Targeted lipid nanoparticle-encapsulated PROTAC delivery inhibits senescent pancreatic ductal adenocarcinoma

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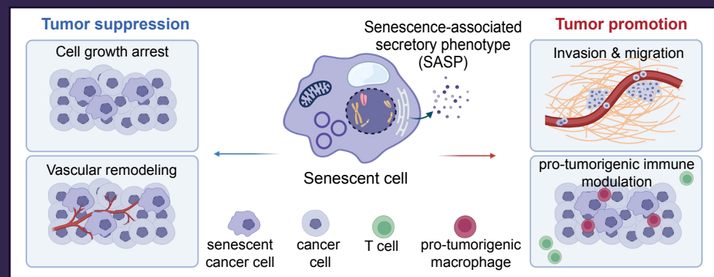
Background

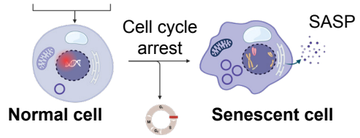
Pancreatic ductal adenocarcinoma (PDAC) arises from malignant modification of the epithelial cells in the duct system. The desmoplastic character of PDAC tumors hinders vascularization and, subsequently, inhibits small molecule drug delivery. Despite recent therapeutic advances, long-term treatments remain ineffective and pancreatic cancer patients face a 5-year survival rate of 12%. Previous work has shown that inducing cell cycle arrest via senescence is able to expose therapeutic vulnerabilities in PDAC through the senescence-associated secretory phenotype (SASP). In SASP, senescent cells secrete pro-angiogenic factors which increase vascularization around the tumor site. However, senescence can also have tumor-promoting effects. In this work, we designed a lipid nanoparticle-based delivery system for a senolytic proteolysis-targeting chimera (PROTAC) molecule which selectively targets senescent cells. PROTACs are an emerging therapeutic strategy which utilizes the ubiquitin-protease system to degrade a target protein and they show particular promise in treating cancers with chemoresistance.

Hypothesis - A one-two punch approach of inducing senescence followed by delivering a senolytic PROTAC degrader to the tumor site will inhibit the growth of PDAC.



Methods: Treatment Strategy

Trametinib MEK inhibitor + **Palbociclib** CDK 4/6 inhibitor



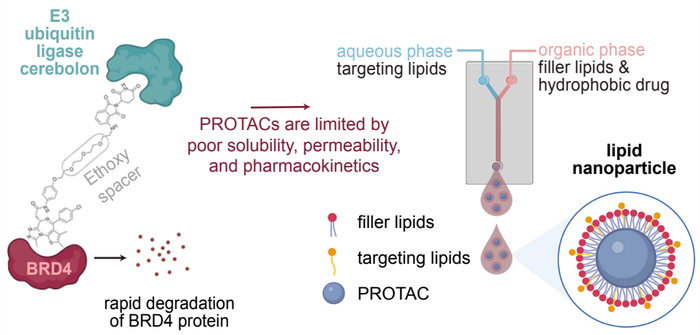
I. Senescence induction - Cell cycle arrest is induced through MEK 1/2 and CDK 4/6 inhibitors to interrupt cell-cell signaling and cell division, respectively

II. Lipid nanoparticle synthesis - LNPs are made by microfluidics with added targeting moieties to NP exterior to aid drug localization to sites of P-selectin and galectin-3 overexpression

- Encapsulated drug - A senolytic PROTAC BRD4 degrader capable of selectively clearing senescent cells

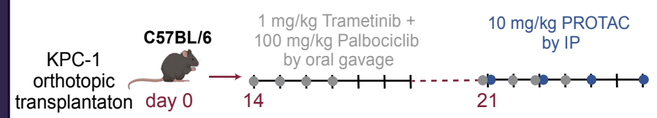
Proteolysis-targeting chimera (PROTAC)
degrading the 'undruggable protein'

Nanoformulation
Improving drug delivery



Methods: *In Vivo* Efficacy

III. Animal model - Efficacy and pharmacodynamics of free drug and LNPs are measured in a murine model of senescent pancreatic cancer



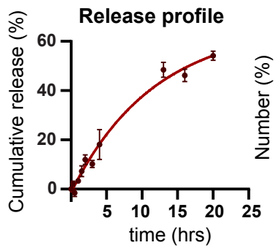
Treatment groups
Free drug
Untargeted LNPs
Gal-3 targeting LNPs
P-sel targeting LNPs
Vehicle

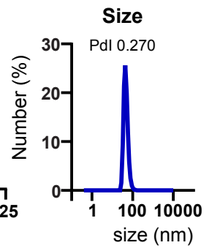
Efficacy study metrics

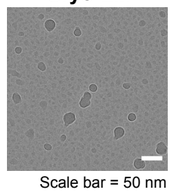
- Ultrasound for tumor growth assessment
- Body weight and blood biomarkers measurments to assess drug toxicity
- Animal survival
- Histological analysis for BRD4 degradation & cleaved caspase-3 activity

Results & Discussion

I. Lipid nanoparticle characterization



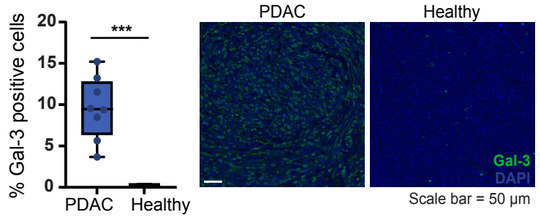


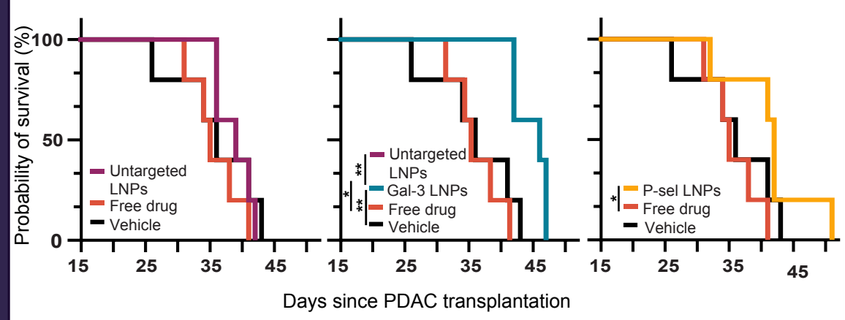


PROTAC-encapsulated LNPs form with sizes between 25 and 50 nm with high drug encapsulation efficiencies of 80% and drug loading of 13%.

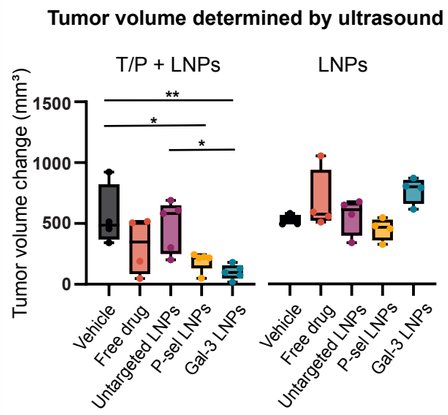
II. Efficacy in a PDAC murine model

Senescent PDAC tumors exhibit galectin-3 overexpression when compared to the pancreases of healthy mice, as confirmed by immunofluorescence. Quantified by QuPath, *** p < 0.001 by Welch's t-test.

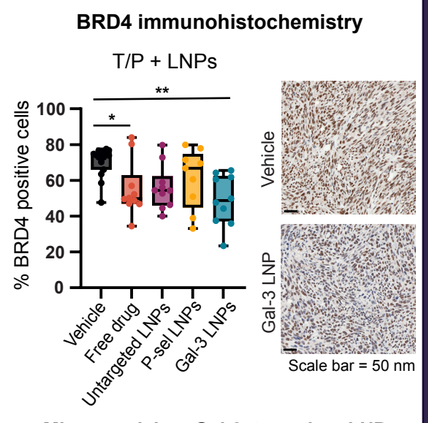




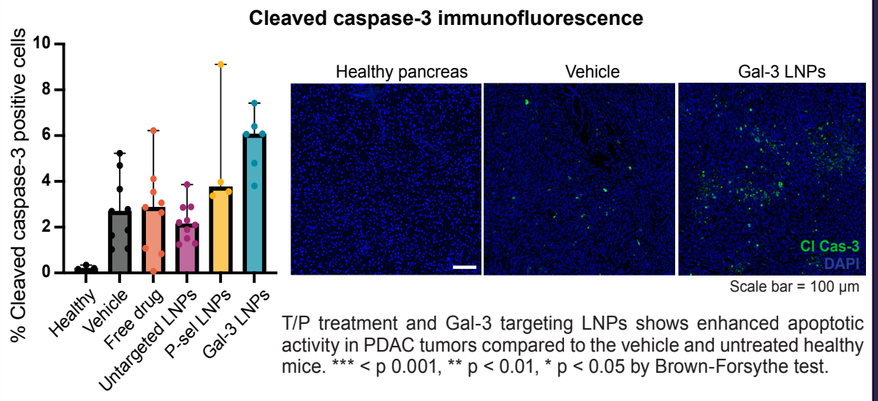
Mice receiving Gal-3 targeting LNPs survive significantly longer than those receiving free drug, untargeted LNPs, or no treatment. ** p < 0.01, * p < 0.05 by Log-rank (Mantel-Cox) test, n=5.



Gal-3 targeting LNPs receiving mice had significant tumor growth inhibition in senescent PDAC between days 14 and 31 post-transplantation. ** p < 0.01, * p > 0.05 by ordinary one-way ANOVA analysis.



Mice receiving Gal-3 targeting LNPs had significantly less BRD4 positive cells compared to the vehicle. ** p < 0.01, * p > 0.05 by ordinary one-way ANOVA analysis.



T/P treatment and Gal-3 targeting LNPs shows enhanced apoptotic activity in PDAC tumors compared to the vehicle and untreated healthy mice. *** < p 0.001, ** p < 0.01, * p < 0.05 by Brown-Forsythe test.

Conclusions

We have investigated the efficacy of treating murine PDAC by combining senescence induction with the targeted delivery of a senolytic degrader to the tumor site. Of all nanoparticle formulations tested, gal-3 LNPs showed superior performance in terms of pharmacodynamics, tumor growth, and survival.

References & Acknowledgements

1. Ruscetti, Marcus, et al. "Senescence-induced vascular remodeling creates therapeutic vulnerabilities in pancreas cancer." *Cell* 181.2 (2020): 424-441.

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D. A. Heller is a cofounder and officer with equity interest of Lime Therapeutics Inc., cofounder with equity interest of Selectin Therapeutics Inc. and Resident Diagnostics, Inc., and a member of the scientific advisory board of Concarlo Therapeutics Inc., Nanorobotics Inc., and Mediphage Bioceuticals Inc.