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Fibroblast-associated protein (FAP) as immunohistochemical prognostic marker after neoadjuvant therapy in Pancreatic Carcinoma – an exploratory study

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Abstract

Purpose

Induction chemotherapy is a rather new approach for therapy of Pancreatic adenocarcinoma (PDAC), enabling secondary resectability and an R0-resection as only curative approach to this highly lethal disease. However, chemotherapy-response varies greatly between individuals and is difficult to evaluate by conventional imaging. Molecules within PDACs extensive stroma, such as Fibroblast activation protein (FAP), offer the possibility of molecular markers for evaluation of chemotherapy response.

Methods

All patients enrolled in our retrospective study underwent surgical tumor resection with one cohort undergoing induction chemotherapy (ICTx) and a matched cohort undergoing upfront resection (US). Survival data derived from the surgical database of Heidelberg University Hospital were compared with histopathological data and FAP immunohistochemistry of tumor tissue derived during resection.

Results

The ICTx-cohort showed a lower FAP-Expression than the US-cohort and our results indicated a higher stromal component within the ICTx-cohort. Whilst within US no correlation between FAP-expression and stromal component could be made, within ICTx higher FAP levels correlated with a smaller stromal component. FAP-dependent survival also differed between the cohorts: Within US higher FAP levels were associated with shorter survival, while ICTx showed higher FAP levels correlating with improved survival.

Conclusion

Our results indicate that FAP may be a relevant marker for response to induction chemotherapy in PDAC. It may give significant information on patient survival, depending on clinical context. These preliminary findings need to be confirmed in larger studies.

Introduction

Within the past years, there has been an increasing incidence and a rising number of deaths due to pancreatic cancer. Since pancreatic adenocarcinoma (PDAC) has the worst 5-year-survival rate of all cancers, there is an urgent need for new therapeutic strategies (Siegel, 2023). To date, the only curative approach is a resection of the tumor (Strobel, 2017 and Leonhardt, 2022). However, only 10–20% of patients are diagnosed with a primarily resectable disease (Hackert, 2013). The remaining patients have cancers classified as borderline-resectable or locally-advanced, possibly with distant metastases, which need neoadjuvant therapy to reach a secondary resectability (Hackert, 2016 and Seufferlein, 2022). While adjuvant therapies have long been established as standard of care (Springfeld, 2019 and Oettle, 2018) and have been shown to significantly improve survival (Neoptolemos, 2001 and Neoptolemos, 2012 and Neoptolemos, 2017), neoadjuvant or inductive chemotherapies are a rather new approach. The major advantage of neoadjuvant therapies is a better local tumor control, including down-sizing and down-staging, leading to secondary resectability with higher R0-resection rates and less surgical complications (Ferrone, 2015). However, the chemotherapy regimens used, naturally come with the risk of toxicity and side effects, while prolonging the curative surgery and not all patients seem to respond equally well to this

approach (Springfeld, 2020). Since PDACs consist of a large stromal component, it is furthermore difficult to evaluate the tumors response to chemotherapy and thus qualification for surgery after chemotherapy, as conventional imaging, such as computed tomography (CT)and magnetic resonance imaging (MRI), cannot distinguish between vital tumor stroma and post-chemotherapeutic fibrosis (Ferrone, 2015).

Recent studies raise hope of finding molecular markers, predicting the patients' response to chemotherapy (Muckenhuber, 2018 and Collisson, 2011). Molecular markers could enable upfront patient stratification and allow for a distinction between those, possibly responding well to chemotherapy, and those, who might not benefit from it.

As a marker protein of cancer-associated fibroblasts (CAFs), fibroblast associated protein (FAP) constitutes a promising candidate for stratification of the tumor stroma (Grünwald, 2021). FAP is an enzyme expressed during embryonic development (Niedermeyer, 2001 and Rettig, 1988). In healthy human adults it is found in tissues undergoing remodelling processes, such as wound healing (Rettig, 1988), as well as in the pancreas' Langerhans-islets (Busek, 2015 and Rettig, 1988).

Past experiments have already revealed a correlation between high FAP-expression and a higher clinical stage (Shi, 2012), positive lymph nodes, positive distant metastases, increased growth (Lo, 2017 and Wen, 2019), as well as invasion (Wen, 2019). Moreover, FAP-overexpression has been shown to be a prognostic factor for a worse outcome, particularly a poorer survival and higher recurrence rates (Shi, 2012 and Cohen, 2008 and Wen, 2019 and Lo, 2017). Other studies, however, were able to show a positive correlation between FAP-expression and survival (Park, 2017), suggesting that the contribution of FAP to tumor development is not yet fully understood.

In recent years, FAP has been probed not only as a therapy target (Hamson, 2014 and Loeffler, 2006 and Yu, 2020), but furthermore as radio-labelled tracer for an imaging modality called fibroblast associated protein inhibitor – positron emission tomography/ computed tomography (FAPI-PET/CT) (Kratochwil, 2019 and Giesel, 2019). This imaging modality might revolutionize diagnosis and staging of PDACs, overcoming the weaknesses of CT and MRI (Loktev, 2018 and Röhrich, 2021). Furthermore, it allows to determine the intratumoral FAP-expression. Recent studies on untreated PDAC and its precursors demonstrated a positive correlation between FAPI-PET/CT-parameters and immunohistochemical FAP-expression in pancreatic lesions (Spektor, 2024). Given the high stromal expression of untreated PDAC, it appears that firstly, changes in FAP-expression may display therapy-induced stromal damage and secondly, FAP-expression of primary PDAC may be useful as a predictive marker for the response of PDAC to neoadjuvant chemotherapy.

The aim of this pilot study was to determine FAP-expression of primarily resected PDAC and PDAC after neoadjuvant chemotherapy to gain a potential rationale for the use of FAPI/PET-CT in PDAC in the context of neoadjuvant chemotherapies.

Materials and Methods

3.1. Patient cohorts

For this retrospective study, the surgical database of Heidelberg University Hospital was screened for patients, who presented with a primarily unresectable PDAC and received neoadjuvant chemotherapy prior to their operation between February 2013 and January 2021. A matched cohort of primarily resected patients was found and included as the Upfront Surgery- (US)cohort. Overall, 117 patients were included in the study: 66 as part of the US-cohort and 51 as part of the induction chemotherapy- (ICTx-)cohort. All

procedures performed in studies involving human participants were approved by the local ethics committee and carried out in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study received approval from the internal review board (Ethical Committee) of the University hospital Heidelberg (Study number S-115/2020). Informed consent for participation was obtained from all individual participants included in the study.

Grading, as well as staging of tumors followed the World Health Organizations (WHO) recommendations, using the 8th edition of the TNM-classification (Brierley, 2023, S.218–227). Clinical data were obtained from the surgical database of Heidelberg University Hospital and, if missing, obtained from the patients' general practitioners or relatives. The observation period started with the patients' initial diagnosis and overall survival (OS) was defined as time between initial diagnosis and date of death.

3.2. FAP-IHC

Immunohistochemistry (IHC) was performed on formalin-fixed paraffin-embedded PDAC slides, which were obtained from the Tissue Bank of the National Center for Tumor Diseases (NCT) Heidelberg, the Institute of Pathology of the University Hospital Heidelberg and the Tissue Bank of the European Pancreas Center in Heidelberg (EPZ). Experienced pathologists reassured that tumor samples were PDACs and of high quality. For conduction of immunohistochemistry, the slides were deparaffinized with Roticlear (Carl Roth GmbH + Co KG) and rehydrated with descending alcohol series. A ph9 buffer (Buffer Substrate DAKO 10x, S2375), diluted 1:10, was added for antigen retrieval and after washing the slides, they were incubated with 3% peroxidase (225ml Methanol + 25ml H_2O_2; Methanol Rotipuran ≥ 99,8% for analysis and Hydrogen Peroxide 30% for analysis Labchem international) to block endogenous peroxidase-activity. After further washing with 1xTBS/0,1%BSA, the slides were encircled with Dako Pen (Dako Denmark A/S, S2002) as a hydrophobic barrier. Next, slides were incubated with a blocking solution (Power Block, Universal Blocking Reagent 10x, BioGenex) to prevent unspecific antibody binding.

Following, the primary antibody ab207178 (Abcam Monoclonal rabbit anti-human Fibroblast activation protein [ERP20021]) was diluted 1:200, applied and incubated over night at 4°C. On the next day, after further washing steps, the secondary antibody (Dako Envision+ System-HRP Labelled Polymer Anti-Rabbit, code K4003) was applied and incubated for 45 minutes at room temperature. Following additional washing steps, the color reaction was conducted with liquid 3–3'-diaminobenzidin (Dako Liquid DAB + Substrate Chromogen System, K3468) for 120 seconds. The reaction was stopped with distilled water.

Subsequently counter staining was done with Mayers hemalaun solution (Mayers hemalaun solution, Merck KGaA) for 10 seconds. The counter staining was stopped with tap water and the slides were left under the running sink for another 10 minutes.

Finally, the slides were dehydrated again with an increasing alcohol series and Roticlear, mounted with Permount Mounting Medium (Catalog no. 17986-01) and left to dry overnight at 37°C.

A positive control was included using the Langerhans-islets of the pancreas.

3.3. Analysis of FAP-immunohistochemistry

Evaluation of the stained slides was done by an experienced board-certified pathologist (EG) and a second independent observer (LS), who reached a high level of interobserver reliability.

For evaluating the FAP-expression of the tumor slides, we considered the intensity of staining, as well as the percentage of FAP-positively stained cells within the tumor sample. The intensity was scaled from 0–3: 0 meaning no FAP-positive staining, 1 meaning weak positivity, 2 meaning moderate positivity and 3

meaning strong positive staining. The quantity of FAP-positive cells was evaluated as the percentage of FAP-positive stroma.

3.4. Ethics approval

The study was approved by the Heidelberg University Hospital Institutional Ethical Review Board (S115-2020) and conducted corresponding to the Institutional Ethics Committee guidelines. The study was performed according to the Declaration of Helsinki and all patients gave written informed consent for the scientific use as well as the publication of their data.

3.5. Statistical analysis

Statistical analysis was conducted with SPSS Statistics software (V.27; IBM Corporation, USA) and GraphPad Prism (version 10.0.0; GraphPad Software, San Diego, CA, USA). Survival curves were compared via Kaplan-Meier analysis and log-rank test. Chi-square was used for comparing binominal variables, while Mann-Whitney-U-test was used to compare binominal and ordinal or continuous variables. Spearman correlation coefficient was used for comparing continuous variables with other continuous variables. Kruskal-Wallis-test was used for comparing continuous variables with ordinal-scaled variables. $P < 0,05$ was considered statistically significant.

Results

4.1. Induction chemotherapy increases the stromal component, but reduces the FAP-expression in PDAC

After ICTx, tumors showed a higher average stromal percentage (70%) compared to upfront surgery (60%) (Fig. 1). However, this difference did not reach statistical significance as determined by the Mann-Whitney-U-test ($p = 0.261$).

Next, we evaluated FAP-expression within the ICTx- and the US-cohort by looking at FAP-intensity as well as the percentage of FAP-positive cells.

The patients receiving upfront surgery showed a median FAP percentage of 45%, while patients undergoing induction chemotherapy presented a median FAP percentage of 30%. As depicted in Fig. 2a this indicates a higher percentage of FAP-positive cells within the US-cohort, while Mann-Whitney-U-Test could not reach statistical significance ($p = 0.164$).

Looking at FAP values above, respectively below, the mean of the percentage of FAP-positive cells, 15.2% of US-patients presented FAP values below the mean, while 84.8% showed FAP values above the mean.

Within the ICTx-cohort 31.4% had a FAP expression below the mean while 68.6% presented with a FAP values above the mean. Chi-square test revealed a statistical significance ($p = 0.036$) as visualized in Fig. 2b.

Within the US-cohort 6.1% of patients presented with a FAP-intensity of 0, 10.6% showed a FAP-intensity of 1, 21.2% a FAP-intensity of 2 and 62.1% a FAP-intensity of 3.

Looking at the ICTx-patients 3.9% presented a FAP-intensity of 0, 19.6% a FAP-intensity of 1, 23.5% a FAP-intensity of 2 and 52.9% a FAP-intensity of 3.

While the bar chart pictured in Fig. 2c indicated higher FAP-intensities being more common among US-patients, the chi-square test showed no statistical significance ($p = 0.503$).

Figure 3 exemplifies an immunohistochemical staining for evaluation of FAP-expression of an US- and an ICTx-patient.

4.2. A negative correlation between FAP expression and stromal component can only be shown within the ICTx-cohort

To further investigate differences in FAP expression and stromal content between the two cohorts, we assessed the correlation between FAP expression and stromal percentage within each group. In the US-cohort, no statistically significant correlation was observed between FAP expression and stromal percentage, whether analyzed as continuous variables or by comparing stromal percentage across FAP expression quartiles (Fig. 4a) and 4c)). Neither the Spearman correlation ($p = 0.189$) nor the Kruskal-Wallis test ($p = 0.311$) indicated statistical significance.

In contrast, the ICTx-cohort showed a trend toward higher FAP expression with decreasing stromal percentage, as illustrated in Fig. 4b) and d). While the Spearman correlation did not reach statistical significance ($p = 0.075$), analysis using FAP expression quartiles revealed a statistically significant difference in stromal percentage, as determined by the Kruskal-Wallis test ($p = 0.045$) and shown in Fig. 4d).

4.3. FAP influences survival differently within patients receiving induction chemotherapy and those undergoing upfront resection

As FAPs influence on survival has not yet been entirely understood, especially regarding induction chemotherapy, we wanted to evaluate what the differences in FAP-expression after neoadjuvant chemotherapy meant for patients' survival and thus prognosis.

When comparing FAP-associated overall survival within the US- and ICTx-cohort, opposing trends were observed: In the US-cohort, patients with FAP expression below the mean demonstrated a longer median overall survival (19.8 months; 95% CI: 12.82–26.78) compared to those with FAP expression above the mean (19.07 months; 95% CI: 15.52–22.62). This difference was not statistically significant based on Kaplan–Meier analysis ($p = 0.459$), as depicted in Fig. 5a.

Conversely, in the ICTx-cohort, patients with FAP expression above the mean showed improved overall survival compared to those with FAP expression below the mean. Patients with higher FAP levels showed a median overall survival of 34.37 months (95% CI: 22.32–46.42), whereas those with lower FAP expression had a median overall survival of 23.1 months (95% CI: 16.34–29.86). This difference also did not reach statistical significance in Kaplan–Meier analysis ($p = 0.116$), as depicted in Fig. 5b.

Discussion

In this study, we analyzed PDAC samples by assessing stromal content and FAP expression through immunohistochemical staining. We compared patients undergoing upfront surgery and patients receiving an induction chemotherapy and were able to show that induction chemotherapy increases the stromal content while reducing FAP expression. Further, our results indicated that the patients undergoing upfront surgery showed a different FAP-dependent survival than those receiving induction chemotherapy.

Our results indicated that patients receiving neoadjuvant chemotherapy present with a higher stromal component compared to those undergoing upfront surgery. While this may suggest an increase in vital tumor stroma, it is more likely a consequence of post-chemotherapeutic fibrosis, resulting in a reduction of

vital tumor and stromal cells. As described in the introduction, one of the main challenges in PDAC therapy is evaluating the tumors' response to neoadjuvant therapy using standard imaging techniques, such as CT and MRI, as the vital tumor stroma cannot be distinguished from post-chemotherapeutic fibrosis.

While comparing FAP-intensity and FAP expression as percentage of staining between the two cohorts merely showed a trend towards a higher FAP expression within the US-cohort. When looking at the FAP-percentage divided into values above, respectively below, the mean, however, we were able to show a significantly lower FAP-expression in patients receiving neoadjuvant chemotherapy compared with patients receiving upfront surgery. This indicates, that FAP-expression is reduced by chemotherapy administration and supports our thesis that chemotherapy reduces the amount of vital tumorstroma – represented by FAP-expression as a marker protein of vital stromal fibroblasts in PDAC (Grünwald, 2021).

When analyzing the correlations between FAP expression and stromal content within the two cohorts, distinct patterns emerged:

While no direct correlation or trend was observed between FAP expression and stromal component in the US-cohort, the ICTx-cohort demonstrated a negative correlation. This finding further supports our hypothesis that the increase in stromal content following neoadjuvant chemotherapy is more likely attributable to post-chemotherapeutic fibrosis, as FAP has been described as a marker for vital tumor stroma (Grünwald, 2021), as outlined in the introduction. The absence of a correlation within the US-cohort may be explained by the heterogeneous nature of PDAC stromal cells, which express varying markers and characteristics.

Our results indicate, that while patients undergoing upfront surgery show better survival rates when their FAP-expression is below the mean, patient receiving induction chemotherapy have a better overall survival when presenting with a FAP-expression above the mean.

This trend shown within the US-cohort supports the thesis that FAP is a marker of vital tumor stroma and thus an indicator for worse survival.

The reversal of this trend in the ICTx -cohort exemplifies the impact of induction chemotherapy on tumor stroma and its components. Furthermore, it challenges our hypothesis that FAP serves as a marker for vital tumor stroma in comparison to post-chemotherapeutic fibrosis. If FAP were indeed a marker of vital tumor stroma, a higher FAP percentage would be expected to correlate with worse overall survival, while a lower FAP percentage would suggest increased post-chemotherapeutic fibrosis and consequently better survival.

This observation may suggest, that the role of FAP changes after chemotherapy, with it potentially being tumor-promoting in native tumors and tumor-restraining in tumors following induction chemotherapy. It is possible that FAP acts as a mediator, transmitting tumor-promoting signals in untreated tumors, while after chemotherapy, the signaling pathways shift, causing FAP to mediate a different pathway with tumor-restraining properties.

Other recent studies were able to define different subsets of molecular stroma types, influencing response to chemotherapy and prognosis. Muckenhuber et al (Muckenhuber, 2018) defined three subtypes based on hepatocyte nuclear factor 1 homebox A(HNF1A) and Keratin 81 (KRT81): the HNF1A-positive, the KRT81-positive and the double negative. Comparing the subtypes revealed significant differences in survival and response to chemotherapy. Collisson et al (Collisson, 2011) likewise proposed a scheme regarding three different subtypes, called the classical, quasi-mesenchymal, and exocrine-like subtype. These three subtypes were shown to be predictive for the tumor's response to chemotherapy, with different sensitivities towards Gemcitabine.

Possibly, FAP serves as a marker for vital tumor cells and thus worse survival in chemotherapy-native tumors, while also being a marker for better chemotherapy response. FAP could gain tumor-restraining properties through the influence of chemotherapy and thus correlate with better survival.

ICTx-cohort n = 51; US-cohort n = 66. Mann-Whitney-U-Test p = 0.261

a) Comparison of FAP percentage; b) Comparison of FAP-values above/ below the mean; c) comparison of FAP-intensity. a) ICTx-cohort n = 51, US-cohort n = 66, Mann-Whitney-U-Test p = 0.164; b) ICTx-cohort FAP-value above mean n = 35, ICTx-cohort FAP-value below meann = 16, US-cohort FAP-value above mean n = 56, US-cohort FAP-value below meann = 10, chi-square p = 0.036. c) ICTx-cohort FAP-intensity 0 n = 2, ICTx-cohort FAP-intensity 1 n = 10, ICTx-cohort FAP-intensity 2 n = 12, ICTx-cohort FAP-intensity 3 n = 27, US-cohort FAP-intensity 0 n = 4, US-cohort FAP-intensity 1 n = 7, US-cohort FAP-intensity 2 n = 14, US-cohort FAP-intensity 3 n = 41; chi-square p = 0.503

a) US-cohort n = 66, Spearman-correlation coefficient p = 0.189; b) ICTx-cohort n = 51, Spearman-correlation coefficient p = 0.075; c) US quartiles n = 16, Kruskal-Wallis-test p = 0.311; d) ICTx-quartiles n = 12, Kruskal-Wallis-test p = 0,045

a) US-patients above/below mean of FAP percentage n = 33, log rank test p = 0.459; b) ICTx-patients above/below mean of FAP percentage n = 25, log rank test p = 0.116

Limitations

Although the results of our exploratory study are promising, several limitations must be acknowledged. A major limitation is the use of semiquantitative analysis, which introduces the potential for interobserver variability. Future studies employing computer-assisted evaluation of staining results may offer a more precise approach. Another limitation is the relatively small sample size, particularly when further dividing into subgroups. Additionally, as a single-center study, our findings may not be fully representative of the broader population, given potential differences in chemotherapy protocols and surgical procedures.

In conclusion, while our findings provide valuable directional insights, they should be validated by larger cohort studies and prospective trials, such as controlled clinical studies.

Conclusion

We concluded an exploratory study correlating immunohistochemical FAP-expression with other histopathological and clinical patient characteristics whilst comparing patients receiving an induction chemotherapy and patients undergoing upfront surgery. We were able to show differences in FAP-expression and stromal component between the two cohorts with US-patients showing higher levels of FAP-expression as well as a smaller stromal component. This might indicate that induction chemotherapy reduces FAP expression, as a marker protein for vital tumor stroma, and increases the stromal component, most likely representing post chemotherapeutic fibrosis.

This in turn might indicate FAP as a marker for vital tumor stroma, possibly suitable for distinguishing between post-chemotherapeutic fibrosis and vital tumor stroma, indicating the tumors response to chemotherapy and thus its suitability for secondary resectability.

Declarations

Competing Interests: There were no potential conflicts of interest. The authors did not receive support from any organization for the submitted work. The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: All procedures performed in studies involving human participants were approved by the local internal review board (Ethics committee) and carried out in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study received approval from the Ethical Committee of the University hospital Heidelberg (Study number S-115/2020).

Informed consent: Informed consent for participation was obtained from all individual participants included in the study, and the investigation was conducted according to national regulatory laws.

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Author contribution statement: Data were obtained from the database by L. Schillings und U. Heger. L. Schillings carried out the immunohistochemical stainings. Pathohistological and immunohistochemical evaluation was done by E. Gutjahr and L. Schillings. Statistical analysis was done by L. Schillings and U. Heger. L. Schillings wrote the manuscript with support from M. Röhrich, T. Hackert and A. Spektor. U. Heger, T. Hackert and M. Büchler helped supervise the project. T. Hackert and U. Heger conceived the original idea.

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11. Data Availability Statement

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are in the controlled access surgical database of University Heidelberg Hospital.

References

1. Brierley, J., Asamura, H., Eycken, E. & Rous, B. TNM-Atlas: ein illustrierter Leitfaden zur *TNM Klassifikation maligner Tumoren*, (2023). https://ebookcentral.proquest.com/lib/ubheidelberg/detail.action?docID=30810008, [17.01.2024], 7. Aufl., Wiley-VCH, Weinheim, 611 Seiten. S.218–227.
2. Busek, P., Hrabal, P., Fric, P. & Sedo, A. Co-expression of the homologous proteases fibroblast activation protein and dipeptidyl peptidase-IV in the adult human Langerhans islets. *Histochem. Cell. Biol.* **143**, 497–504. 10.1007/s00418-014-1292-0 (2015).
3. Cohen, S. J. et al. Fibroblast activation protein and its relationship to clinical outcome in pancreatic adenocarcinoma. *Pancreas* **37**, 154–158. 10.1097/MPA.0b013e31816618ce (2008).

4. Collisson, E. A. et al. J. W. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. *Nat Med* 17, 500–503, (2011). 10.1038/nm.2344
5. Ferrone, C. R. et al. Radiological and surgical implications of neoadjuvant treatment with FOLFIRINOX for locally advanced and borderline resectable pancreatic cancer. *Ann. Surg.* **261**, 12–17. 10.1097/sla.0000000000000867 (2015).
6. Giesel, F. L. et al. 68)Ga-FAPI PET/CT: Biodistribution and Preliminary Dosimetry Estimate of 2 DOTA-Containing FAP-Targeting Agents in Patients with Various Cancers. *J. Nucl. Med.* **60**, 386–392. 10.2967/jnumed.118.215913 (2019).
7. Grünwald, B. T. et al. Spatially confined sub-tumor microenvironments in pancreatic cancer. *Cell* **184** (e5518), 5577–5592. 10.1016/j.cell.2021.09.022 (2021).
8. Hackert, T. & Büchler, M. W. Pancreatic cancer: advances in treatment, results and limitations. *Dig. Dis.* **31**, 51–56. 10.1159/000347178 (2013).
9. Hackert, T. et al. Locally Advanced Pancreatic Cancer: Neoadjuvant Therapy With Folfirinox Results in Resectability in 60% of the Patients. *Ann. Surg.* **264**, 457–463. 10.1097/sla.0000000000001850 (2016).
10. Hamson, E. J., Keane, F. M., Tholen, S., Schilling, O. & Gorrell, M. D. Understanding fibroblast activation protein (FAP): substrates, activities, expression and targeting for cancer therapy. *Proteom. Clin. Appl.* **8**, 454–463. 10.1002/prca.201300095 (2014).
11. Kratochwil, C. et al. (68)Ga-FAPI PET/CT: Tracer Uptake in 28 Different Kinds of Cancer. *J. Nucl. Med.* **60**, 801–805. 10.2967/jnumed.119.227967 (2019).
12. Leonhardt, C. S. et al. Prognostic relevance of the revised R status definition in pancreatic cancer: meta-analysis. *BJS Open.* **6** 10.1093/bjsopen/zrac010 (2022).
13. Lo, A. et al. Fibroblast activation protein augments progression and metastasis of pancreatic ductal adenocarcinoma. *JCI Insight*. **2** 10.1172/jci.insight.92232 (2017).
14. Loeffler, M., Krüger, J. A., Niethammer, A. G. & Reisfeld, R. A. Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. *J Clin Invest* 116, 1955 1962, (2006). 10.1172/jci26532
15. Loktev, A. et al. A Tumor-Imaging Method Targeting Cancer-Associated Fibroblasts. *J. Nucl. Med.* **59**, 1423–1429. 10.2967/jnumed.118.210435 (2018).
16. Muckenhuber, A. et al. Pancreatic Ductal Adenocarcinoma Subtyping Using the Biomarkers Hepatocyte Nuclear Factor-1A and Cytokeratin-81 Correlates with Outcome and Treatment Response. *Clin. Cancer Res.* **24**, 351–359. 10.1158/1078-0432.Ccr-17-2180 (2018).
17. Neoptolemos, J. P. et al. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. *Lancet* **358**, 1576–1585. 10.1016/s0140-6736(01)06651-x (2001).
18. Neoptolemos, J. P. et al. Effect of adjuvant chemotherapy with fluorouracil plus folinic acid or gemcitabine vs observation on survival in patients with resected periampullary adenocarcinoma: the ESPAC-3 periampullary cancer randomized trial. *Jama* **308**, 147–156. 10.1001/jama.2012.7352 (2012).
19. Neoptolemos, J. 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* **389**, 1011–1024. 10.1016/s0140-6736(16)32409-6 (2017).
20. Niedermeyer, J. et al. Expression of the fibroblast activation protein during mouse embryo development. *Int. J. Dev. Biol.* **45**, 445–447 (2001).
21. Oettle, H. et al. Pankreaskarzinom. URL: (2018). https://www.onkopedia.com/de/onkopedia/guidelines/pankreaskarzinom/@@guideline/html/index.html [Stand:31.08.2021].

22. Park, H. et al. The prognostic significance of cancer-associated fibroblasts in pancreatic ductal adenocarcinoma. *Tumour Biol.* **39**, 1010428317718403. 10.1177/1010428317718403 (2017).
23. Rettig, W. J. et al. Cell-surface glycoproteins of human sarcomas: differential expression in normal and malignant tissues and cultured cells. *Proc. Natl. Acad. Sci. U S A.* **85**, 3110–3114. 10.1073/pnas.85.9.3110 (1988).
24. Röhrich, M. et al. Impact of (68)Ga-FAPI PET/CT Imaging on the Therapeutic Management of Primary and Recurrent Pancreatic Ductal Adenocarcinomas. *J. Nucl. Med.* **62**, 779–786. 10.2967/jnumed.120.253062 (2021).
25. Seufferlein, T. et al. S3-Leitlinie zum exokrinen Pankreaskarzinom – Kurzversion 2.0 – Dezember 2021. *AWMF Z Gastroenterol.* **60**, 991–1037. 10.1055/a-1771-6811 (2022).
26. Shi, M. et al. Expression of fibroblast activation protein in human pancreatic adenocarcinoma and its clinicopathological significance. *World J. Gastroenterol.* **18**, 840–846. 10.3748/wjg.v18.i8.840 (2012).
27. Siegel, R. L., Miller, K. D., Wagle, N. S. & Jemal, A. Cancer statistics, 2023. *CA Cancer J. Clin.* **73**, 17–48. 10.3322/caac.21763 (2023).
28. Spektor, A. M. et al. Immunohistochemical FAP Expression Reflects (68)Ga-FAPI PET Imaging Properties of Low- and High-Grade Intraductal Papillary Mucinous Neoplasms and Pancreatic Ductal Adenocarcinoma. *J. Nucl. Med.* **65**, 52–58. 10.2967/jnumed.123.266393 (2024).
29. Springfeld, C., Hackert, T., Jäger, D., Büchler, M. W. & Neoptolemos, J. P. Neoadjuvant and adjuvant treatment of pancreatic cancer. *Chirurg* 10.1007/s00104-020-01169-9 (2020).
30. Springfeld, C. et al. Chemotherapy for pancreatic cancer. *Presse Med.* **48**, e159–e174. 10.1016/j.lpm.2019.02.025 (2019).
31. Strobel, O. et al. Pancreatic Cancer Surgery: The New R-status Counts. *Ann. Surg.* **265**, 565–573. 10.1097/sla.0000000000001731 (2017).
32. Wen, Z. et al. Fibroblast activation protein α-positive pancreatic stellate cells promote the migration and invasion of pancreatic cancer by CXCL1-mediated Akt phosphorylation. *Ann. Transl Med.* **7**, 532. 10.21037/atm.2019.09.164 (2019).
33. Yu, Q. et al. Targeting cancer associated fibroblasts by dual-responsive lipid-albumin nanoparticles to enhance drug perfusion for pancreatic tumor therapy. *J. Control Release.* **321**, 564–575. 10.1016/j.jconrel.2020.02.040 (2020).

Figures

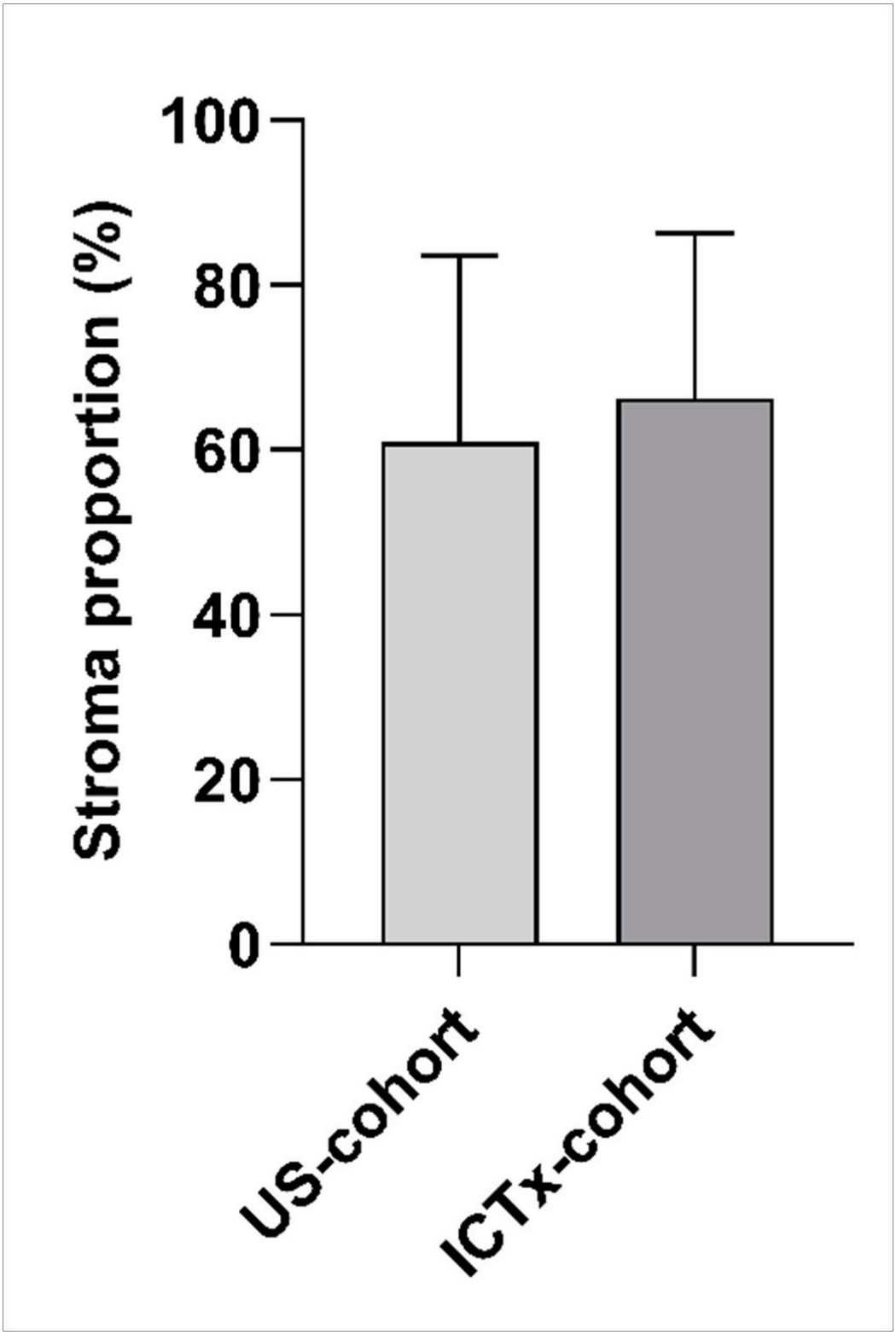


Figure 1

Comparison of tumor stromal content between cohorts

ICTx-cohort n= 51; US-cohort n= 66. Mann-Whitney-U-Test p=0.261

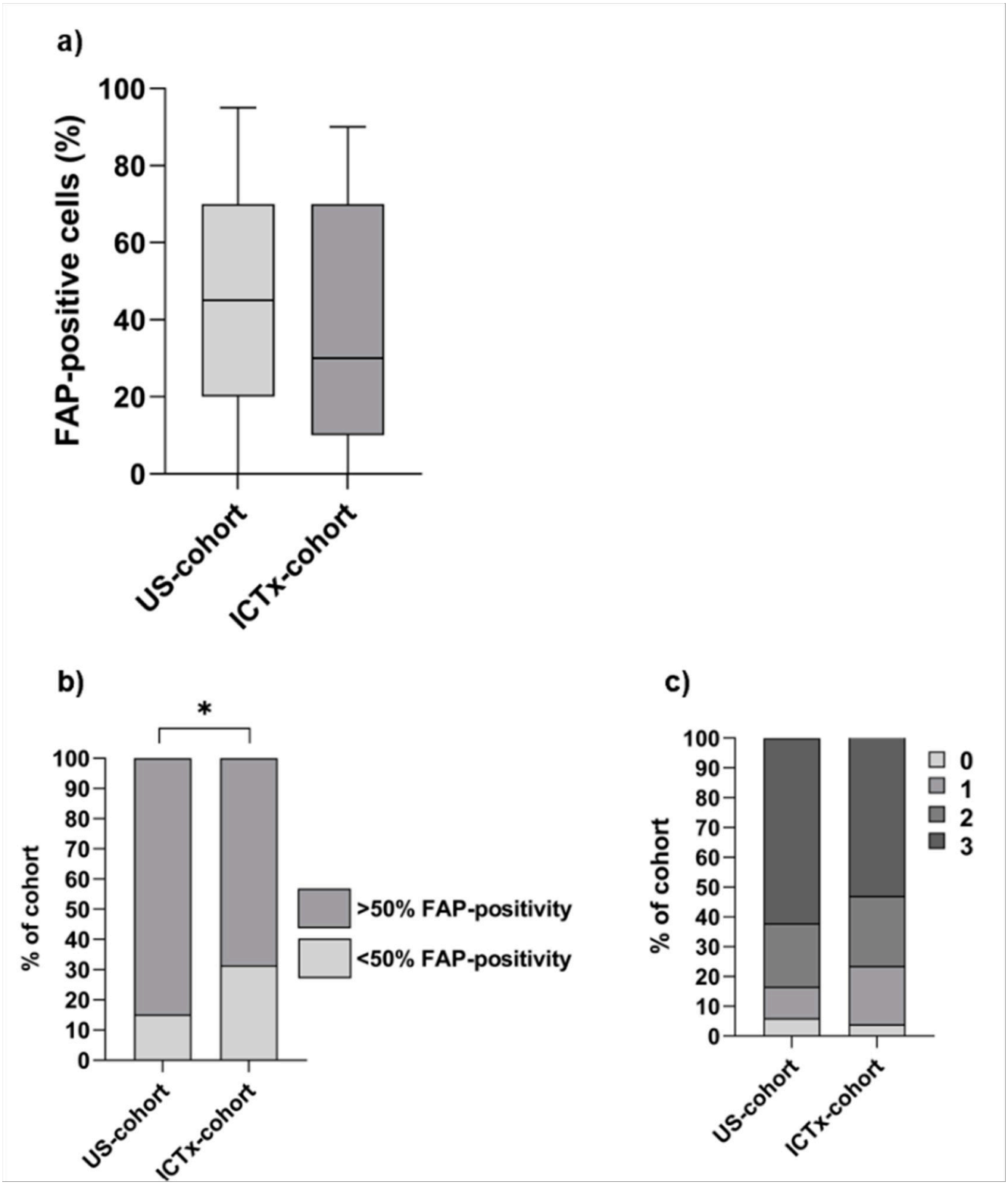


Figure 2

Comparison of FAP-Expression between the cohorts

a) Comparison of FAP percentage; b) Comparison of FAP-values above/ below the mean; c) comparison of FAP-intensity. a) ICTx-cohort n=51, US-cohort n=66, Mann-Whitney-U-Test p=0.164; b) ICTx-cohort FAP-value above mean n= 35, ICTx-cohort FAP-value below meann= 16, US-cohort FAP-value above mean n= 56, US-cohort FAP-value below meann= 10, chi-square p=0.036. c) ICTx-cohort FAP-intensity 0 n= 2, ICTx-cohort FAP-intensity 1 n= 10, ICTx-cohort FAP-intensity 2 n= 12, ICTx-cohort FAP-intensity 3 n= 27, US-

cohort FAP-intensity 0 n= 4, US-cohort FAP-intensity 1 n= 7, US-cohort FAP-intensity 2 n= 14, US-cohort FAP-intensity 3 n= 41; chi-square p=0.503

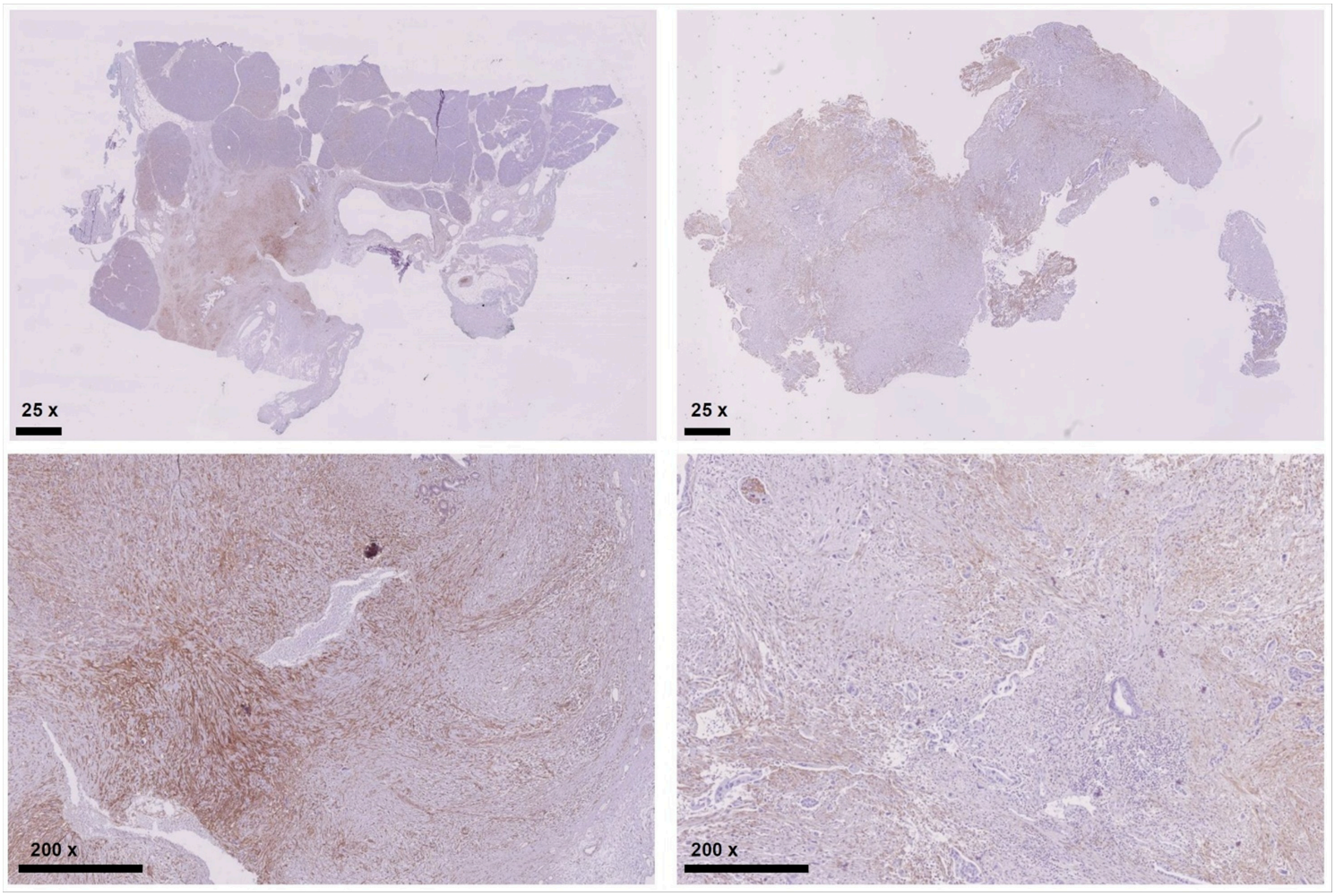


Figure 3

Representative immunohistochemical staining against FAP from a tumor after upfront resection (left) and a tumor receiving induction chemotherapy (right)

FAP expression is indicated by brown staining. All scale bars indicate 1 mm

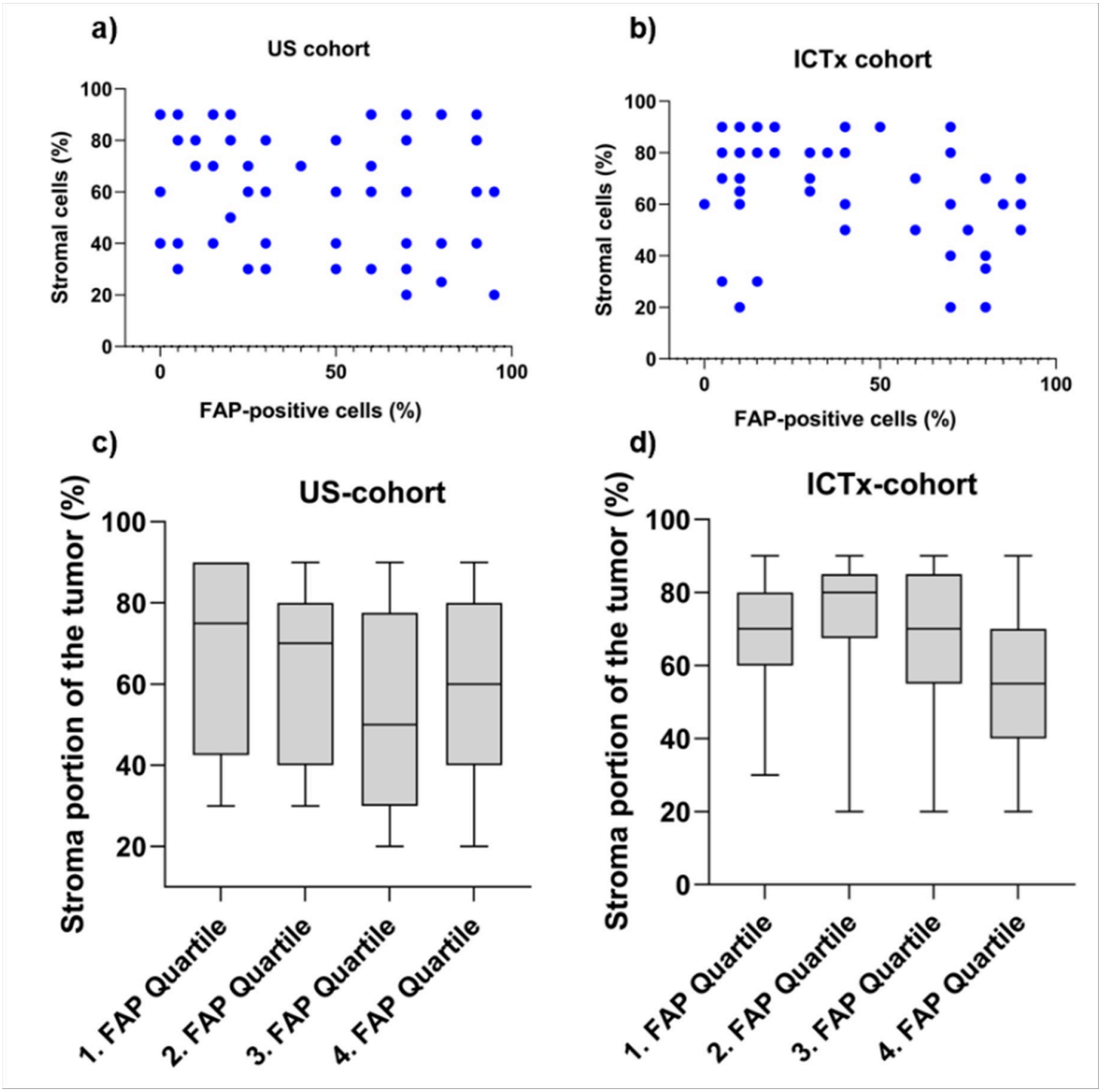


Figure 4

Correlation of tumor stromal content and FAP percentage within the cohorts

a) US-cohort n=66, Spearman-correlation coefficient p=0.189; b) ICTx-cohort n=51, Spearman-correlation coefficient p=0.075; c) US quartiles n=16, Kruskal-Wallis-test p=0.311; d) ICTx-quartiles n=12, Kruskal-Wallis-test p=0,045

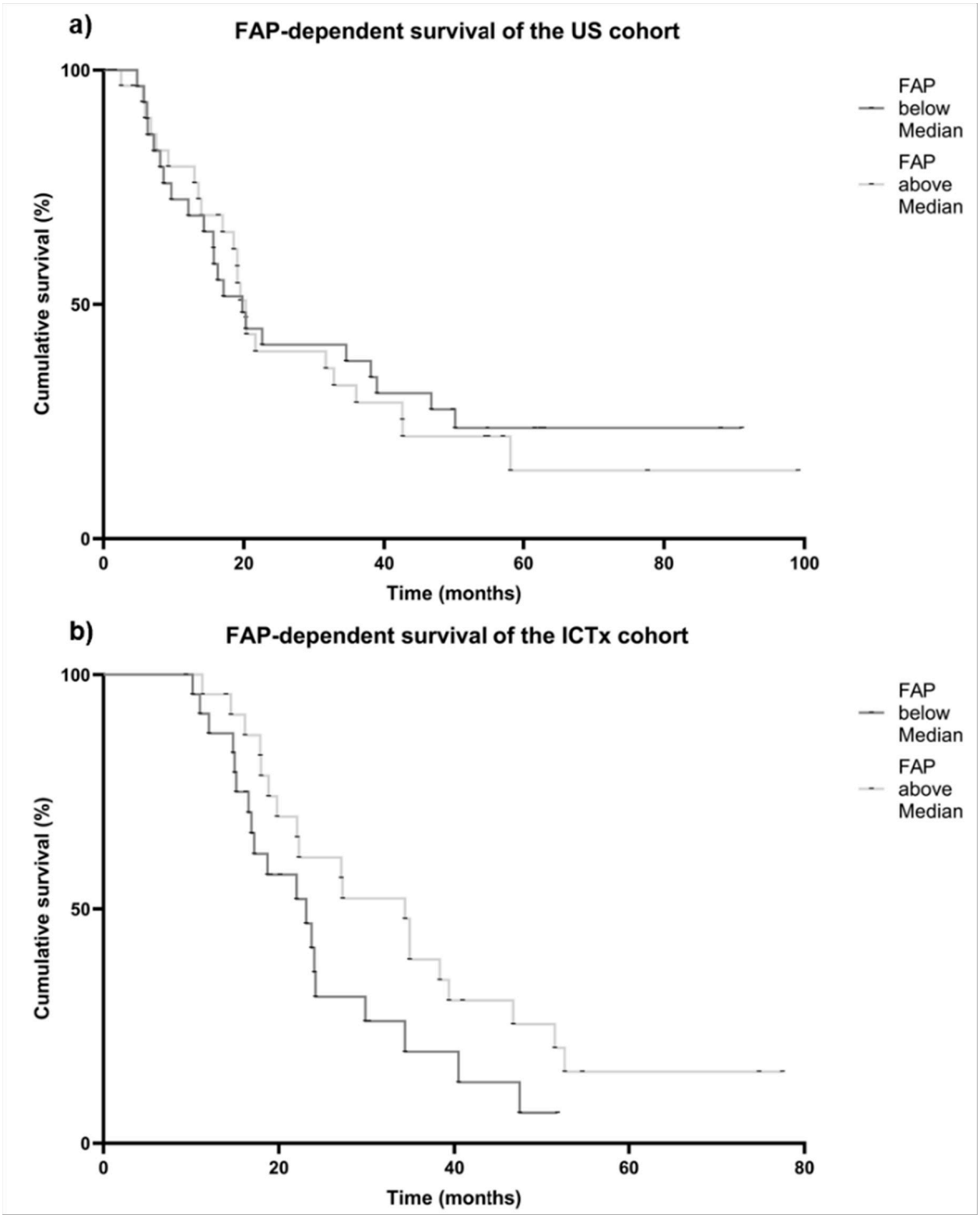


Figure 5

Correlation of tumor stromal content and FAP percentage within the cohorts

a) US-patients above/below mean of FAP percentage n=33, log rank test p=0.459; b) ICTx-patients above/below mean of FAP percentage n=25, log rank test p=0.116