Abstract: LB-43

M402, a Novel Heparan Sulfate Mimetic, Synergizes with Gemcitabine to Improve Survival and Reduce Metastasis and Epithelial-to-Mesenchymal Transition (EMT) in a Genetically Engineered Mouse Model for Pancreatic Cancer

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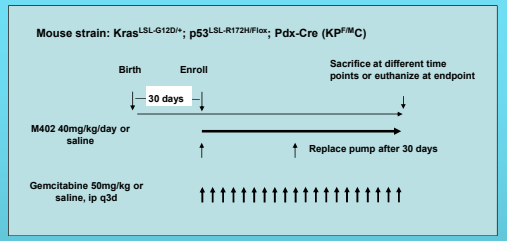
Introduction

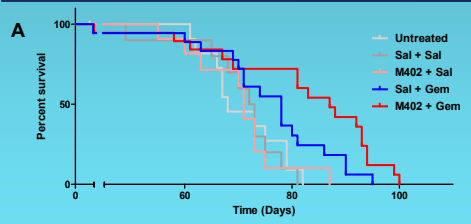
Heparan sulphate proteoglycans (HSPGs) play a central role in tumor progression and metastasis by presenting and modulating growth factors, cytokines, and other soluble factors. A novel heparan sulfate mimetic (M402), engineered to have low anti-coagulant activity, has shown promising anti-tumor efficacy in several pre-clinical tumor models. This study was designed to probe the efficacy and mechanism of action of M402 in a genetically engineered mouse (GEM) model for pancreatic cancer.

Methods



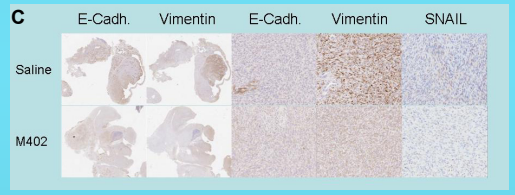
The Kras$^{LSl-G12D}$ has a stop codon flanked by Lox sites in the promotor region; the p53$^{LSL-R172H}$ allele has a stop codon in the first exon flanked by Lox sites and the p53^{Lox} allele has a Lox site in intron 1 and 10 that results in a null allele upon recombination. All alleles are activated using Cre Recombinase under control of the pancreas specific Pdx promotor. The M402 was delivered using a subcutaneously implanted osmotic pump.

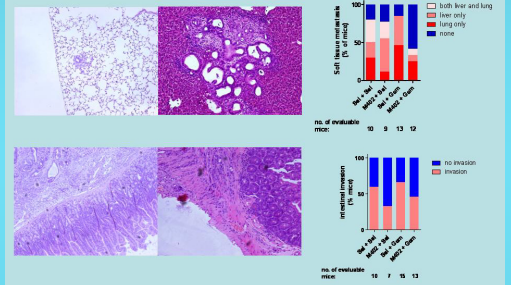
Results: Survival Study



B

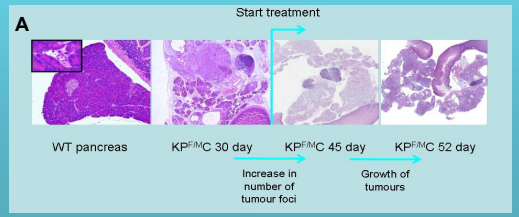
Survival comparisons between treatment groups			
	Median Survival (days)	Log-Rank test p-value	HR (95%CI)
Sal/Sal vs. Sal/Gem	72.5 vs 78	0.088	0.43 (0.17- 1.13)
Sal/Sal vs. M402/Gem	72.5 vs 87	0.0029 *	0.19 (0.062-0.56)
Sal/Gem vs M402/Gem	78 vs 87	0.049 *	0.46 (0.21- 0.99)

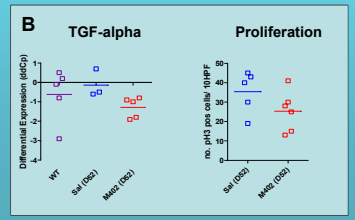




A: Survival curve of KP$^{F/M}$C mice in different treatment groups B: survival statistics of essential comparisons C: IHC for EMT related proteins on tissues derived from mice on survival studies. M402-treated tumors displayed reduced Vimentin staining and SNAIL translocation to the nucleus, suggesting reduced EMT. D: Pictures and quantification of metastasis frequency and invasiveness. Left 2 panels: representative pictures of lung (upper left), liver (upper middle), and intestines (lower left and middle) from mice on survival studies step-sectioned at 50-100 µm. Right panels: presence of metastasis (upper) or invasions to the intestines (lower) was scored using HE stains

Results: Short Term studies





A:HE stained slides from pancreas derived from KP$^{F/M}$C mice at the different time points used for short term studies. B: left panel presents RT-qPCR for TGF-α from RNA derived from D52 tumors; right panel shows quantification of pH3 (proliferation) IHC positive cells, each square represents a tumor sample from a single animal.

Conclusions

- M402 in combination with Gemcitabine significantly prolongs survival as compared to saline or Gemcitabine-treated groups.
- M402 in combination with Gemcitabine leads to less metastases and less local invasion
- M402 inhibits Epithelial-to-Mesenchymal Transition (EMT)
- Micro-array analyses reveal TGF-alpha as potential treatment target
- M402 treatment prevents a rise in TGF-alpha in these mice between 45 and 52 days of age, and this correlates with a decrease in proliferation

Discussion

The combination of M402 and Gemcitabine was the most effective anti-tumor regimen in this study, as manifested by increased survival and, interestingly, decreased incidence of metastasis. One potential mechanism is that the observed reduction in EMT may be due to the reduced expression of TGF-alpha, a cognate ligand for EGFR. These data suggest that the EGFR pathway is active in KRAS mutant tumors. Overall, these results provide a rationale for investigating the clinical use of M402 in combination with Gemcitabine in the treatment of human pancreatic cancer.

Acknowledgements

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