Box 3 Modeling metastasis

Almost all drugs tried in humans work against subcutaneous xenografts in mice. The problem is this hardly ever translates to the clinic. According to a 2004 study, as few as 3.8% of patients in phase 1 cancer drug trials between 1991 and 2002 achieved an objective clinical response[18]. Placing tumor cells under the skin, although commonly used in drug testing settings because the tumors are easy to establish and measure, does not take into account tumor tropism—the predilection for tumors to grow in only certain environments. In seminal work conducted over 20 years ago describing the behavior of tumor cells in different microenvironments, MD Anderson's Fidler confirmed this principle and went on to develop orthotopic models for studying metastasis, in which cells derived from a variety of human tumors were implanted into correct anatomical sites in nude mice[19]. The difference is night and day. Measuring metastasis from tumors planted subcutaneously, Fidler reported zero successes in 700 tries, whereas orthotopic placement of tumor tissue in mice produced metastases in every tumor type attempted.

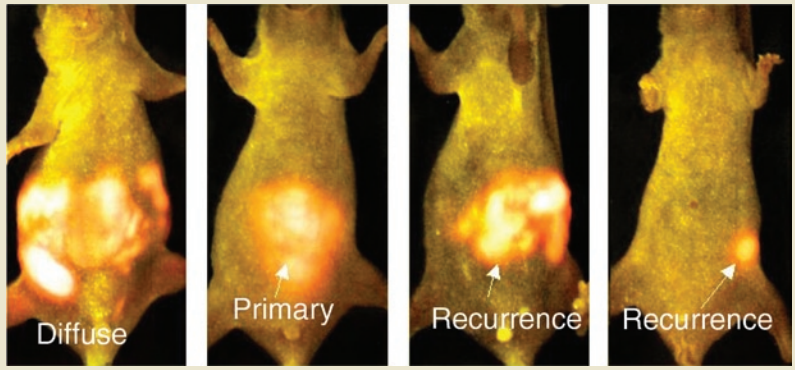

Figure 3 AntiCancer's MetaMouse allows visualization of tumors in whole animals following orthotopic implantation of human pancreatic tumor cells expressing red fluorescent protein. The mice in the different panels received different treatments. Reprinted with permission from ref. 29.

Echoing that sentiment is Robert Hoffman, president and CEO of AntiCancer (San Diego) and professor of surgery at the University of California at San Diego. "An intact tumor microenvironment is necessary for a good cancer model," he says. Hoffman's company has commercialized the concept by creating a set of mice in which tumor tissue is implanted orthotopically at various sites to follow tumor progression and dissemination[20]. Hoffman, along with his then post-doc Takashi Chisima, in 1996 had the idea to make the tumors fluorescent so that imaging could be used to follow tumor cells to distant tissues and organs. It would be the first time cancer metastases would be observed through expression of green fluorescent protein[21]. Today, the company offers a set of organ-specific animal models, called MetaMouse, which have tumor tissue implanted in different organs (e.g., breast, brain, prostate), that allow stromal cells and tumor cells to be labeled with different fluorescent labels so that interactions between the two cell types can be studied *in vivo*. Instead of killing and opening the animal to see where tumors developed, researchers can see the tumors and their progress in real time with an imaging system (**Fig. 2**).

Taking a different approach to dissecting metastasis, the Canadian company Innovascreen (New Glasgow, Newfoundland, Canada) has developed an *in vivo* system to observe and quantify tumor cell behavior and treatment response using a shell-less (*ex ovo*) avian embryo system. Innovascreen founders John Lewis, the CEO, and CSO Andries Zijlstra originated an intravital imaging system while at Scripps Clinic (La Jolla, CA, USA)[22], which is sold as a service by Innovascreen. By injecting tumor cells into the chorioallantoic membrane where they form a tumor, or into the vasculature of the chorioallantoic membrane, they are able to monitor the migration of cells away from the primary tumor, the invasion of the vasculature by tumor cells as well as extravasion from the vessels using three-dimensional time-lapse photography. The imaging system can visualize as many as six different molecules, including fluorescently labeled therapeutics that target vasculature and fluorescent proteins transduced into the tumor cells. Using this direct observation approach, preclinical drug candidates can be quickly assessed for their ability to affect each step in metastasis. "These assays are all designed to be completed within 3 weeks," Lewis says. "It allows for flexibility in planning and refinement of dosing and other experimental parameters to evaluate investigational drugs." *LD* and *GM*

ing through basement membranes. Although S100A4 has been accepted for years as a useful prognostic biomarker—when it is not expressed aberrantly, many breast cancer patients can survive to live out a full life[4]—until recently, it hasn't attracted much attention as a therapeutic target.

This is set to change as Supratek Pharma (Montreal) pushes ahead with a S100A4-targeting drug that was originally approved two decades ago. Supratek has modified the antiallergy product azaxanthone with an excipient that enhances solubility to create an orally bioavailable drug, SP-MET-X1. The intention is to administer SP-MET-X1 in larger doses than labeled for the allergy indication, but as the safety of azaxanthone has been long established, the company hopes that it will be a good candidate for long-term chronic treatment, administered several times daily, potentially for years, as a prophylaxis and maintenance therapy.

Thus far, supporting data from preclinical animal models show that the drug inhibits or delays metastasis formation, implantation and progression in animal models. Moreover, Supratek investigators believe SP-MET-X1 can be synergistic with other forms of therapy, such as chemotherapy. The company is planning to begin a phase 1 trial with SP-MET-X1 this year, but the first indication for which the company will file is not yet settled.

Inhibiting urokinase

Another enzyme that is able to proteolytically degrade the ECM and basement membrane around primary tumors is the secreted 54 kDa serine protease urokinase-type plasminogen activator (uPA). In research that originated at the Technical University of Munich two decades ago, investigators Manfred Schmitt, Viktor Magdolen, Nadia Harbeck and Olaf Wilhelm were searching for reasons why a subset of post-surgical breast cancer patients did particularly poorly in terms of survival. By studying patient plasma and tumor samples, they discovered that levels of uPA are inversely correlated with survival. Their findings ultimately revealed that uPA can trigger