Addition of an ECM remodeling drug improves target engagement of immunotherapy in solid pancreatic cancer tumors

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ABSTRACT

Pancreatic ductal adenocarcinoma continues to be one of the most lethal cancers today with an abysmal ~8% 5-year survival rate that has remained relatively constant over time. This is thought to be largely due the desmoplastic stroma in the extracellular matrix of these tumor types, inhibiting both the penetration as well as target engagement of treatments. Here we present a methodology for evaluating a monoclonal antibody's drug target engagement in the presence of an extracellular matrix remodeling drug using paired-agent imaging principles and a subcutaneous tumor mouse model.

1. INTRODUCTION

Pancreatic ductal adenocarcinoma is an almost uniformly lethal disease that commonly presents clinically with fibrotic desmoplastic solid tumors. Due to the dense stroma surrounding the cancer cells, drug penetration and target binding are limited, leading to an unfavorable therapeutic response. Recent studies have seen that patients being cotreated with the antihypertensive drug, losartan potassium, have higher survival statistics compared to those who are not.[1,2] This has spurred research into the effects of losartan and how its extracellular matrix (ECM) remodeling capabilities can improve treatment outcomes.[3–5] Here we present a mouse model for the determination of an anti-epidermal growth factor receptor (EGFR) monoclonal antibody's receptor occupancy (RO) when treated with an ECM remodeling drug, compared to a control group using paired-agent imaging (PAI) principles.[6–9] A mixed human cell-line (PANC-1 and AsPC1) is subcutaneously implanted into nude athymic mice, and two groups are treated with losartan potassium daily via intraperitoneal injection, while two groups are not. Upon the tumor reaching the desired diameter, two groups are injected with bolus cetuximab followed by tracers ABY-029, and IRDye700DX-control affibody 24 hours later, while two groups are injected with only ABY-029 and IRDye700DX-control affibody. The tumor is then cryosectioned at 200-micron thickness and imaged on the PEARL fluorescence imager. Population-based RO can then be found by calculating the difference in binding potential (BP) between cetuximab and non-cetuximab injected groups. ECM remodeling can be inferred by the difference in collagen content between losartan-treated and non-treated mice, imaged via second harmonic generation microscopy as well as scanning x-ray diffraction. Group RO measurements can be compared alongside correlation with collagen mappings.

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2. METHODS

A diagram depicting the experimental design can be seen in **Fig. 1.** Mice (n = 24) are injected subcutaneously in the flank with $1x10^6$ 1:1 mixture of AsPC1 and PANC-1 human pancreatic ductal adenocarcinoma cells. After the seventh day of tumor growth, losartan-treated mice are given 20 mg/kg/day of losartan potassium via i.p. injection. Once the tumors reached ~1 cm in diameter, cetuximab-treated mice were given a 20 mg/kg bolus injection of cetuximab i.p. 24 hours later, mice were administered 1 nmol each ABY-029 and IRDye700-control affibody via tail vein injection. After waiting 40 minutes post-injection with imaging agents, mice were sacrificed via an intracardiac KCl injection and the tumors were harvested. Tumors were bisected and embedded in OCT, followed by serial cryosectioning a 10-micron thick slice followed by a 200-micron thick slice. The 200-micron thick slice was then used for fluorescence imaging followed by scanning x-ray diffraction, while the 10-micron slice was stained via hematoxylin and eosin followed by collagen imaging via second-harmonic generation microscopy. Via the fluorescence intensity maps, average binding potential (BP) in the tumor can first be calculated by taking the ratio of average ABY-029 to IRDye700DX-control affibody signals, minus 1. These binding potential values in cetuximab dosed and non-dosed mice can then be used to calculate population-based RO by taking the difference in BP between non-cetuximab dosed mice and dosed mice, divided by BP in non-dosed mice. The corresponding RO measurements in losartan-treated and untreated mice can be the compared to determine if a statistically significant difference exists.
To determine the contribution of collagen density to cetuximab's ability to bind to its target, scanning XRD heatmaps and second-harmonic generation microscopy images can be coregistered with prior calculated BP maps, and the existence of any correlation between local collagen density and cetuximab binding can be elucidated.

3. CONCLUSIONS

This work presents a methodology for the direct investigation of an ECM remodeling drug's ability to modulate both collagen expression along with corresponding drug target engagement of a commonly used immunotherapy. The methods outlined in this manuscript may be useful in assisting drug design and implementation in clinical trials, while also providing valuable insight into a drug's ability to interact with its molecular target under differing conditions of tumor ECM.

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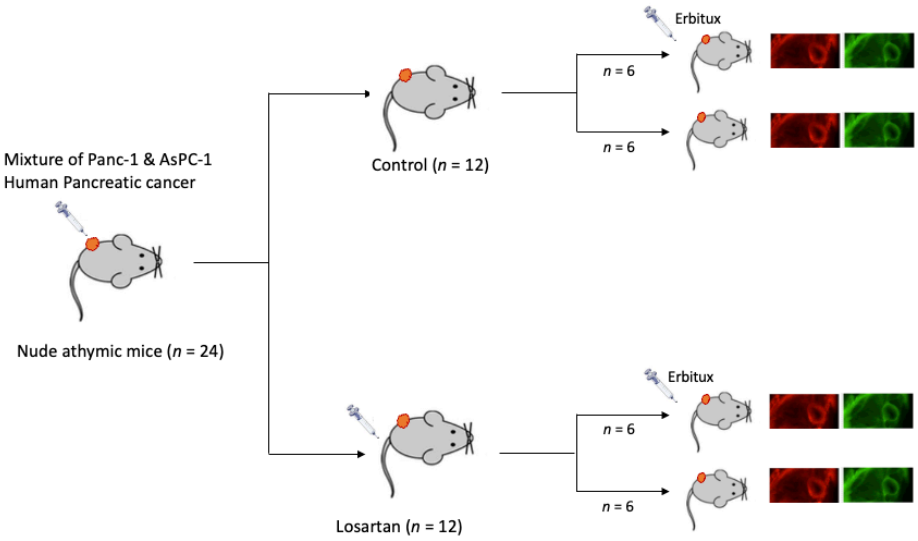

Fig. 1: Experimental setup. From left to right, mice are first implanted with $1x10^6$ of human pancreatic ductal adenocarcinoma cells in the flank. Half of these mice are given 20 mg/kg/day of losartan potassium, while half are not. In both the losartan-treated and non-treated groups, half the mice are given a bolus injection of 20 mg/kg cetuximab 24 hours prior to sacrifice and tumor harvesting.

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