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Pentoxifylline and Pirfenidone sensitize pancreatic cancer cells to gemcitabine treatment

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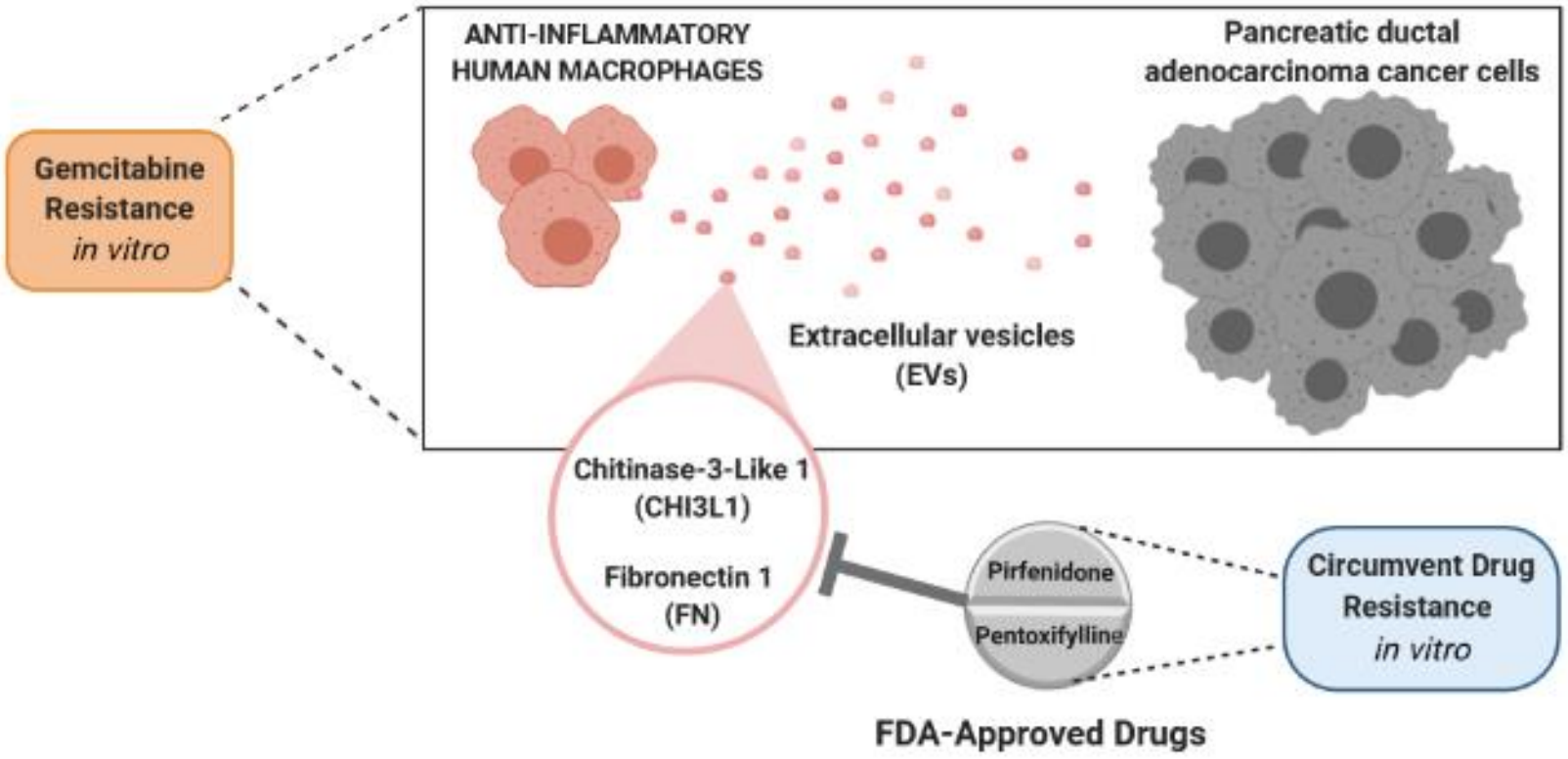
Pentoxifylline and Pirfenidone sensitize pancreatic cancer cells to gemcitabine treatment



Abstract

Repurposing “old” drugs is an attractive strategy for cancer drug discovery, particularly for cancers with limited chemotherapeutic options and/or high therapy resistance, such as pancreatic ductal adenocarcinoma (PDAC). Drug resistance in PDAC is highly influenced by the tumor microenvironment, especially by macrophages. Extracellular vesicles (EVs) released by macrophages might have a role in PDAC drug resistance, by carrying cargo from their donor macrophages. Therefore, we aimed to i) identify, in the cargo of EVs shed by macrophages, proteins responsible for decreasing sensitivity to gemcitabine (standard-based chemotherapy in PDAC) and ii) study in PDAC cells the antitumor effect of drugs clinically approved for other diseases and known to inhibit those protein targets as an off-target effect.

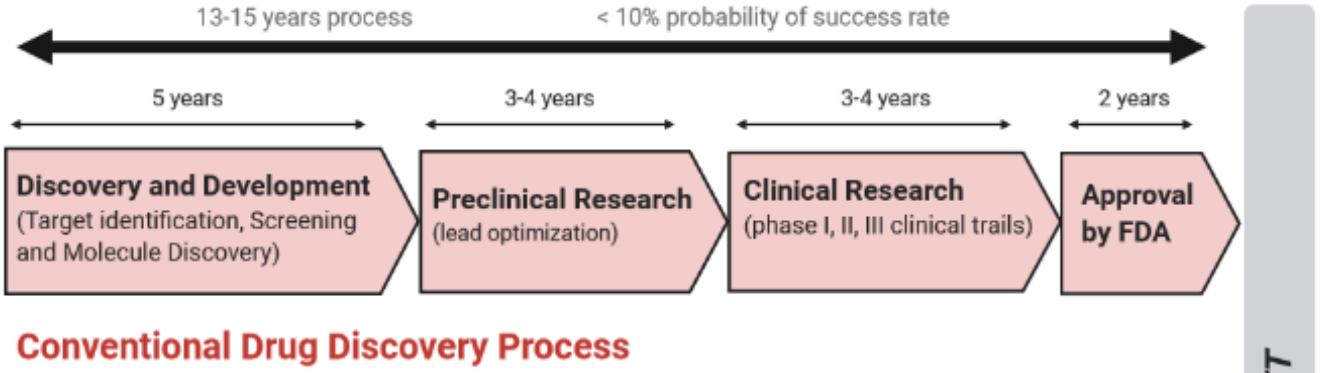
Proteomic analysis identified Fibronectin (FN1) and Chitinase 3-like 1 (CH3IL1) as abundantly present in EVs cargo released by human macrophages. Recombinant human proteins, rhCHI3L1 and rhFN, reduced PDAC cellular sensitivity to gemcitabine through the ERK pathway. Immunohistochemistry of PDAC tumor patient samples showed that expression of FN1 and CH3IL1 was associated to high presence of macrophages. The Cancer Genome Atlas confirmed an association of *CHI3L1* and *FN1* gene expression with PDAC patients overall survival, gemcitabine response and macrophage infiltration. Inhibition of CHI3L1 by pentoxifylline (approved drug for peripheral arterial disease) and of FN1 by pirfenidone (an antifibrotic drug for the treatment of idiopathic pulmonary fibrosis), partially reverted gemcitabine resistance induced by the respective recombinant proteins.

Additional work will be performed in xenograft mice models of PDAC, to further study the possibility of repurposing pirfenidone and pentoxifylline for PDAC treatment.

Keywords: Chitinase 3-like 1, Fibronectin, Gemcitabine resistance, Pancreatic cancer, Drug repurposing, Pirfenidone, Pentoxifylline

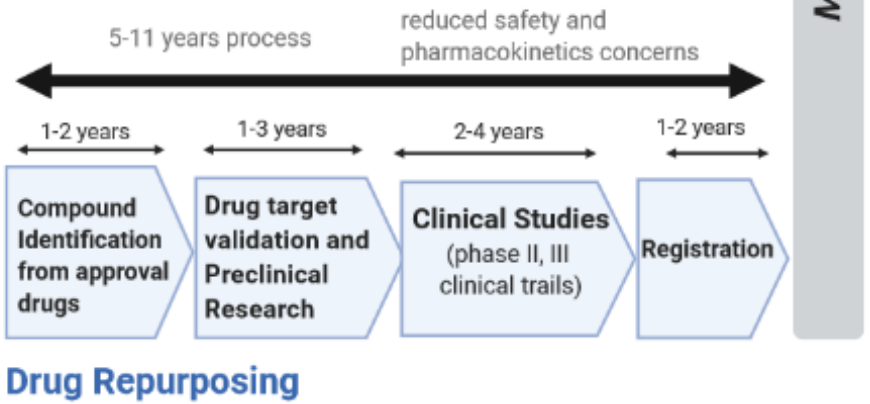
Drug repurposing

An attractive strategy for identification of **antitumor therapeutic potential on drugs previously clinically approved** for other diseases



Advantages of clinically-approved drugs:

- Previously established safety and toxicity
- Reduce the time necessary for drug development
- Less investment required
- Less economic risk (less failure in the clinic)
- Provide cheaper medicines



Drug repurposing in PDAC

An attractive strategy for cancers with limited chemotherapeutic options and/or high therapy resistance, such as **pancreatic ductal adenocarcinoma (PDAC)**

- A very aggressive type of cancer
- Low survival rate
- High rate of resistance to chemotherapy in the majority of PDAC patients undergoing gemcitabine treatment

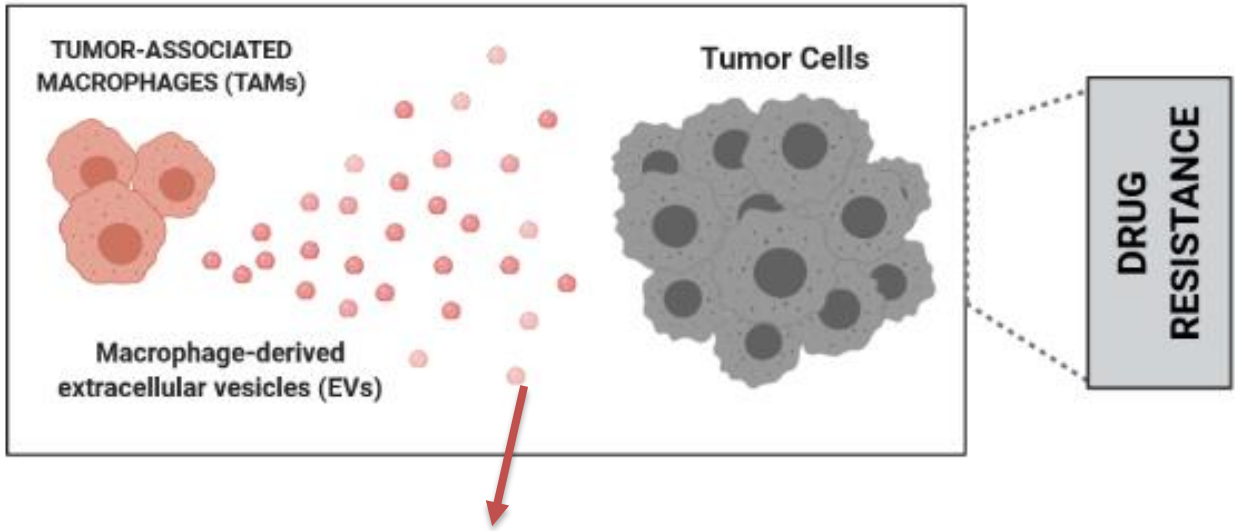
✓ **Study the possibility of repurposing clinically-approved drugs to increase the sensitivity of PDAC to conventional chemotherapy**

✓ **Find new molecular therapeutic targets for pharmacological inhibition**

Drug resistance in PDAC

- ✓ Highly influenced by the **tumor microenvironment**
- ✓ Macrophages are the most frequent immune cells present in the stroma of PDAC influencing **therapy response,** possibly through **Extracellular Vesicles (EVs)**

EVs released by macrophages might have a role in **PDAC drug resistance**, by carrying cargo from their donor macrophages



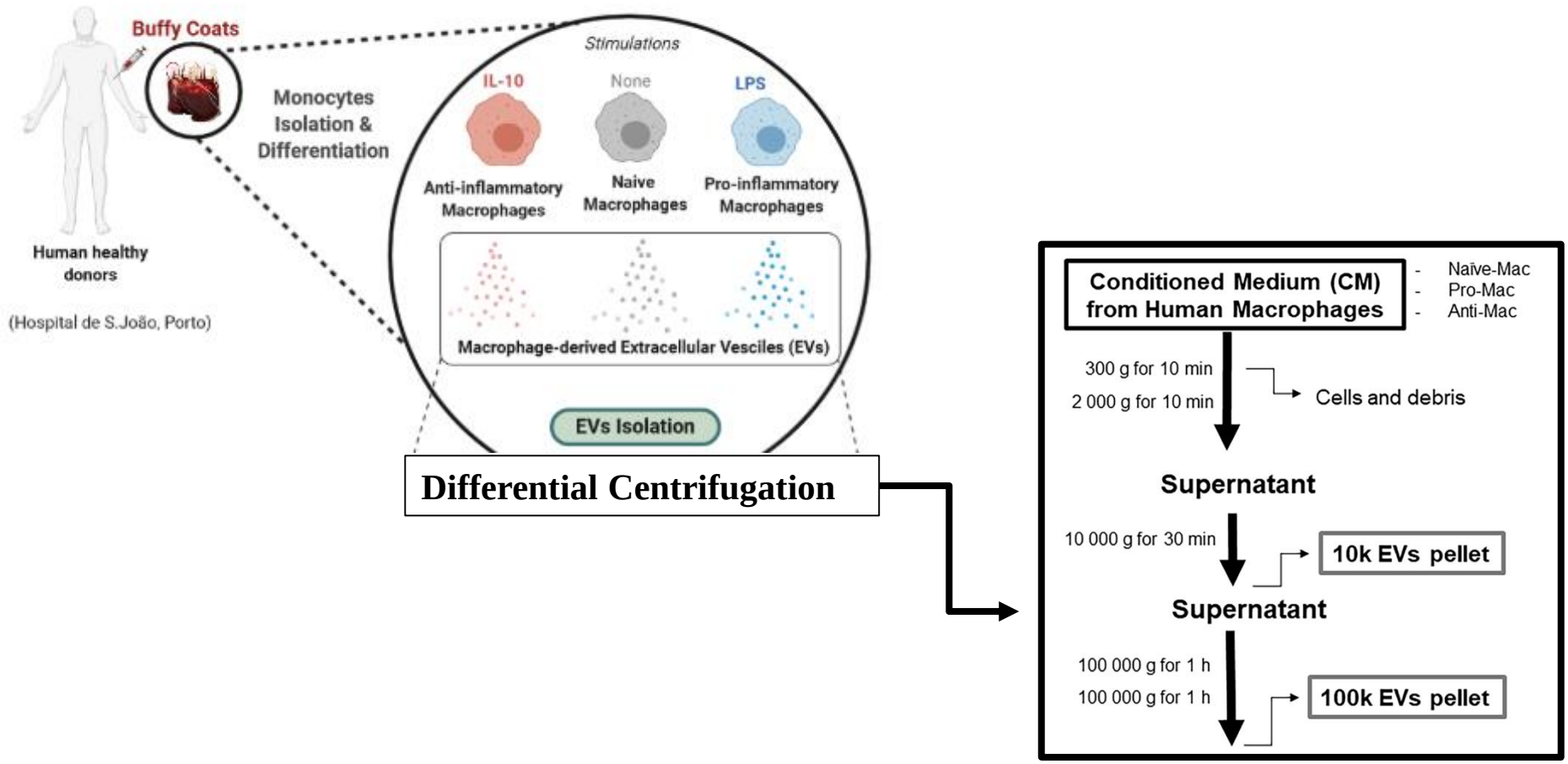
EVs are small size particles released by all cells, containing molecules and genetic material from their donor cells, which can be horizontally transferred to recipient cells, being **important mediators of drug resistance**

- **Identify, in the cargo of EVs shed by human anti-inflammatory macrophages, proteins responsible for decreasing sensitivity to gemcitabine (standard-based chemotherapy in PDAC)**

- **Study in pancreatic cancer cells the antitumor effect of drugs clinically approved for other diseases and known to inhibit those protein targets as an off-target effect**

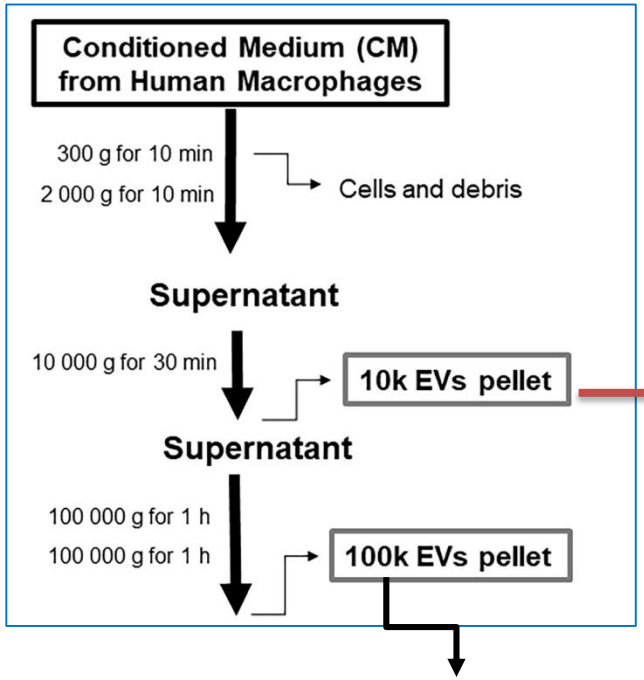
Successful characterisation of EVs released from human macrophages

(isolated from healthy blood donors)



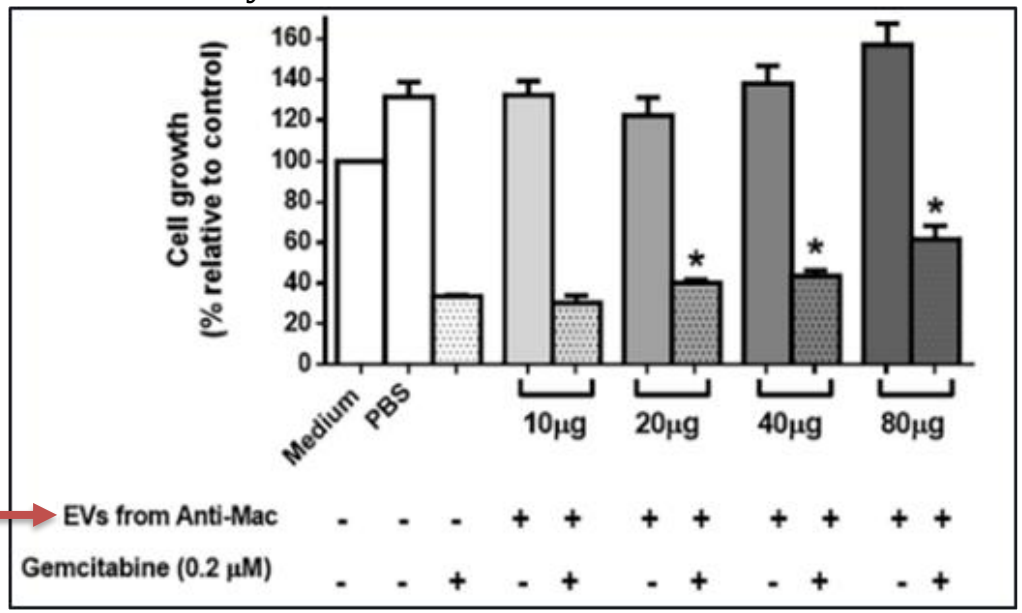
(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

Large EVs shed by macrophages decreased PDAC cellular response to gemcitabine in a concentration-dependent manner



Did not affect response to gemcitabine treatment

SRB assay



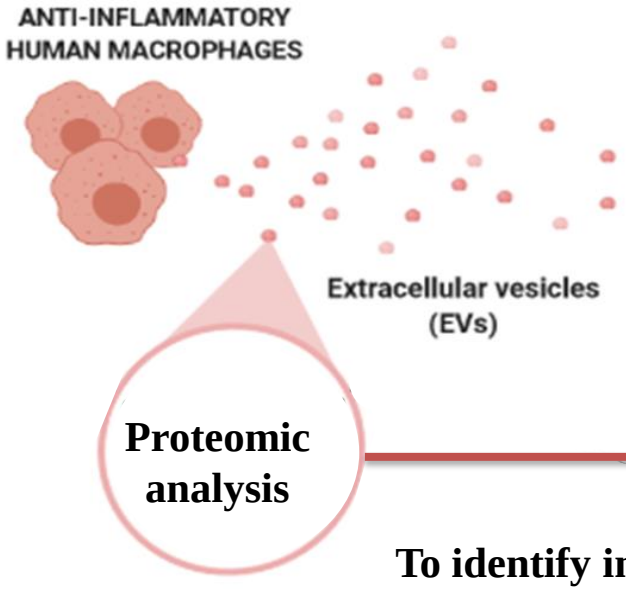
Co-culture experiments of Large EVs with BCPC3 cells

*compared with gemcitabine alone

Anti-Mac: anti-inflammatory human macrophages

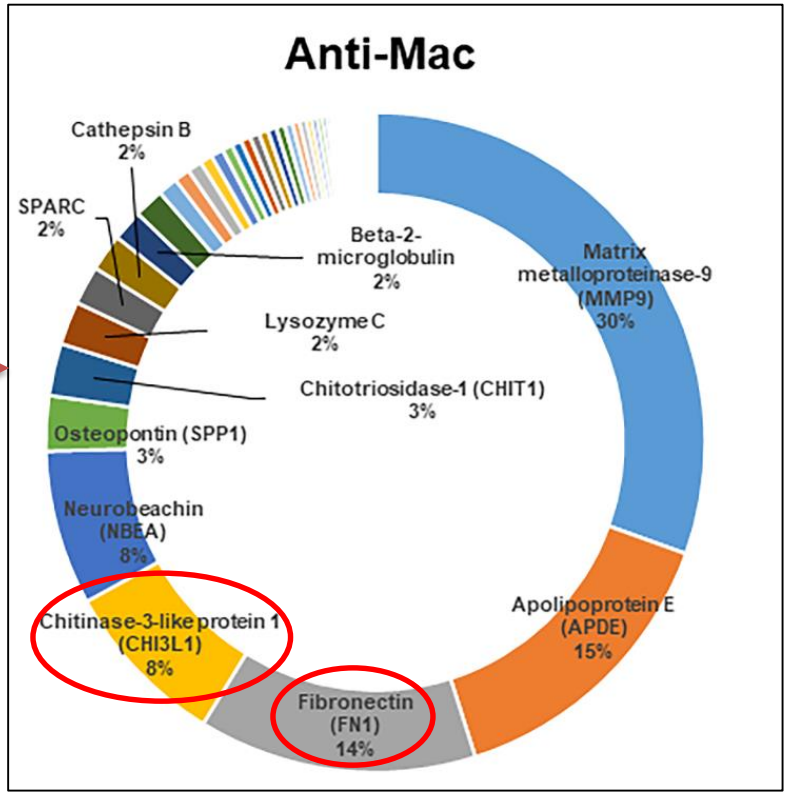
(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

Identification of Fibronectin (FN1) and Chitinase 3-like 1 (CH3IL1) as abundantly present in EVs cargo released by Anti-Mac



Liquid chromatography-mass spectrometry (LC-MS/MS)

To identify in the EVs cargo proteins responsible for decreased PDAC cellular response to gemcitabine



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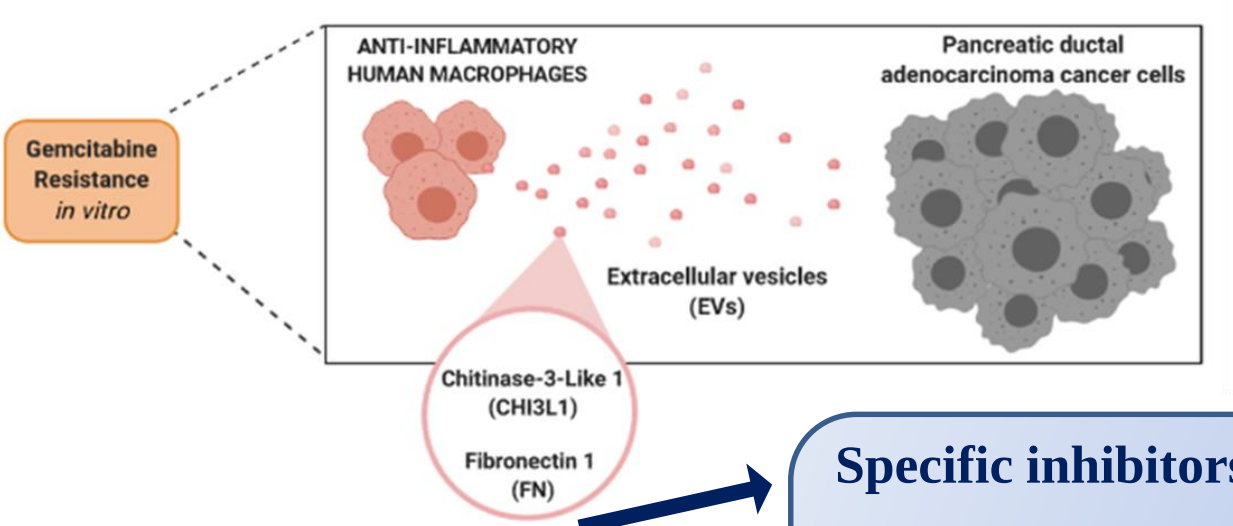
CHI3L1 and FN1 as molecular targets to overcome gemcitabine resistance in PDAC



Specific inhibitors – FDA-approved drugs

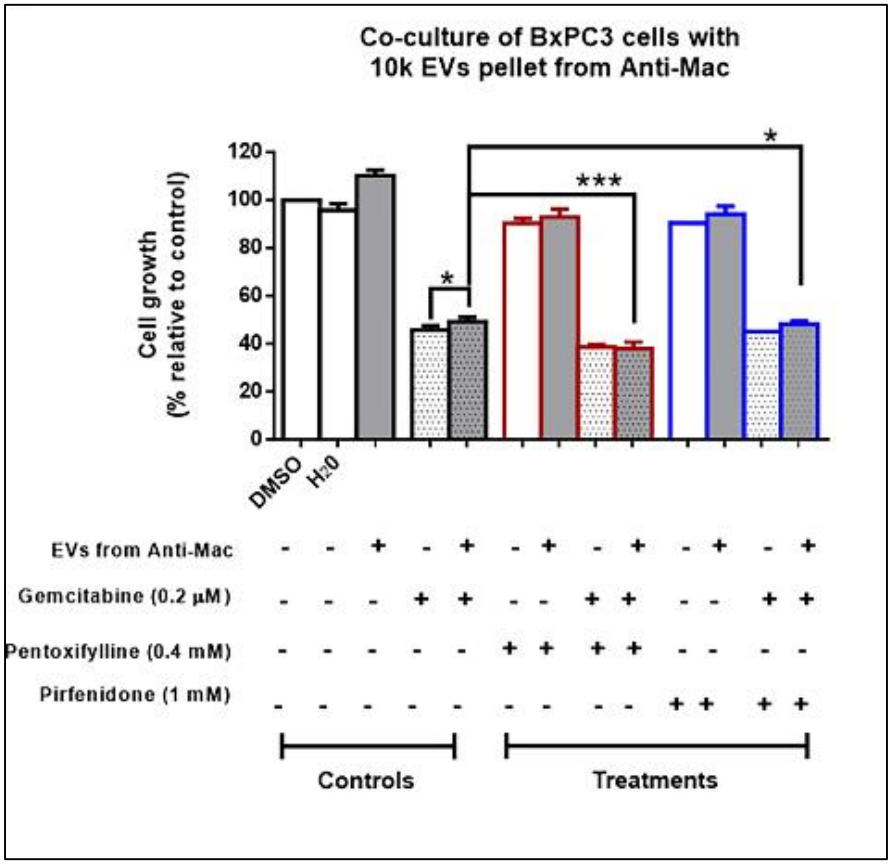
- **Inhibition of CHI3L1**: Pentoxifylline (approved drug for peripheral arterial disease)
- **Decrease FN1**: Pirfenidone (an antifibrotic drug for the treatment of idiopathic pulmonary fibrosis)

Recombinant human (rh) proteins

- **rhCHI3L1**
- **rhFN1**

(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

CHI3L1 and FN1 as molecular targets for pharmacological inhibition with Pentoxifylline or Pirfenidone to overcome gemcitabine resistance



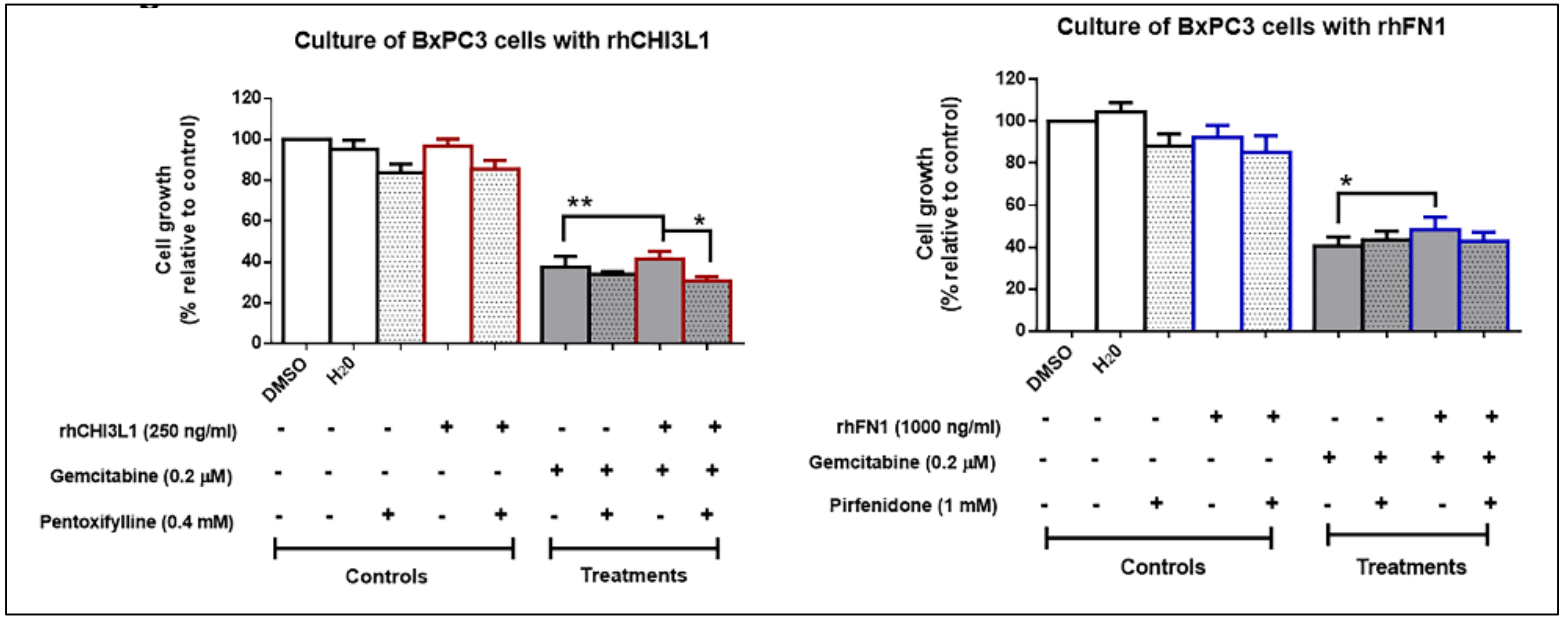
- BxPC3 cells incubated with **EVs** released by Anti-Mac were **less sensitive to gemcitabine treatment**
- **Concomitant treatment** of gemcitabine with Pentoxifylline or Pirfenidone counteracted this drug resistance

Pentoxifylline and Pirfenidone decreased gemcitabine resistance in PDAC cells

(which have been induced by EVs secreted by anti-inflammatory macrophages)

(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

CHI3L1 and FN1 as molecular targets for pharmacological inhibition with Pentoxifylline or Pirfenidone to overcome gemcitabine resistance

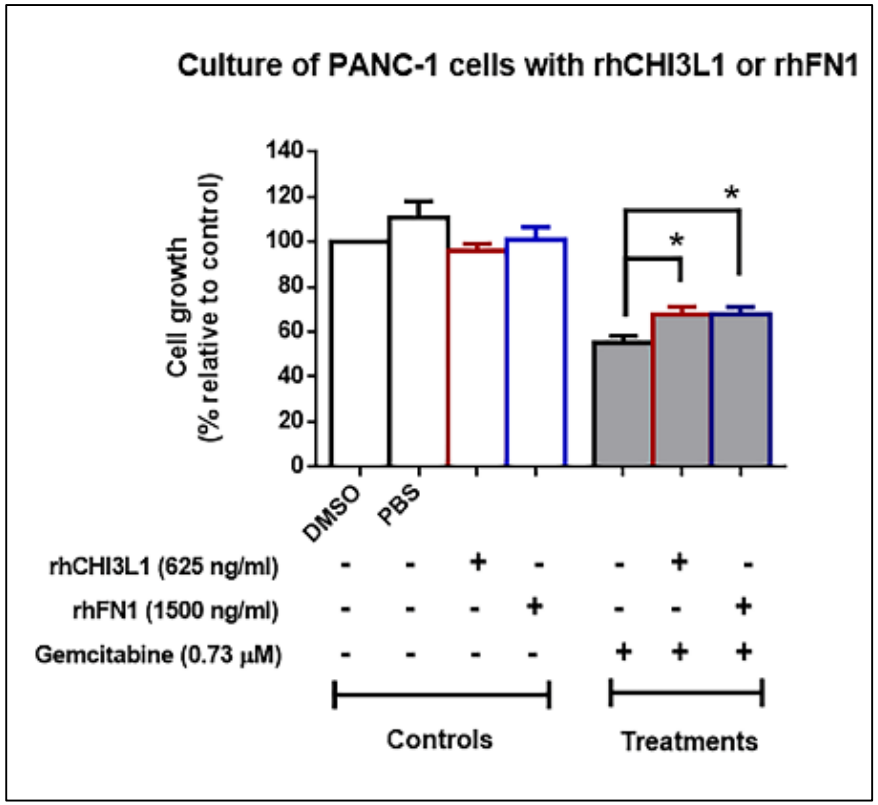


rhCHI3L1 or rhFN1 significantly **enhanced gemcitabine resistance** in BxPC3 cells, which was partially reverted **by Pentoxifylline and Pirfenidone**, respectively

CHI3L1 and FN1 are involved in gemcitabine resistance and the combination of gemcitabine with Pentoxifylline or Pirfenidone could improve PDAC treatment

(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

CHI3L1 and FN1 as molecular targets to overcome gemcitabine resistance

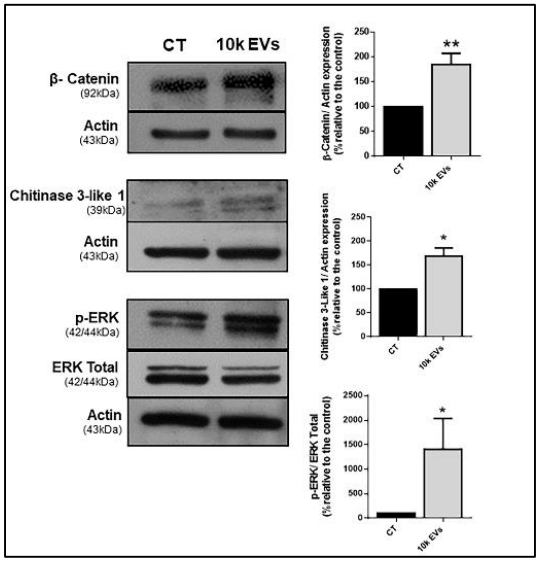


rhCHI3L1 and rhFN also reduced PDAC cellular sensitivity to gemcitabine in another PDAC cell line

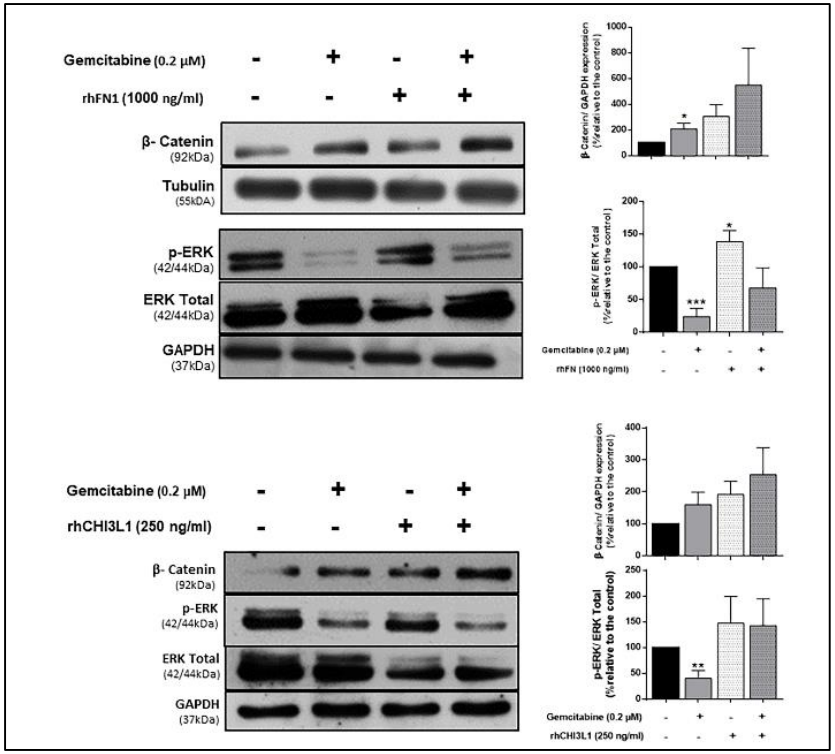
(PANC-1 cells: more resistant cells and harboring a KRAS mutation)

(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

Role of ERK pathway in gemcitabine resistance



BxPC3 cells incubated with large EVs presented higher expression of CHI3L1, β-catenin, and p-ERK

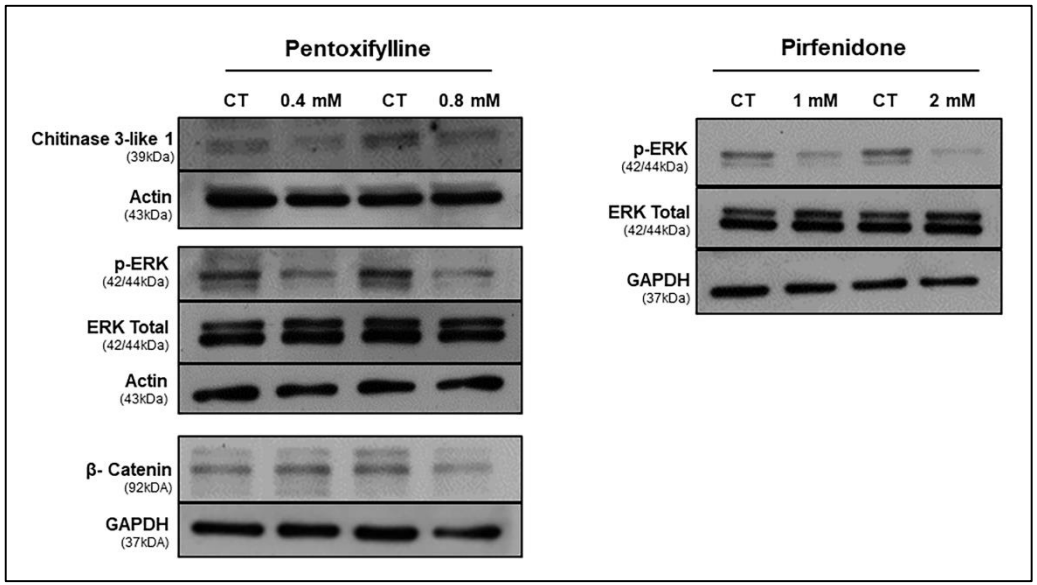


BxPC3 cells incubated with rhFN1 or rhCHI3L1 under gemcitabine treatment presented higher expression of p-ERK and β-catenin

CHI3L1 and FN1 in the cargo of EVs shed by human Anti-Mac induce gemcitabine resistance through the activation of ERK signalling

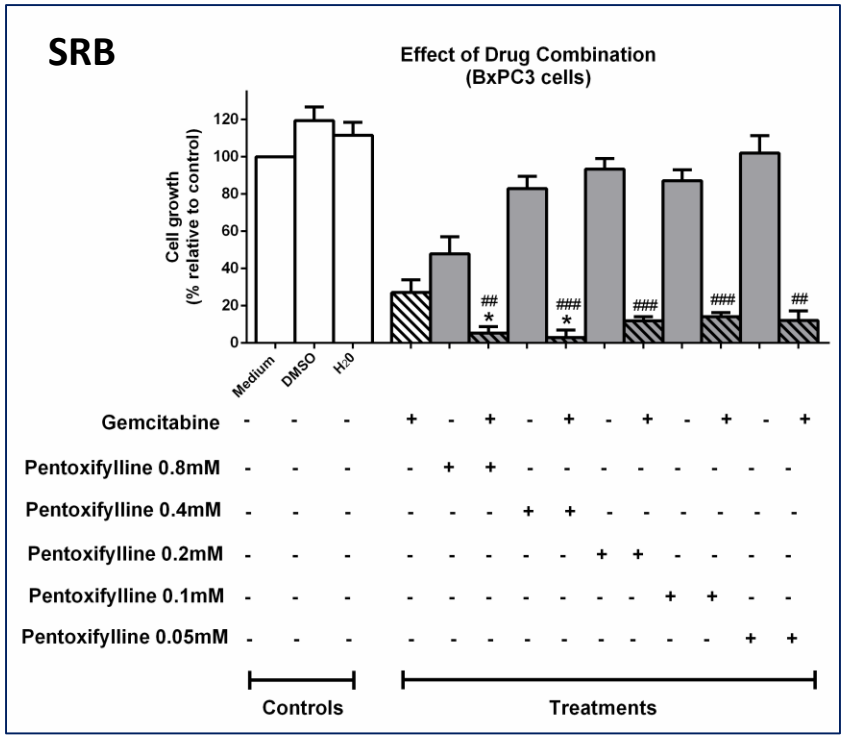
(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

Effect of Pentoxifylline and Pirfenidone in PDAC cells



Pentoxifylline and Pirfenidone decreased ERK signaling in BxPC3 PDAC cells

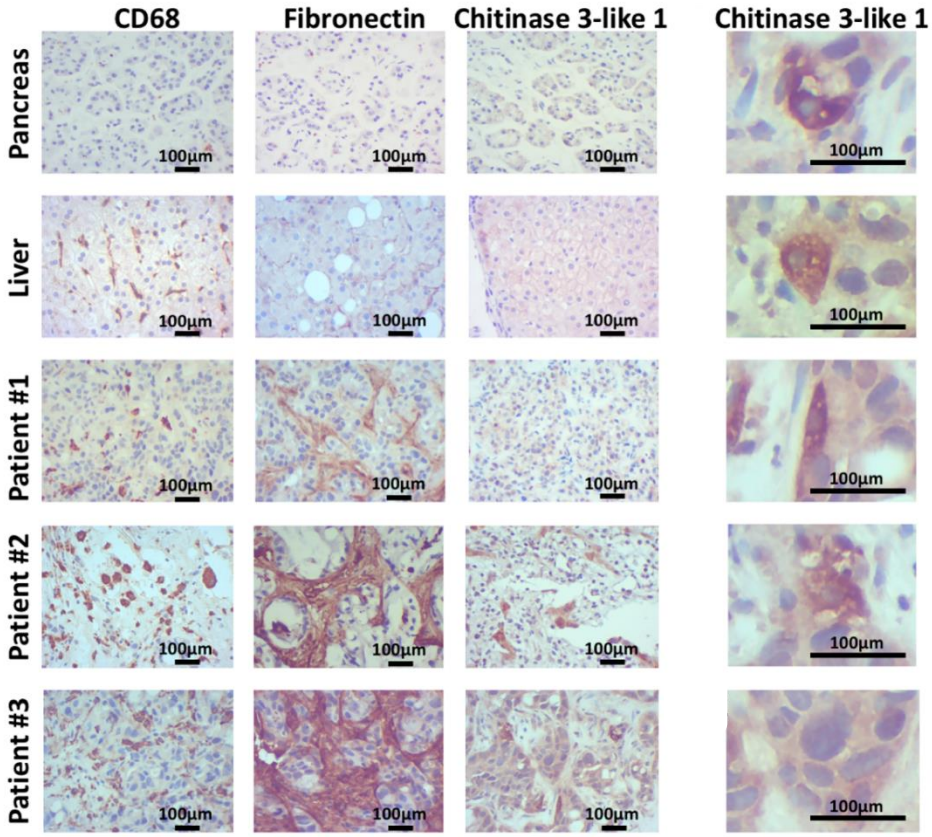
* compared to gemcitabine alone
\# compared to Pentoxifylline alone



Pentoxifylline, at different concentrations, combined with gemcitabine reduced PDAC cell growth

(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

Expression of FN1 and CH3IL1 was associated to high presence of macrophages



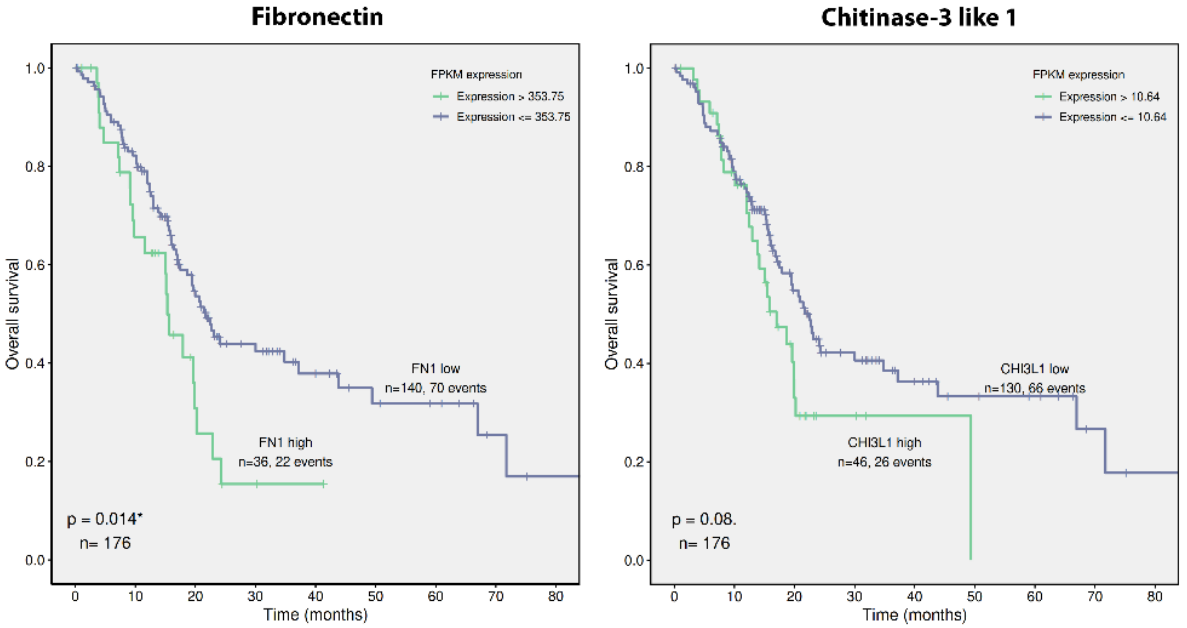
Immunohistochemistry of three metastatic PDAC tumor patient samples

- **Higher CD68** expression (a macrophage lineage marker) associated with **higher FN1 expression**
- **FN1** not detected inside the tumor cells but detected in the extracellular matrix
- **CHI3L1** presented reduced cytoplasmic expression in tumor cells and moderate cytoplasmic expression in stromal cells, especially **in CD68 positive cases**
- Reduced CHI3L1 expression, and no relevant expression of CD68 and FN1 was found in **healthy pancreas**

(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

Association of *CHI3L1* and *FN1* gene expression with PDAC patients overall survival

176 PDAC patients was investigated using the TCGA database

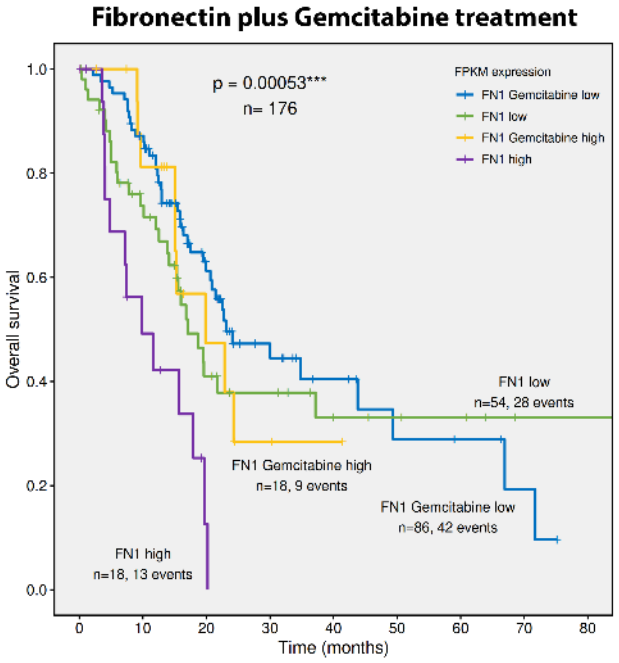


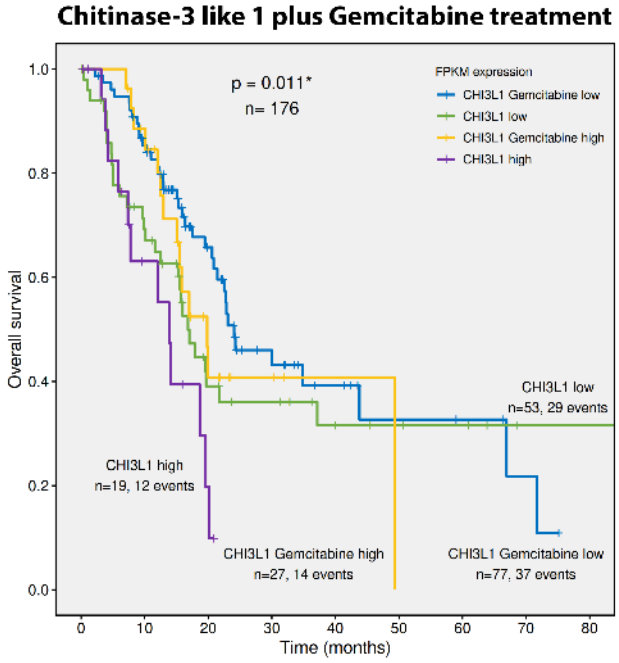
A **higher *FN1*** and ***CHI3L1*** expression were significantly associated with **poorer survival** (although not statistically significant for *CHI3L1*)

(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

Association of *CHI3L1* and *FN1* gene expression with PDAC patients gemcitabine response

176 PDAC patients was investigated using the TCGA database



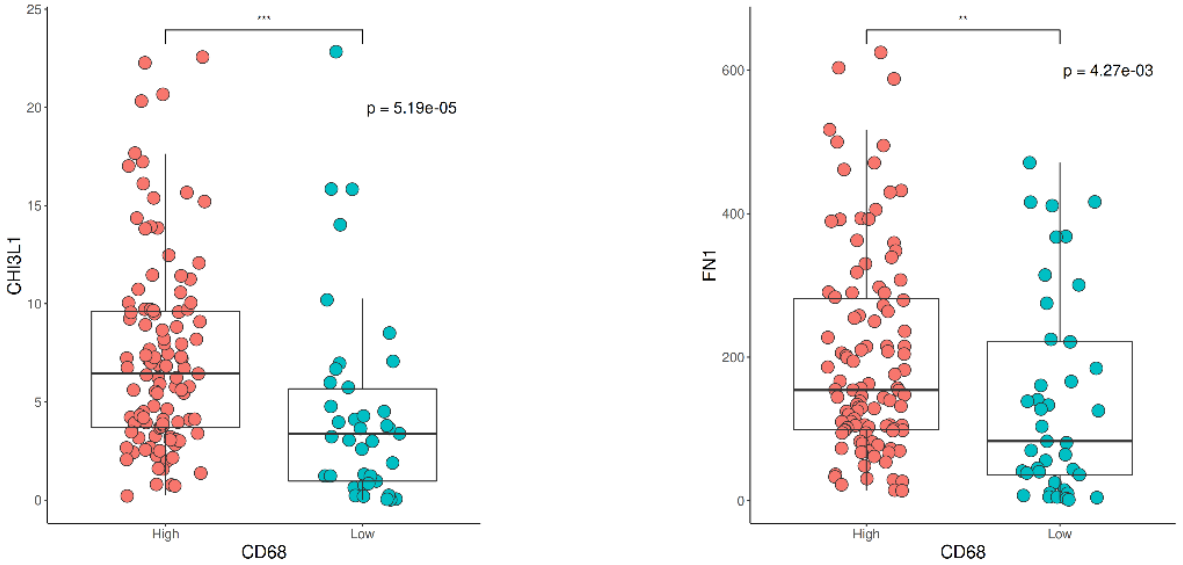


No overall survival improvement was observed in PDAC patients treated with gemcitabine with **high expression levels of *FN1* or *CHI3L1***

(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

Association of *CHI3L1* and *FN1* gene expression with macrophage infiltration

176 PDAC patients was investigated using the TCGA database



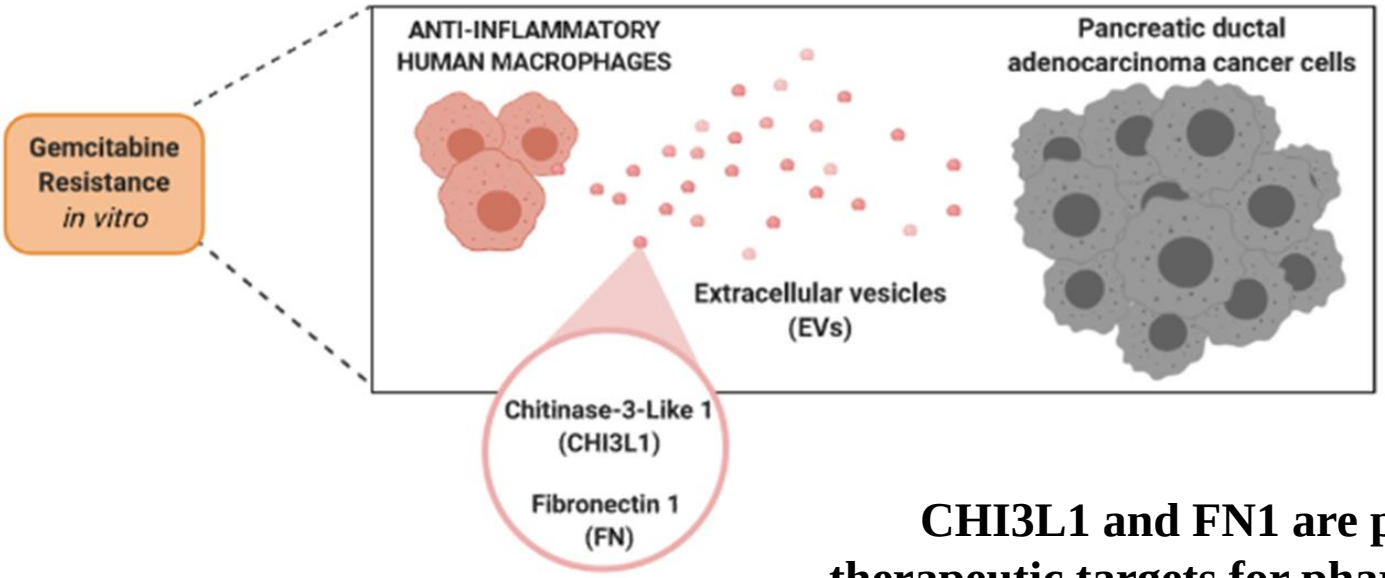
Tumours expressing **higher levels of *CD68*** also expressed **higher levels of *CHI3L1* or *FN1***

(Cancer Letters, *in Press*, DOI: 10.1016/j.canlet.2020.11.013)

Conclusions

- ✓ ***In vitro*** data showed that **CHI3L1 and FN1** are responsible, at least in part, for gemcitabine resistance of PDAC cells through activation of the ERK signalling

- ✓ **Clinical data** supports that CHI3L1 and FN1 counteract cellular response to gemcitabine in PDAC patients



CHI3L1 and FN1 are potential therapeutic targets for pharmacological inhibition in PDAC

Conclusions

- ✓ **Pentoxifylline**, approved for peripheral arterial disease and a known inhibitor of CHI3L1, reversed the chemoresistant effect of the EVs or of rhCHI3L1 on gemcitabine response

- ✓ **Pirfenidone,** an antifibrotic agent used for the treatment of idiopathic pulmonary fibrosis, decreased the effect of the EVs shed by macrophages on gemcitabine resistance

Possibility of repurposing Pirfenidone and Pentoxifylline for PDAC treatment

Additional work will be performed in xenograft mice models of PDAC

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