized chemoradiotherapy for borderline resectable pancreatic adenocarcinoma: a phase 2 clinical trial. JAMA Oncol 2018;4(7):963–9.
[11] Yamaguchi J, Yokoyama Y, Fujii T, Yamada S, Takami H, Kawashima H, et al. Results of a phase II study on the use of neoadjuvant chemotherapy (FOLFIRINOX or GEM/nab-PTX) for Borderline-resectable pancreatic cancer (NUPAT-01). Ann Surg 2022;275(6):1043–9.
[12] Macedo FI, Ryon E, Maithel SK, Lee RM, Kooby DA, Fields RC, et al. Survival outcomes associated with clinical and pathological response following neoadjuvant FOLFIRINOX or Gemcitabine/Nab-Paclitaxel chemotherapy in resected pancreatic cancer. Ann Surg 2019;270(3):400–13.
[13] Suker M, Beumer BR, Sadot E, Marthey L, Faris JE, Mellon EA, et al. FOLFIRINOX for locally advanced pancreatic cancer: a systematic review and patient-level meta-analysis. Lancet Oncol 2016;17(6):801–10.
[14] Tong YT, Lai Z, Katz MHG, Prakash LR, Wang H, Chatterjee D, et al. Prognosticators for patients with pancreatic ductal adenocarcinoma who received neoadjuvant FOLFIRINOX or Gemcitabine/Nab-Paclitaxel therapy and pancreatectomy. Cancers 2023;15(9).
[15] Wang YJ, Fletcher R, Yu J, Zhang L. Immunogenic effects of chemotherapy-induced tumor cell death. Genes Dis 2018;5(3):194–203.
[16] Mota Reyes C, Teller S, Muckenhuber A, Konukiewitz B, Safak O, Weichert W, et al. Neoadjuvant therapy remodels the pancreatic cancer microenvironment via depletion of protumorigenic immune cells. Clin Cancer Res 2020;26(1):220–31.
[17] Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell 2015;28(6):690–714.
[18] Feig C, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. Clin Cancer Res 2012;18(16):4266–76.
[19] Balachandran VP, Beatty GL, Dougan SK. Broadening the Impact of Immunotherapy to Pancreatic Cancer: Challenges and Opportunities. Gastroenterology 2019;156(7):2056–72.
[20] van Eijck CWF, de Koning W, van der Sijde F, Moskie M, Groot Koerkamp B, Homs MYV, et al. A multigene circulating biomarker to predict the lack of FOLFIRINOX response after a single cycle in patients with pancreatic ductal adenocarcinoma. Eur J Cancer 2023;181:119–34.
[21] Peng H, James CA, Cullinan DR, Hogg GD, Mudd JL, Zuo C, et al. Neoadjuvant FOLFIRINOX therapy is associated with increased effector T cells and reduced suppressor cells in patients with pancreatic cancer. Clin Cancer Res 2021;27(24):6761–71.
[22] Wu MY, Shen M, Xu MD, Yu ZY, Tao M. FOLFIRINOX regulated tumor immune microenvironment to extend the survival of patients with resectable pancreatic ductal adenocarcinoma. Gland Surg 2020;9(6):2125–35.
[23] Hwang WL, Jagadeesh KA, Guo JA, Hoffman HI, Yadollahpour P, Reeves JW, et al. Single-nucleus and spatial transcriptome profiling of pancreatic cancer identifies multicellular dynamics associated with neoadjuvant treatment. Nat Genet 2022;54(8):1178–91.
[24] Farren MR, Sayegh L, Ware MB, Chen HR, Gong J, Liang Y, et al. Immunologic alterations in the pancreatic cancer microenvironment of patients treated with neoadjuvant chemotherapy and radiotherapy. JCI Insight 2020;5(1).
[25] Tesniere A, Schlemmer F, Boige V, Kepp O, Martins I, Ghiringhelli F, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene 2010;29(4):482–91.
[26] Stojanovska V, Prakash M, McQuade R, Fraser S, Apostolopoulos V, Sakkal S, et al. Oxaliplatin treatment alters systemic immune responses. Biomed Res Int 2019;2019:4650695.
[27] Rojas LA, Sethna Z, Soares KC, Olcese C, Pang N, Patterson E, et al. Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. Nature 2023:1–7.
[28] Conroy T, Hammel P, Hebbar M, Ben Abdelghani M, Wei AC, Raoul JL, et al. FOLFIRINOX or gemcitabine as adjuvant therapy for pancreatic cancer. N Engl J Med 2018;379(25):2395–406.
[29] Perri G, Prakash L, Qiao W, Varadhachary GR, Wolff R, Fogelman D, et al. Response and survival associated with First-line FOLFIRINOX vs gemcitabine and nab-Paclitaxel chemotherapy for localized pancreatic ductal adenocarcinoma. JAMA Surg 2020;155(9):832–9.
[30] van der Sijde F, Vietsch EE, Mustafa DAM, Besselink MG, Groot Koerkamp B, van Eijck CHJ. Circulating biomarkers for prediction of objective response to chemotherapy in pancreatic cancer patients. Cancers 2019;11(1).
[31] Luo G, Jin K, Deng S, Cheng H, Fan Z, Gong Y, et al. Roles of CA19-9 in pancreatic cancer: biomarker, predictor and promoter. Biochim Biophys Acta Rev Cancer 2021;1875(2):188409.
[32] van der Sijde F, Dik WA, Mustafa DAM, Vietsch EE, Besselink MG, Debets R, et al. Serum cytokine levels are associated with tumor progression during FOLFIRINOX chemotherapy and overall survival in pancreatic cancer patients. Front Immunol 2022;13:898498.
[33] Rapisuwon S, Vietsch EE, Wellstein A. Circulating biomarkers to monitor cancer progression and treatment. Comput Struct Biotechnol J 2016;14:211–22.
[34] Vietsch EE, Graham GT, McCutcheon JN, Javaid A, Giaccone G, Marshall JL, et al. Circulating cell-free DNA mutation patterns in early and late stage colon and pancreatic cancer. Cancer Genet 2017;218–219:39–50.
[35] Macaire P, Paris J, Vincent J, Ghiringhelli F, Bengrine-Lefevre L, Schmitt A. Impact of granulocyte colony-stimulating factor on FOLFIRINOX-induced neutropenia prevention: a population pharmacokinetic/pharmacodynamic approach. Br J Clin Pharm 2020;86(12):2473–85.
[36] Assarsson E, Lundberg M, Holmquist G, Bjorkesten J, Thorsen SB, Ekman D, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One 2014;9(4):e95192.
[37] Whelan CD, Mattsson N, Nagle MW, Vijayaraghavan S, Hyde C, Janelidze S, et al. Multiplex proteomics identifies novel CSF and plasma biomarkers of early Alzheimer's disease. Acta Neuropathol Commun 2019;7(1):169.
[38] OlinkProteomics. White paper - Data normalization and standardization. 2021.
[39] Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. Nat Commun 2017;8(1):1826.
[40] Sams L, Kruger S, Heinemann V, Bararia D, Haebe S, Alig S, et al. Alterations in regulatory T cells and immune checkpoint molecules in pancreatic cancer patients receiving FOLFIRINOX or gemcitabine plus nab-paclitaxel. Clin Transl Oncol 2021;23(11):2394–401.
[41] Buchan SL, Rogel A, Al-Shamkhani A. The immunobiology of CD27 and OX40 and their potential as targets for cancer immunotherapy. Blood 2018;131(1):39–48.
[42] Zhang Q, Rui W, Jiang Y, Yuan F, Chen Y, Guo X, et al. Tumor-infiltrating OX40(+) lymphocytes is an independent positive prognostic factor for patients with pancreatic ductal adenocarcinoma. Clin Transl Oncol 2022;24(10):2029–38.
[43] Chen X, Ma H, Mo S, Zhang Y, Lu Z, Yu S, et al. Analysis of the OX40/OX40L immunoregulatory axis combined with alternative immune checkpoint molecules in pancreatic ductal adenocarcinoma. Front Immunol 2022;13:942154.
[44] Lutz V, Hellmund VM, Picard FSR, Raifer H, Ruckenbrod T, Klein M, et al. IL18 Receptor signaling regulates tumor-reactive CD8+ T-cell exhaustion via activation of the IL2/STAT5/mTOR pathway in a pancreatic cancer model. Cancer Immunol Res 2023;11(4):421–34.
[45] Steel JC, Waldmann TA, Morris JC. Interleukin-15 biology and its therapeutic implications in cancer. Trends Pharmacol Sci 2012;33(1):35–41.
[46] Van Audenaerde JRM, De Waele J, Marcq E, Van Loenhout J, Lion E, Van den Bergh JMJ, et al. Interleukin-15 stimulates natural killer cell-mediated killing of both human pancreatic cancer and stellate cells. Oncotarget 2017;8(34):56968–79.
[47] Costache MI, Ioana M, Iordache S, Ene D, Costache CA, Saftoiu A. VEGF Expression in pancreatic cancer and other malignancies: a review of the literature. Rom J Intern Med 2015;53(3):199–208.
[48] Sato M, Matsumoto M, Saiki Y, Alam M, Nishizawa H, Rokugo M, et al. BACH1 Promotes pancreatic cancer metastasis by repressing epithelial genes and enhancing epithelial-mesenchymal transition. Cancer Res 2020;80(6):1279–92.
[49] Yang X, Ren H, Guo X, Hu C, Fu J. The expressions and mechanisms of sarcomeric proteins in cancers. Dis Markers 2020;2020:8885286.
[50] Reader CS, Vallath S, Steele CW, Haider S, Brentnall A, Desai A, et al. The integrin alphavbeta6 drives pancreatic cancer through diverse mechanisms and represents an effective target for therapy. J Pathol 2019;249(3):332–42.
[51] Park MH, Jo M, Kim YR, Lee CK, Hong JT. Roles of peroxiredoxins in cancer, neurodegenerative diseases and inflammatory diseases. Pharmacol Ther 2016;163:1–23.
[52] Arndt B, Krieger T, Kalinski T, Thielitz A, Reinhold D, Roessner A, et al. The transmembrane adaptor protein SIT inhibits TCR-mediated signaling. PLoS One 2011;6(9):e23761.

[53] Sanchez ML, Covenas R. The Galaninergic system: a target for cancer treatment. Cancers 2022;14(15).
[54] Chistiakov DA, Chistiakov AP. Is FCRL3 a new general autoimmunity gene? Hum Immunol 2007;68(5):375–83.
[55] Swainson LA, Mold JE, Bajpai UD, McCune JM. Expression of the autoimmune susceptibility gene FcRL3 on human regulatory T cells is associated with dysfunction and high levels of programmed cell death-1. J Immunol 2010;184(7):3639–47.
[56] McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, et al. REporting recommendations for tumour MARKer prognostic studies (REMARK). Eur J Cancer 2005;41(12):1690–6.