A “Mouse Hospital” for Preclinical Testing of Diagnostic and Treatment Modalities in Pancreatic Ductal Adenocarcinoma (PDA)

Background and rationale:

Drug development for cancer is a time consuming and costly endeavor. One of the major challenges is choosing which compounds to bring forward to clinical trials, given the expense and time involved in human testing. Mouse models have provided a helpful pre-screening tool, with xenotransplantation of human tumor cell lines serving as the conventional method for assessing in vivo efficacy prior to embarking on Phase I clinical trials. While this traditional approach has been useful, it is also associated with a high failure rate: it is estimated that 90-95% of cancer drugs that enter clinical trials do not make it to market. Thus, xenograft models are poorly predictive of a treatment’s efficacy against a naturally-occurring tumor.

There may be multiple reasons that xenografts would respond to a test drug while bona fide tumors may be resistant. Principal among these is the fact that xenografted cell lines do not reproduce the tumor microenvironment. Most epithelial tumors are encased by a dense and complex stroma that includes fibroblasts, blood vessels, and immune cells. This stromal microenvironment can influence the ability of a drug to penetrate and reach tumor cells, drug metabolism, and the overall metabolic state of a tumor. Tumors generated by injection of human cell lines recapitulate few features of the normal tumor microenvironment, which likely contributes to their poor predictive power. Another specific drawback of xenografts is a failure to appropriately model the contribution of the host immune system during cancer treatment. Tumor models employing human cells require the use of immunodeficient mice as recipients to prevent recognition of cross-species antigens. Thus a xenograft’s response to a candidate anti-tumor agent cannot adequately interrogate this component of the anti-tumor response. A third significant limitation of such traditional models is that tumor cell lines used for xenograft tumor formation have a relatively fixed and homogenous genetic makeup. This is in contrast to the heterogenous composition of a naturally-occurring tumor, which may have a dramatically different effect on a tumor’s response to drugs. Hence, a preclinical model that more accurately reproduces all the features of a tumor, including its microenvironment, may provide a more accurate readout for response to therapy.

The Pancreatic Cancer “Mouse Hospital” at the Abramson Cancer Center – an innovative concept for preclinical testing:

Pancreatic ductal adenocarcinoma (PDA) is an almost uniformly lethal disease for which novel effective therapies are critically needed. PDA is currently the 3rd leading cause of cancer death in the United States, but recent projections suggest that pancreas cancer will overtake colon and breast cancer to become the second leading cause of cancer death by 2020 [1]. PDA is an excellent example of the poor predictive value of traditional preclinical models: despite a number of successful preclinical trials with novel agents using
xenograft models, the standard of care for advanced pancreas cancer has not changed significantly since 1997, and remains gemcitabine or, for patients with excellent performance status, combination therapy with the FOLFIRINOX regimen.

To avoid these limitations, genetically engineered mouse (GEM) models of PDA have been developed [2-4]. These models exploit the fact that virtually all PDAs exhibit activating mutations in Kras (exon 12) and that over 75% have mutations in p53. These models use a pancreas-specific Cre recombinase (“C”) to create mutations in Kras (“K”) and p53 (“P”) in the pancreatic epithelium. Genetically engineered mice harboring a pancreas specific Cre allele along with conditional mutant alleles in p53 and Kras are collectively referred to as the “KPC model”.

Unlike their counterparts with grafted tumors, KPC mice recapitulate the salient clinical, histopathologic, and molecular features of the human disease. These features include the development of premalignant Pancreatic Intraepithelial Neoplastic lesions (“PanIN” lesions), acquisition of a dense desmoplastic stroma (which renders KPC tumors histologically indistinguishable from their human counterparts), and metastasis (particularly to the liver). Furthermore, KPC mice are immune-competent. This is of critical importance, as we have previously shown that progression to PDA in these mice is intimately associated with a suppressive immune reaction, particularly in the myeloid compartment, such that adaptive anti-tumor immunity is essentially non-existent [5,6].

Available models - KPC, KPCY, orthotopic:

The KPC mice described above serve as the ‘bread and butter’ model for the mouse hospital, including basic studies investigating tumor progression and preclinical testing of novel compounds in a chemo-preventive or therapeutic setting. KPC mice develop premalignant PanIN lesions and adenocarcinomas with reproducible kinetics (at 7-10 weeks of age for PanIN lesions; 17-19 weeks for PDA) and high penetrance. Screening for tumors is done by weekly abdominal palpation and ultrasound starting at approximately 11 weeks of age (Fig. 1) and, when tumors have reached an appropriate size (typically a volume of 30mm$^3$), animals are enrolled in the study as they would be in a human trial. Ultrasound exams are used throughout the trial period to measure tumor responsiveness (primarily tumor volume, but assessment of tumor vascularization and density are feasible.
using the Vevo 2100 ultrasound). In addition to overall survival and tumor volumetric measurements, endpoints for analysis include serum chemistries, histology, tissue collection for pharmacodynamics studies, immune subtyping, etc. as would be performed in a human clinical trial.

In addition to the KPC model, we have developed the KPCY model to be able to study the parameters of metastasis [7]. In addition to the aforementioned Kras and p53 mutations, KPCY mice have a fluorescent lineage label in the epithelial cells of the pancreas, causing them to appear green under light (Fig. 2). This in turn allows us to follow pancreatic cells as they move out of the pancreas and into the bloodstream and other tissues, prior to becoming established (and easily detectable) metastases. KPCY mice will be used for experiments aimed at detecting effects on metastatic incidence or growth from a given compound or intervention.

Both the KPC and KPCY animals produced in the Mouse Hospital are fully backcrossed on the C57 BL/6 strain background.

Syngeneic models provide less labor-intensive tools for evaluating efficacy of cancer therapies in the presence of a functional immune system. The Mouse Hospital performs orthotopic implantation of murine PDA cell lines (derived from KPC and KPCY mice) in the pancreas of C57 BL/6 mice, both by laparotomy and by ultrasound guided injection. The ultrasound guided technique is faster and less invasive, and it requires less recovery time than the traditional laparotomy approach.

**Infrastructure and Previous Accomplishments:**

The Pancreatic Cancer Mouse Hospital of the Abramson Cancer Center is housed in the vivarium located on the 6th floor of the Smilow Center for Translational Research at the University of Pennsylvania. Both institutional and philanthropic funds were used to establish this facility. The space consists of a 700 cage holding room and two procedure rooms housing two state-of-the-art VisualSonics Vevo 2100 imaging systems for ultrasound studies.

The activities of the Mouse Hospital are overseen by an Executive Committee that consists of Drs. Ben Stanger (chair), Robert Vonderheide, Gregory Beatty, and Anil Rustgi. The Executive Committee will be responsible for decisions regarding resource
allocation and ensuring quality standards for work done within the Mouse Hospital. Operations are overseen by Cynthia Clendenin, VMD, the scientific director of the facility. In addition, the activities within the Mouse Hospital are monitored by University Laboratory Animal Resources (ULAR) and the Institutional Animal Care and Use Committee (IACUC) to ensure that all activities are in compliance with institutional and federal regulations governing the use of animals in experiments.

The Mouse Hospital serves several different types of “clients,” supporting the research activities of investigators at the University of Pennsylvania (including the laboratories of members of the Executive Committee), academic collaborators at other institutions, and Industry collaborators. Such relationships are managed through grant subcontracts or sponsored research agreements as appropriate. In addition to basic studies involving administration of drugs or biologics to KPC and KPCY animals (with standard survival and tumor measurement endpoints), we are also able to pursue more refined studies involving serum and tissue analysis (including immunostaining, flow cytometry, and molecular analyses). The use of such resources will be discussed, as appropriate, at the outset of any collaboration.

The Mouse Hospital paradigm has been successfully deployed in several preclinical trials performed here at Penn and in centers on other campuses. In one example, we performed a preclinical trial with a γ-secretase inhibitor (GSI), a class of drugs which has inhibitory activity against the Notch pathway. In this study, we found that treating animals with GSI during the PanIN stage resulted in a dramatic delay in progression, resulting in a complete inhibition of tumor formation in the treatment group (compared to a 35% tumor incidence in the vehicle-treated control group) over the time period examined [8]. In another study, the efficacy of a CD40 agonist antibody was tested in animals with established tumors (identified by ultrasound) for anti-tumor efficacy. In this study, approximately a third of the animals exhibited tumor regression, a nearly unprecedented response rate in KPC animals (as alluded to above, KPC animals show minimal responsiveness to agents that are effective in xenografts). In a parallel clinical trial, a third of human patients showed a response when treated with a humanized CD40 agonist antibody, further bolstering the notion that the Mouse Hospital will provide a predictive readout for clinical responsiveness [9].
Parties interested in utilization of the Mouse Hospital can contact Drs. Clendenin, Stanger or Vonderheide. Our collaborations with industry are typically carried out under sponsored research agreements. A detailed project description can be assembled for consideration following discussions with Dr. Clendenin and the project leader.

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