Imaging guided trials of the angiogenesis inhibitor sunitinib in mouse models predict efficacy in pancreatic neuroendocrine but not ductal carcinoma

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AUTHOR SUMMARY

An important step in the drug development process is the demonstration of efficacy in models of human disease in mice. However, many compounds that succeed in mice fail in human clinical trials (1). This disconnect demonstrates the need for better preclinical models and methodologies that can identify drugs with the best chance of success before resource-intensive clinical trials are pursued. In this study, we drew upon the promising field of genetically engineered mouse models (GEMMs) of human diseases. Specifically, we evaluated sunitinib—an antitumor drug that targets aberrant blood vessel growth (tumor angiogenesis)—in mice with pancreatic cancers. Our preclinical trials in such mouse models showed that sunitinib was effective against pancreatic neuroendocrine tumors, but not a second type pancreatic cancer, ductal adenocarcinoma. This study therefore exemplifies the value of preclinical trials in GEMMs, as these models were able to predict both the success as well as the failure of clinical trials in these two distinct forms of human pancreatic cancer.

The comparatively rare form of pancreatic neuroendocrine cancer is modeled by one of the first mouse models of cancer, called RIP1-Tag2, in which targeted expression of a viral oncoprotein in the insulin-producing pancreatic b-cells results in the formation of highly vascularized islet cell tumors (insulinomas) that are quite similar to the human counterpart. The second and more prevalent form of ductal adenocarcinoma is represented by a series of GEMMs that resemble human tumors. In particular, both the tumor vasculature and the desmoplastic stroma (a tumor-supportive infrastructure composed of inflammatory cells, fibroblasts, and fibrous matrix) of mouse pancreatic ductal adenocarcinoma (PDAC) tumors have histopathological similarities to those of the human cancer, likely as a consequence of recapitulating causally implicated mutations from human PDAC into the mouse genome. Both PDAC and pancreatic neuroendocrine tumor (PNET) models have proved timely, because angiogenesis inhibitors targeting the proangiogenic VEGF signaling circuit have been (and continue to be) broadly tested in clinical trials, including trials focused on both PNET and PDAC.

In previous studies, we had shown that sunitinib was effective at inhibiting tumor angiogenesis and impairing tumor growth in the PNET model. Our rationale for comparatively testing sunitinib in PDAC involved several considerations. First was its demonstrable antiangiogenic activity in the pancreas, where its particular benefits, and those of similar drugs, could be ascribed to its ability to doubly target the tumor vasculature via inhibiting both VEGF receptor signaling in tumor endothelial cells and PDGF receptor signaling in the supporting pericytes. Second, it has been known for some time that PDAC tumors are poorly vascularized compared to normal pancreas and to the PNET tumors, suggesting that PDAC would be all the more dependent on what little vasculature was present. Third, we envisioned a potentially broader impact: Cancer-associated fibroblasts express the PDGF receptors and are emerging as important contributors to tumor progression, and thus their therapeutic targeting might prove beneficial.

Surprisingly, however, we observed a lack of efficacy when sunitinib was tested in preclinical trials in PDAC GEMMs. Two possible explanations were prominent: Either the compound failed to inhibit angiogenesis in this tumor type or the drug successfully inhibited angiogenesis, but the tumor was refractory to the loss of vascularity (2). To distinguish between these possibilities, we employed microbubble contrast-enhanced ultrasound imaging, which uses an infusion of tiny bubbles into the circulatory system to noninvasively visualize

![Ultrasound imaging following infusion of microbubbles into the circulatory system of tumor-bearing mice reveals reduced blood flow following antiangiogenic therapy in a genetically engineered mouse model of pancreatic ductal adenocarcinoma. Cine loops (approximately 1-min movies) were taken while injecting the microbubble contrast agent into the circulation (shown as green) before and after 12 d of treatment with the sunitinib drug. Tumor area is outlined in blue.](Image)

Fig. P1. Ultrasound imaging following infusion of microbubbles into the circulatory system of tumor-bearing mice reveals reduced blood flow following antiangiogenic therapy in a genetically engineered mouse model of pancreatic ductal adenocarcinoma. Cine loops (approximately 1-min movies) were taken while injecting the microbubble contrast agent into the circulation (shown as green) before and after 12 d of treatment with the sunitinib drug. Tumor area is outlined in blue.

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blood flow using an ultrasound instrument similar to ones used routinely in the clinic. The ultrasound method also allows determination of tumor size and hence provides a measure of the effectiveness of the drug in disrupting tumor growth. Blood flow and tumor volume were serially monitored during the course of sunitinib therapy in a PDAC model, in comparison with a similar analysis performed in the PNET model, wherein we had previously demonstrated efficacy using standard measurements of angiogenesis inhibition and tumor shrinkage. Strikingly, sunitinib caused a reduction in blood flow in the PDAC tumors, as it did in the PNET tumors, indicative of a disrupted tumor vasculature (Fig. P1), an interpretation that was confirmed by histopathological analyses of biopsied tumors. Although such vascular impairment from sunitinib therapy was associated with shrinkage of PNET tumors, there was no discernible effect on the growth of the PDAC tumors, suggesting that this tumor type has the remarkable capability to grow without the concomitant support of a dense neovasculature produced by VEGF-induced angiogenesis. Notably, clinical trials of sunitinib have demonstrated convincing efficacy in human PNET, leading to its recent approval both in the United States and Europe as a treatment for this form of pancreatic cancer (3). In contrast, clinical trials in PDAC of two other drugs that target the same proangiogenic (VEGF) signaling circuit failed (4, 5).

The concordance between success or failure in our preclinical trials and results from clinical trials for PNET and PDAC presents an important lesson, both for pancreatic cancer research in particular and for cancer drug development in general. First, the failure of this drug in a PDAC GEMM, despite effective on-target activity in disrupting the tumor vasculature, suggests that such antiangiogenic therapies do not have a promising future for this tumor type, consistent with two clinical failures of such drugs. It is unclear whether this unexpected conclusion would have been accepted based on the human trials alone, which monitored tumor shrinkage (“responses”), time to resumption of tumor growth, and overall survival. The use of a mouse model that recapitulated tumor biology and therapeutic outcome, and allowed pre- and posttreatment biopsies and visualization of the tumors during therapy with a noninvasive vascular imaging technology, has added important insights into the unexpected basis for the failure of this class of drug. This realization may stimulate future studies aimed at better understanding how PDAC tumors thrive despite such surprisingly poor vascularization, knowledge which may in turn suggest new therapeutic strategies that exploit their unusual physiology. Perhaps a broader lesson from this work is its illustration of the value of preclinical trials using sophisticated genetically engineered mouse models. Despite the challenges and expense of GEMM-based preclinical trials, the benefit of avoiding clinical failure arguably outweighs the up-front costs. The concordant lack of efficacy in therapeutic trials in mouse models and human PDAC suggests that carefully documented failures in preclinical trials involving representative mouse models could be used to inform decisions about drug development. By this approach, it may be possible to avoid expensive clinical trials that are likely destined to fail with a particular drug, instead focusing on other cancer types where preclinical trials in appropriate GEMMs predict benefit to the cancer patient.