Inhibition of Heparan sulfate proteoglycans enhanced signaling in Pancreatic Ductal Adenocarcinoma reduces EMT

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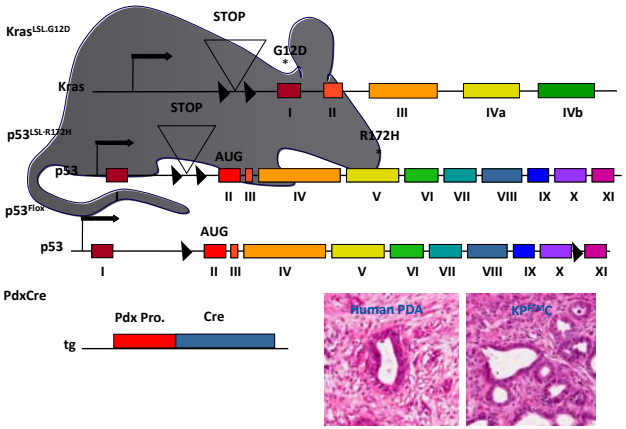
BACKGROUND

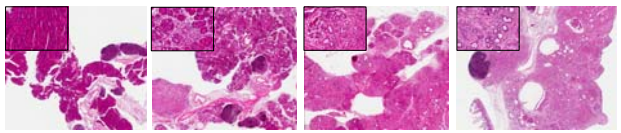
Pancreatic Ductal Adenocarcinoma (PDA) is an almost uniformly lethal disease. For more than 80 percent of the patients with PDA, surgery is not an option. For these patients, palliative chemotherapy using gemcitabine is considered standard of care. Even with palliative chemotherapy, the median overall survival for patients with metastatic PDA is dismal. This is mainly caused by the stromal cells, such as stellate cells and fibroblasts which are creating a favorable niche for tumor cells to proliferate and disseminate. Heparin binding growth factors (HBGFs) and the transmembrane heparan sulfate proteoglycans, to which these growth factors bind, are ubiquitously expressed in pancreatic tumors and contribute to the recruitment of stromal and immune cells. A novel heparan sulfate mimetic (M402), engineered to have low anti-coagulant activity and high binding affinity to growth factors, cytokines and soluble factors, reduces tumor growth and metastasis formation in preclinical tumor models.

GEM MODEL FOR PDA

We created the KPFMC mouse model by interbreeding pdx-Cre; Trp53Flox with KrasG12D; Trp53R172H mice. Pancreas specific pdx1-Cre recombines flox sites in Trp53, inducing loss of heterozygosity (LOH) of the wild type Trp53, and a flox stop cassette in the mutated Kras and Trp53 allele inducing expression of KrasG12D and Trp53R172H respectively. Tumors of these mice recapitulate the biology and histology of human pancreatic cancer.



Tumor development in these mice is not a stochastic event and occurs on Day 30 after birth of the mice. The median survival of these mice is approximately 60 days.



WT pancreas

KPF/MC Day30
Multi-focal dysplasia with small tumours and some remaining normal pancreatic tissue

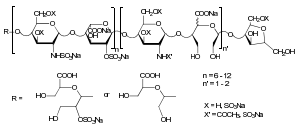
KPF/MC Day 45
Multi-focal tumours, clear consolidation, almost no normal tissue left

KPF/MC Day 51
Variable tumour load but no normal tissue left and more solid areas of tumour

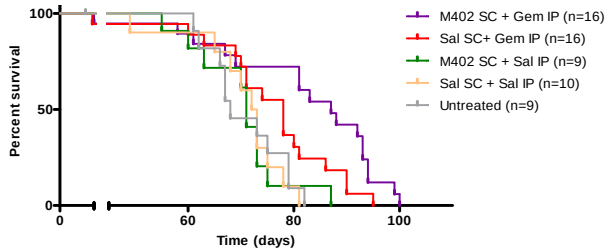
Increase in number of tumour foci

Growth of tumours

SURVIVAL, APOPTOSIS AND PROLIFERATION

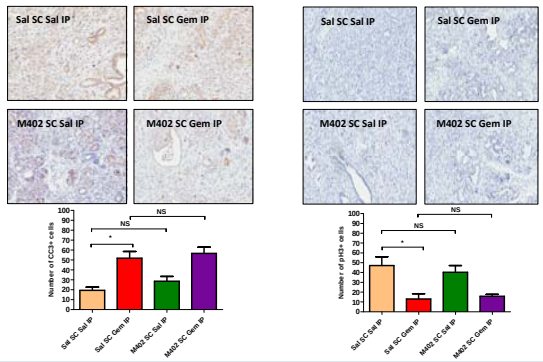


The heparan sulfate mimetic M402 was created by glycol-splitting the non-sulfated uronic acid residues after depolymerization of unfractionated heparin. M402 has low anti-coagulant activity (<2 IU/mg anti-IIa and anti-Xa activity) and high affinity to FGF-2, VEGF, SDF-1, HGF, SHH, P-selectin. M402 in combination with the standard of care treatment gemcitabine, increased survival of KPFMC mice.



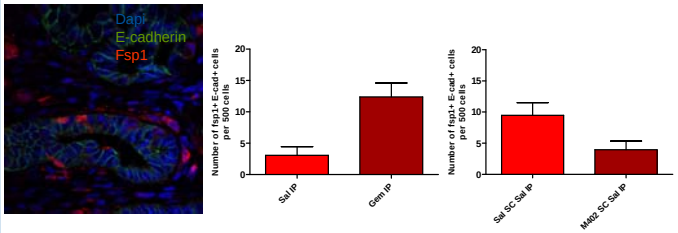
Survival comparisons between treatment groups			
	Median Survival (days)	Log-Rank test p-value	HR (95%CI)
Sal/Sal vs. Sal/Gem	72.5 vs 78	0.088	0.43 (0.17- 1.13)
Sal/Sal vs. M402/Gem	72.5 vs 87	0.0029 *	0.19 (0.062-0.56)
Sal/Gem vs. M402/Gem	78 vs 87	0.049 *	0.46 (0.21- 0.99)

M402 has no influence on changes in apoptosis and proliferation induced by gemcitabine.

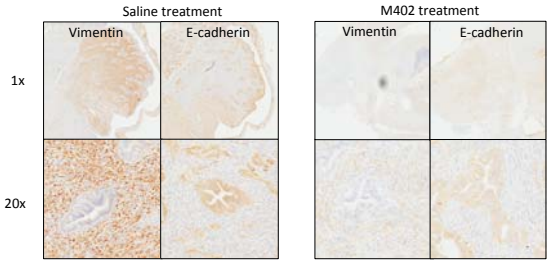


EMT

The effect of M402 on EMT was confirmed by observation of E-cadherin and Fsp1 double positive cells. Gemcitabine treatment increased EMT as indicated by an increase in E-cadherin Fsp1 double positive cells in pancreatic tumors. In contrast, M402 treatment resulted in a decrease in E-cadherin and Fsp1 double positive cells.



Immunohistological stainings with the epithelial marker E-cadherin and the mesenchymal marker Vimentin suggest that M402 inhibits EMT



CONCLUSION

The heparan sulfate mimetic M402 extended survival of a genetically engineered mouse model for pancreatic cancer when it is combined with the current standard of care chemotherapy gemcitabine. The combined therapy had no effect on apoptosis and proliferation in the primary tumor. Interestingly, M402 inhibited EMT in pancreatic tumors of KPFMC mice. In contrast, gemcitabine monotherapy resulted in an increase in EMT as indicated by an increase in E-cadherin Fsp1 double positive cells. The inhibition of gemcitabine-mediated EMT induction by M402 slows tumor progression resulting in prolonged survival. The key targeted pathways are under investigation.