Pulsed priming with the FAK inhibitor narmafotinib enhances both gemcitabine/Abraxane and FOLFIRINOX chemotherapy response in pancreatic cancer

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Chemotherapy induces fibrosis

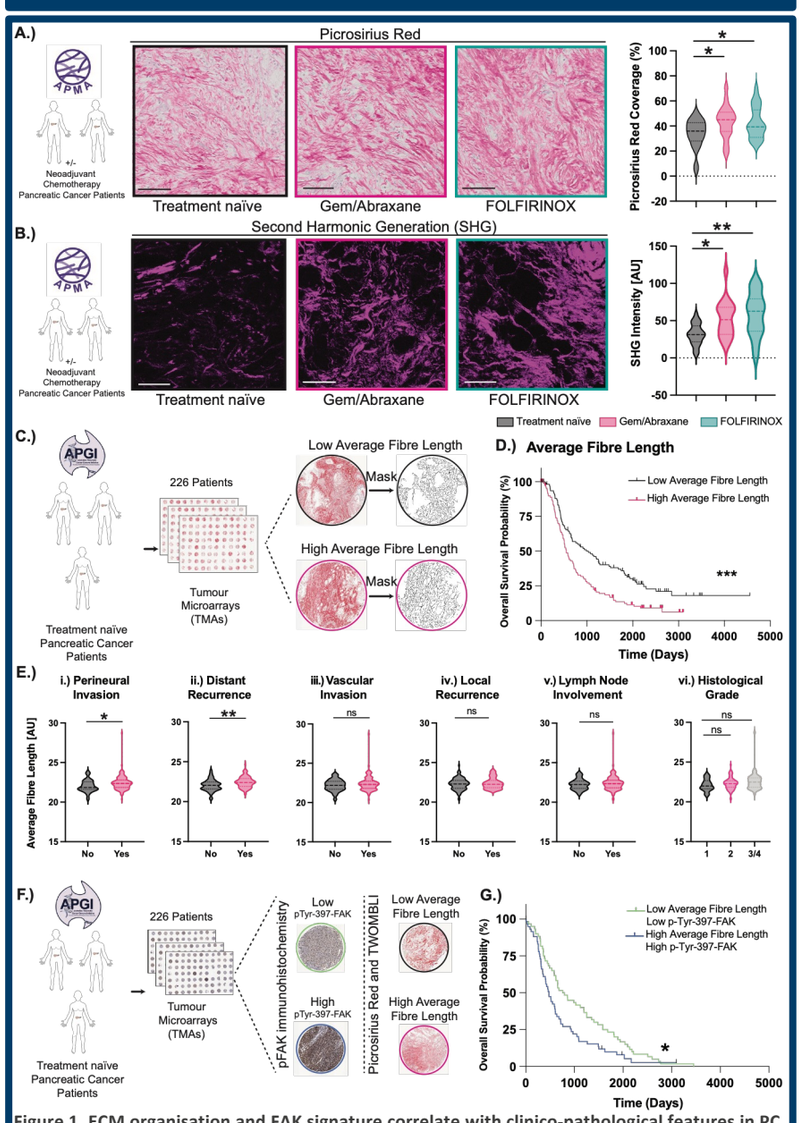

Figure 1. ECM organisation and FAK signature correlate with clinico-pathological features in PC patients. (**A,B**) Representative images and quantification of Picrosirius Red brightfield (**A.**) or SHG (**B.**) from treatment-naïve *versus* neoadjuvant gemcitabine/Abraxane- or FOLFIRINOX-treated PC patients in the Royal North Shore Hospital/APMA cohort. Scale bar, 50 µm (**A.**), 100 µm (**B.**). n=22 treatment-naïve, n=20 neoadjuvant gemcitabine/Abraxane, n=19 neoadjuvant FOLFIRINOX patients, with ≥5 ROIs per tumour. (**C.**) Schematic of treatment-naïve patient TMA staining with Picrosirius Red (three cores per patient). (**D.**) Kaplan-Meier analysis of human PC patient overall survival based on high (pink, n=113) *versus* low average fibre length (black, n=113). (**E.**) Analysis of clinico-pathological differences (**i.**) perineural invasion, **ii.**) distant recurrence, **iii.**) vascular invasion, **iv.**) local recurrence, **v.**) lymph node involvement, **vi.**) histological grade) between patients with high and low average fibre length (n=186 patients). (**F.**) Schematic of treatment-naïve patient TMA with high or low Picrosirius Red or pTyr-397-FAK. (**G.**) Kaplan-Meier analysis of human PC patient overall survival based on fibre length and pFAK levels (high [blue, n=56] *versus* low [green, n=60]).

Narmafotinib, a highly selective FAK inhibitor

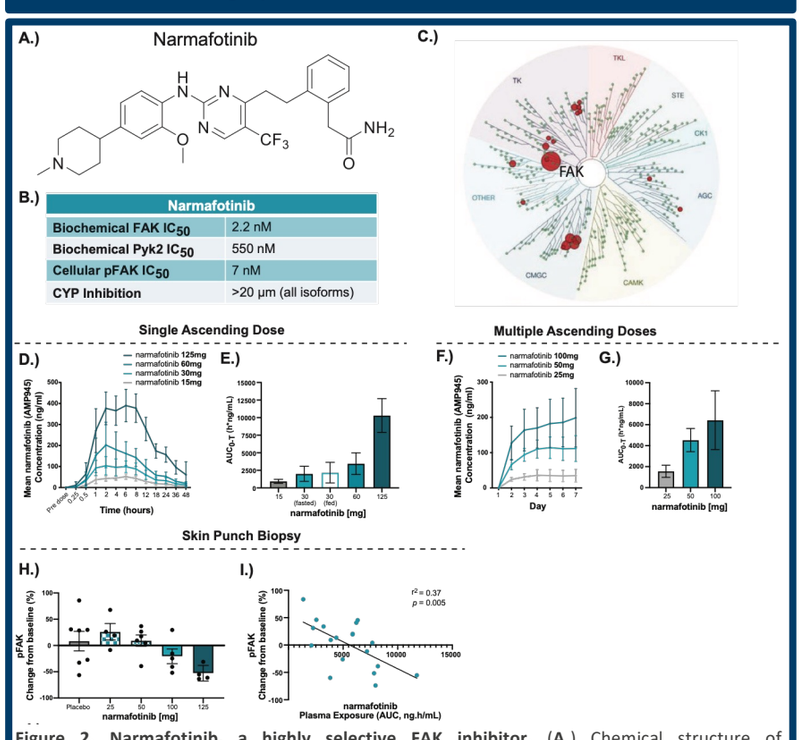

Figure 2. Narmafotinib, a highly selective FAK inhibitor. (**A.**) Chemical structure of narmafotinib. (**B.**) Determination of IC_{50} values for narmafotinib against FAK, and Pyk2, cellular pFAK inhibition (n=12). (**C.**) Kinase array data for narmafotinib against 468 kinases (**D-G**) Pharmacokinetics of narmafotinib in healthy volunteers following SAD (**D,E**) or MAD administration of narmafotinib (**F,G**). (**H.**) pTyr-397-FAK levels in skin punch biopsies from healthy volunteers. (**I.**) Linear relationship between change in FAK activity from baseline and $AUC_{0\text{-}inf}$ following narmafotinib dosing. $AUC_{0\text{-}T}$: Area under the plasma concentration-time curve from the time of dosage administration over the dosage interval. n ≥ 6.

Narmafotinib reduces ECM remodelling and KPC cancer cell invasion

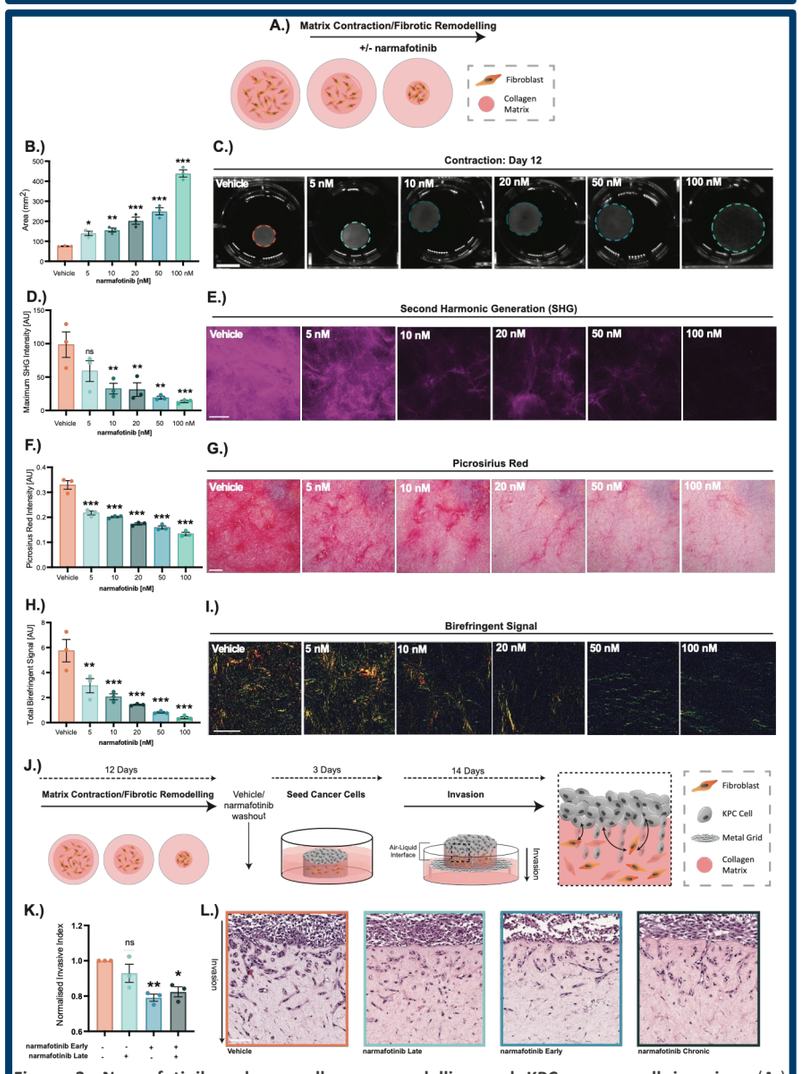

Figure 3. Narmafotinib reduces collagen remodelling and KPC cancer cell invasion. (**A.**) Schematic of 3D organotypic contraction assays. (**B,C**) Quantification (**B.**) and representative images (**C.**) of matrix contraction (Day 12, scale bar, 1 cm). (**D-I**) Quantification (**D,F,H**) and representative images (**E,G,I**) of SHG **(D,E),** Picrosirius Red intensity **(F,G)** and birefringence signal (**H,I**) and representative birefringence images (**I.**) of Picrosirius Red stained matrices (Day 12). (**J.**) Schematic of 3D organotypic invasion assays. (**K,L**) Quantification (**K.**) and representative images (**L.**) of KPC cell invasion upon treatment with vehicle or 20 nM narmafotinib during invasion, matrix contraction or matrix contraction and invasion (chronic). Scale bar, 100 µm (**E,G,I,L**).

Narmafotinib improves chemotherapy response *in vivo*.

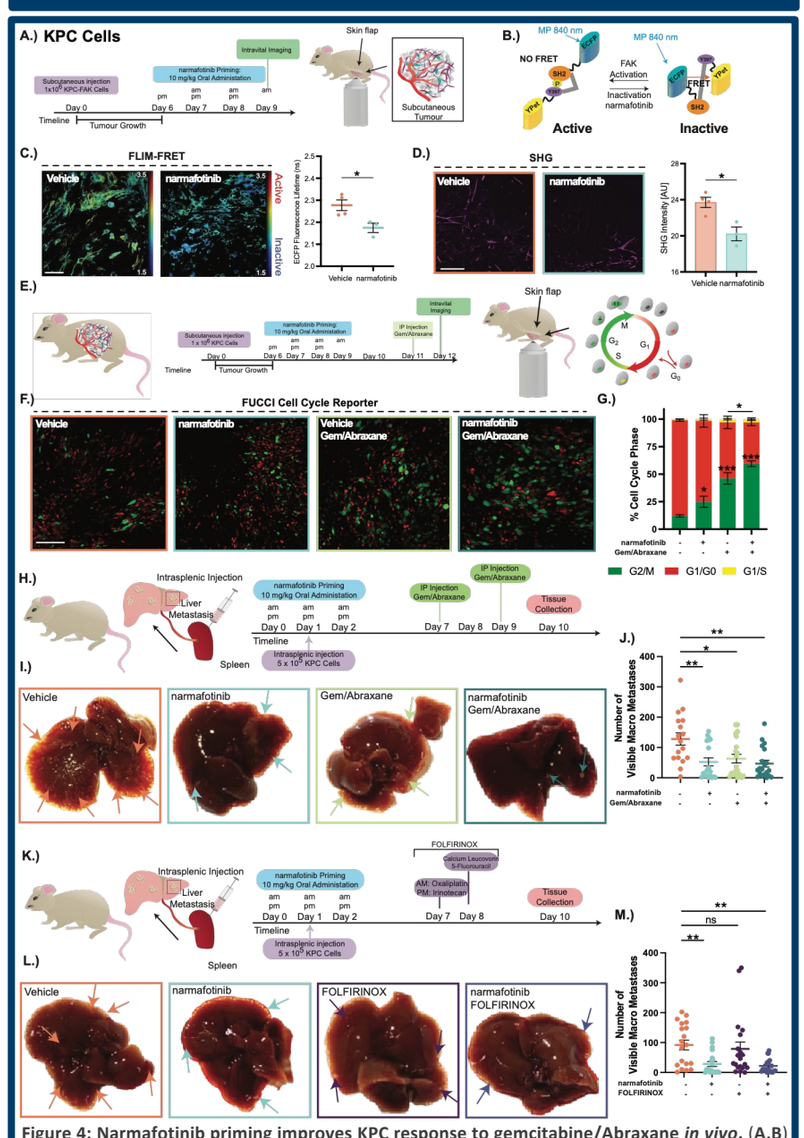

Figure 4: Narmafotinib priming improves KPC response to gemcitabine/Abraxane *in vivo*. (**A,B**) Timeline of KPC-FAK-FRET intravital imaging study (**A.**) using the Lyn-FAK-FRET biosensor (**B.**). (**C.**) Images and quantification of fluorescence lifetime (nanoseconds) in mice treated with vehicle or narmafotinib (10 mg/kg). n=50 cells per animal. (**D.**) Representative images and quantification of SHG in tumours treated with vehicle or narmafotinib. 5 FOV per animal, n=4 vehicle and n=3 narmafotinib animals (**C,D**). (**E.**) Timeline of KPC-FUCCI intravital imaging study. (**F,G**) Representative FUCCI images (**F.**) and quantification of cell cycle distribution (**G.**) in tumours treated with vehicle or narmafotinib followed by saline or gemcitabine/Abraxane. n=10 FOV per animal, 4 animals per treatment group. (**H-J**) Timeline (**H.**), representative liver images (**I.**) and quantification of visible liver macrometastases (**J.**) following intrasplenic KPC cell injection into mice. n ≥ 17 mice per treatment group. (**K-M**) Timeline (**K.**), representative liver images (**L.**) and quantification of visible liver macrometastases (**M.**) following intrasplenic KPC cell injection into mice. n ≥ 18 mice per treatment group. Arrows point at macrometastases in the liver tissue (**I,L**). Scale bar, 50 µm (**C.**), 100 µm (**D,F**).

Narmafotinib priming is superior over chronic treatment

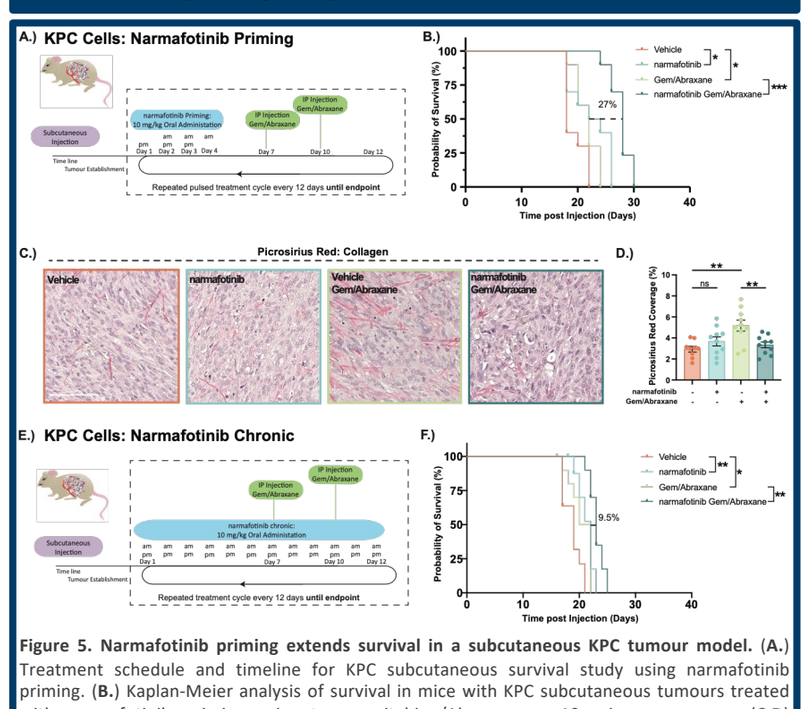

Figure 5. Narmafotinib priming extends survival in a subcutaneous KPC tumour model. (**A.**) Treatment schedule and timeline for KPC subcutaneous survival study using narmafotinib priming. (**B.**) Kaplan-Meier analysis of survival in mice with KPC subcutaneous tumours treated with narmafotinib priming prior to gemcitabine/Abraxane. n=10 mice per group. (**C,D**) Representative images of Picrosirius Red staining (**C.**) and quantification of Picrosirius Red coverage (**D.**). n=6 FOV per animal, n≥9 animals per group. Scale bar, 100 µm. (**E.**) Treatment schedule and timeline for KPC subcutaneous survival study using chronic daily narmafotinib treatment. (**F.**) Kaplan-Meier analysis of survival in mice with KPC subcutaneous tumours treated with chronic narmafotinib prior to gemcitabine/Abraxane. n=10 mice per group.

Narmafotinib priming improves gemcitabine/Abraxane in subcutaneous patient-derived models

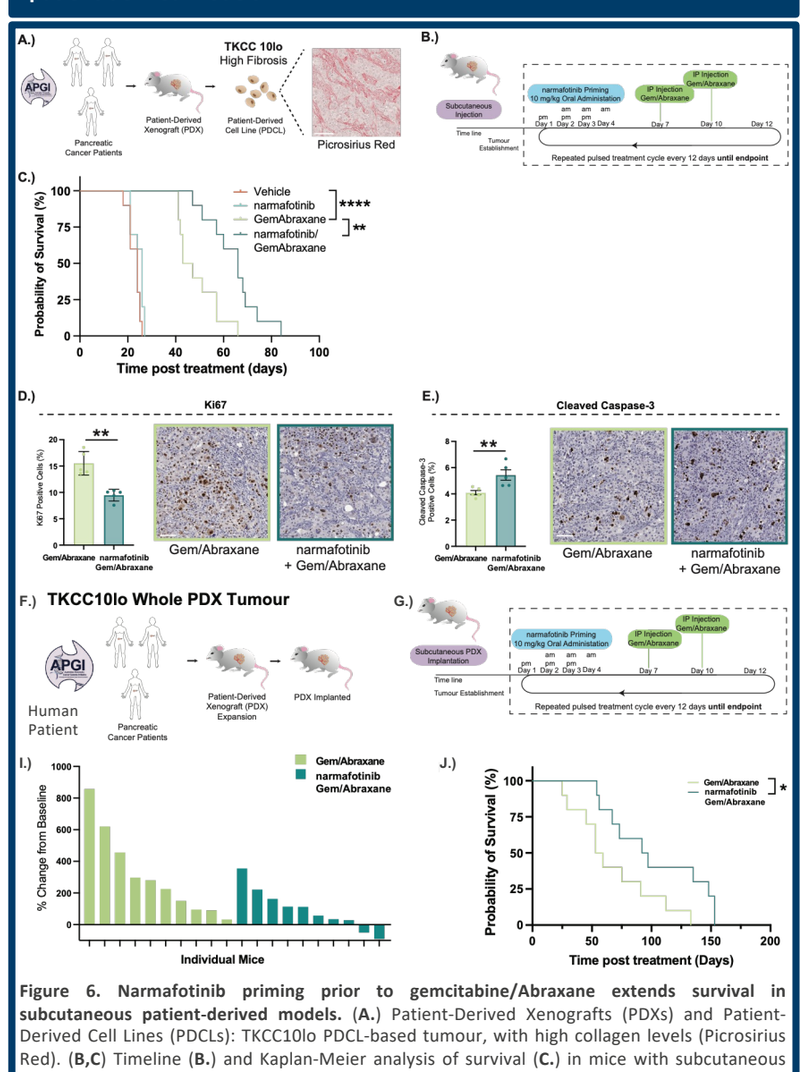

Figure 6. Narmafotinib priming prior to gemcitabine/Abraxane extends survival in subcutaneous patient-derived models. (**A.**) Patient-Derived Xenografts (PDXs) and Patient-Derived Cell Lines (PDCLs): TKCC10lo PDCL-based tumour, with high collagen levels (Picrosirius Red). (**B,C**) Timeline (**B.**) and Kaplan-Meier analysis of survival (**C.**) in mice with subcutaneous TKCC10lo tumours. n=10 mice per treatment group. (**D,E**) Quantification and representative images of TKCC10lo tumours stained with Ki67 (**D.**) and cleaved caspase-3 (**E.**) at timed endpoint after 3 treatment cycles. Scale bar, 100 µm. n=5 mice per treatment group, 6 FOV per tumour. (**F,G**) Schematics depicting the generation of patient-derived xenografts (PDXs) *via* the Australian Pancreatic Genome Initiative (APGI) (**F.**) as well as treatment schedule and timeline for the TKCC10lo PDX model (**G.**). (**H.**) Waterfall plot of PDX tumour growth as a change from baseline following three cycles of treatment. (**I.**) Kaplan-Meier analysis of survival in mice with implanted TKCC10lo PDX tumours. n=10 mice per treatment group.

Narmafotinib priming prior to FOLFIRINOX extends survival in subcutaneous patient-derived models

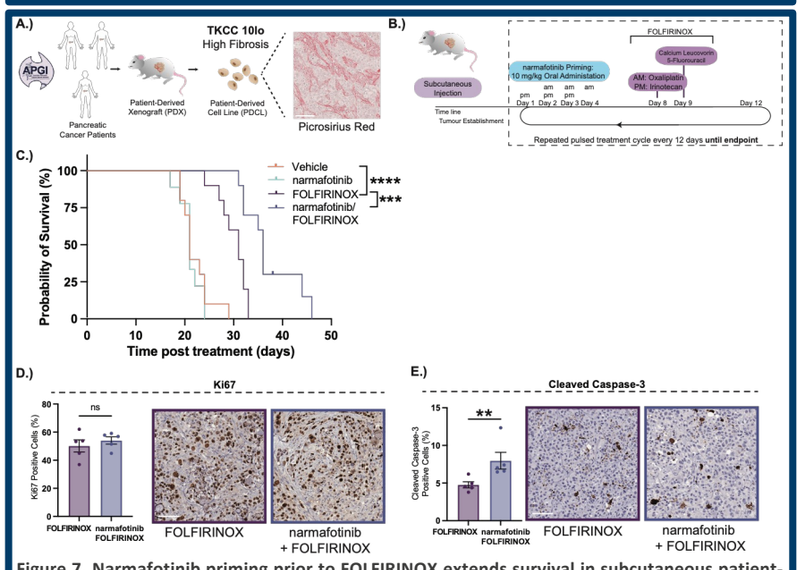

Figure 7. Narmafotinib priming prior to FOLFIRINOX extends survival in subcutaneous patient-derived models. (**A.**) Patient-Derived Xenografts (PDXs) and Patient-Derived Cell Lines (PDCLs): TKCC10lo PDCL-based tumour. (**B,C.**) Timeline (**B.**) and Kaplan-Meier analysis of survival (**C.**) in mice with TKCC10lo subcutaneous tumours. n=10 mice per group. (**D,E**) Quantification and representative images of TKCC10lo tumours stained with Ki67 (**D.**) and cleaved caspase-3 (**E.**) at timed endpoint after two treatment cycles. Scale bar, 100 µm. n=5 mice per treatment group, 6 FOV per tumour.

Narmafotinib priming prior to chemotherapy extends survival in long-term orthotopic patient-derived models

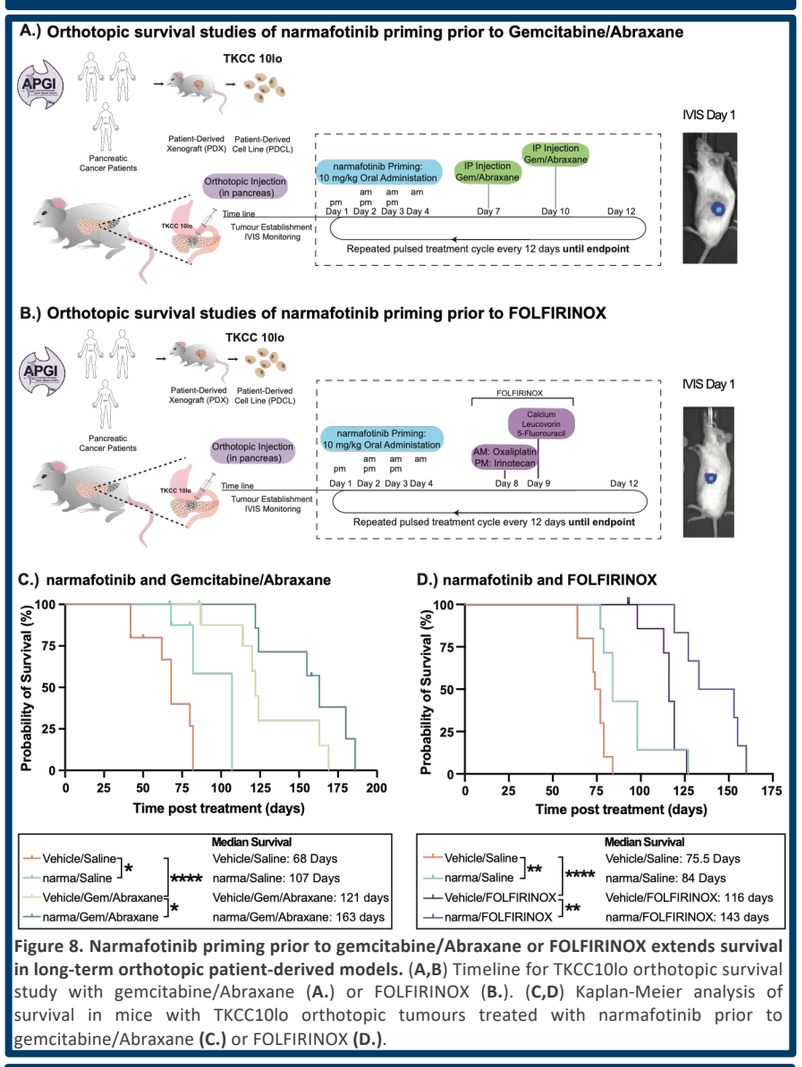

Figure 8. Narmafotinib priming prior to gemcitabine/Abraxane or FOLFIRINOX extends survival in long-term orthotopic patient-derived models. (**A,B**) Timeline for TKCC10lo orthotopic survival study with gemcitabine/Abraxane (**A.**) or FOLFIRINOX (**B.**). (**C,D**) Kaplan-Meier analysis of survival in mice with TKCC10lo orthotopic tumours treated with narmafotinib prior to gemcitabine/Abraxane (**C.**) or FOLFIRINOX (**D.**).

Narmafotinib priming reduces collagen remodelling and deposition and increases chemotherapy performance

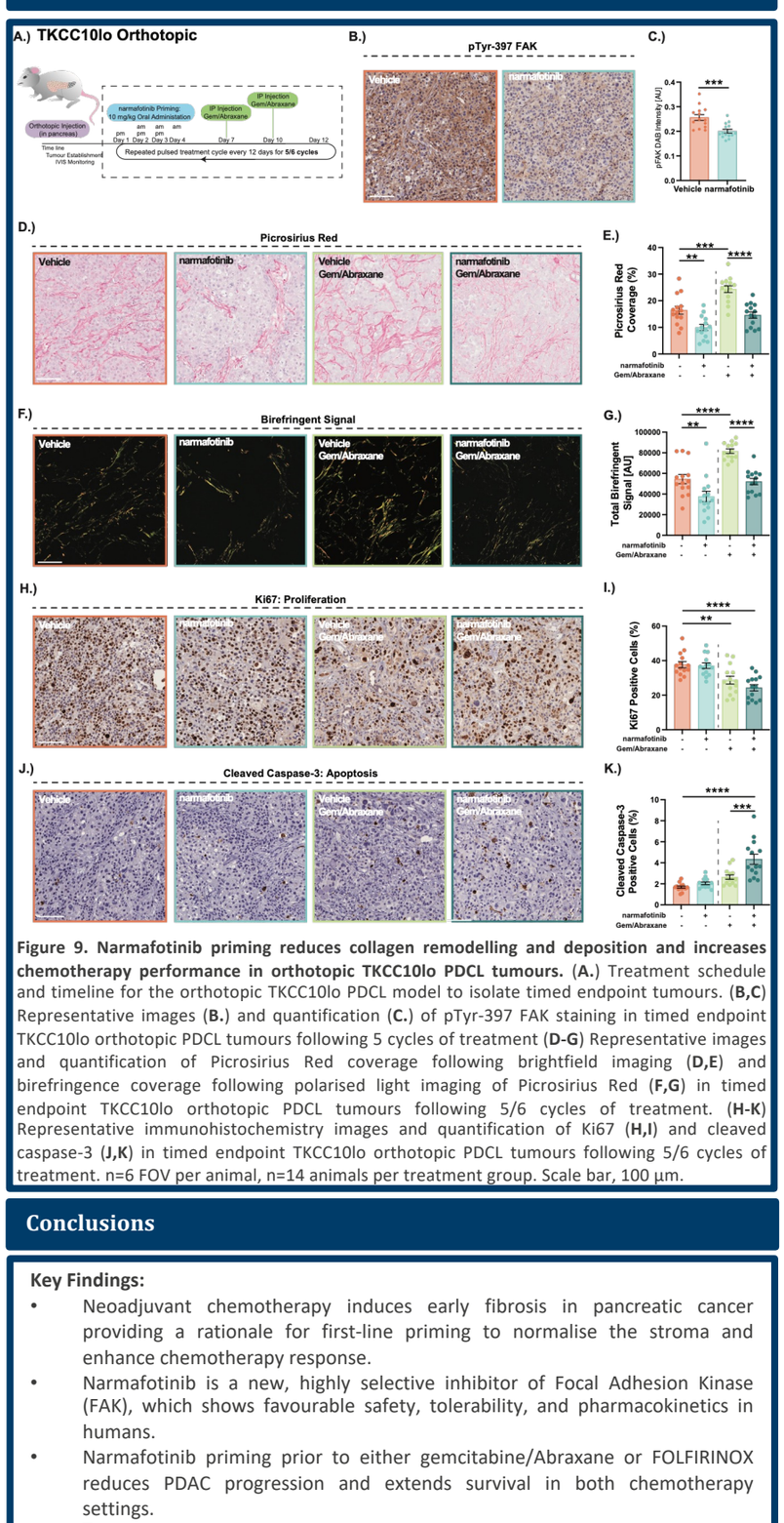

Figure 9. Narmafotinib priming reduces collagen remodelling and deposition and increases chemotherapy performance in orthotopic TKCC10lo PDCL tumours. (**A.**) Treatment schedule and timeline for the orthotopic TKCC10lo PDCL model to isolate timed endpoint tumours. (**B,C**) Representative images (**B.**) and quantification (**C.**) of pTyr-397 FAK staining in timed endpoint TKCC10lo orthotopic PDCL tumours following 5 cycles of treatment (**D-G**) Representative images and quantification of Picrosirius Red coverage following brightfield imaging (**D,E**) and birefringence coverage following polarised light imaging of Picrosirius Red (**F,G**) in timed endpoint TKCC10lo orthotopic PDCL tumours following 5/6 cycles of treatment. (**H-K**) Representative immunohistochemistry images and quantification of Ki67 (**H,I**) and cleaved caspase-3 (**J,K**) in timed endpoint TKCC10lo orthotopic PDCL tumours following 5/6 cycles of treatment. n=6 FOV per animal, n=14 animals per treatment group. Scale bar, 100 µm.

Conclusions

Key Findings:

- Neoadjuvant chemotherapy induces early fibrosis in pancreatic cancer providing a rationale for first-line priming to normalise the stroma and enhance chemotherapy response.
- Narmafotinib is a new, highly selective inhibitor of Focal Adhesion Kinase (FAK), which shows favourable safety, tolerability, and pharmacokinetics in humans.
- Narmafotinib priming prior to either gemcitabine/Abraxane or FOLFIRINOX reduces PDAC progression and extends survival in both chemotherapy settings.
- This first-line priming strategy may be applicable to other combination therapies in pancreatic cancer.

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