The objective of this study was to test the ability of necuparanib to reduce proliferation of pancreatic tumor cells in patients. Necuparanib in combination with gemcitabine and nab-paclitaxel is currently being evaluated in a Phase 2 clinical trial in patients with metastatic pancreatic cancer (ClinicalTrials.gov identifier: NCT01621243).

The objective of this study was to test the ability of necuparanib to reduce proliferation of pancreatic tumor cells in 2D cultures and to reduce invasion of pancreatic tumor cells into surrounding matrix in 3D cultures confirming that an anti-tumor effect previously observed with other heparins was maintained with necuparanib.

**METHODS**

**2D Experiments**

In 2D cultures, a resazurin assay was used to measure cell proliferation and a TUNEL assay was used to determine apoptosis.

**3D Culture Set-up**

Pancreatic cancer is one of the most aggressive cancers, with only about 5% of patients surviving 5 years past the initial diagnosis. Despite advances with new chemotherapy combinations, overall survival outcomes are still dismal and require novel therapeutic approaches. Necuparanib (previously M4802) is a heparin-sulfate-like molecule that binds and inhibits multiple heparin-binding growth factors, chemokines, adhesion molecules in cancer progression and metastasis and is such, is musculoskeletal and extracellular components of the tumor microenvironment. In order to further explore how epithelial-stromal interactions are affected by necuparanib, a 3-dimensional (3D) culture system mimicking pancreatic cancer was developed containing a co-culture of pancreatic tumor cells and pancreatic stellate cells, the most abundant stromal cell in the pancreas. Necuparanib inhibited the invasive behavior of tumor spheroids in a dose-dependent manner. We demonstrate an inhibitory effect of necuparanib on proliferation and invasion of pancreatic tumor cells. These studies aid in our understanding of the key biological targets and pathways responsible for necuparanib's anti-tumor effects and support biomarker discovery in the ongoing Phase 2 efficacy study in metastatic pancreatic cancer.

**RESULTS**

Pancreatic cancer cells such as AsPC-1 and Capan2, are only found to be invasive in vitro when co-cultured with pancreatic stellate cells (PSCs) in 3D culture. Necuparanib inhibited invasion of AsPC-1 in a 3D co-culture system in a dose-dependent manner.

**CONCLUSIONS**

- Necuparanib was shown to inhibit tumor cell proliferation in 2D culture.
- Pancreatic tumor cells such as AsPC-1 and Capan2, are only found to be invasive in vitro when co-cultured with pancreatic stellate cells (PSCs) in 3D culture.
- Necuparanib inhibited invasion of AsPC-1 in a 3D co-culture with stellate cells in a dose-dependent manner.
- Necuparanib and Fragmin inhibited invasion in a dose-dependent manner confirming that anti-tumor activity, which had been previously observed with heparins, was maintained by necuparanib while low anti-coagulant activity allows for higher concentrations to be administrated to patients.

In summary, our in vitro data indicate that necuparanib affects tumor cell invasion, which is likely driven by modulating tumor-stromal cell interactions.