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 involves 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.

There are likely to be several reasons that xenografts may 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 from human cell lines recapitulate few features of the normal tumor microenvironment, which likely contributes to their poor predictive power. One 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 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. Another significant drawback 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 is likely to have dramatic effects 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 4th 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 2025 [1]. PDA represents an excellent example of the poor predictive value of traditional preclinical models, for despite a number of successful preclinical trials with novel agents using xenograft models, the standard of care for advanced pancreas cancer remains gemcitabine or for patients with excellent performance status, combination therapy with the FOLFIRINOX regimen.

Based on these limitations, genetically engineered mouse (GEM) models of this disease 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 grafted counterparts, KPC mice recapitulate the salient clinical, histopathologic, and molecular features of the human disease. These features include the development of premalignant Pancreatic Intrepithelial 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:

The KPC mice described above serve as the “bread and butter” model for the mouse hospital, including basic studies investigation of 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 (7-10 weeks for PanIN lesions; 14-18 weeks for PDA) and high penetrance. Screening for tumors is done by weekly ultrasound starting at approximately week 13 (Fig. 1), and when tumors have reached an appropriate size (typically 5mm) 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 (mainly tumor volume at this
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 in this model [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 (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 an impact on metastatic incidence or growth from a given compound or intervention.

Fig. 2. The KPCY model. In this model, a fluorescent lineage tracer (YFP) is introduced into the pancreatic epithelium at the same time as Kras and p53 mutations, resulting in a pancreas that appears green (right panels). These cells can be traced, quantified, captured, and analyzed as the spread from a pancreatic tumor through the bloodstream to distant sites.

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 and two Vivo 2100 state-of-the-art small animal ultrasound machines. We anticipate that when operating at full capacity, the Mouse Hospital will be able to conduct 10-15 large-scale preclinical trials per year.

The activities of the Mouse Hospital are overseen by an Executive Committee that consists of Drs. Ben Stanger (chair), Robert Vonderheide, and Gregory Beatty, all of whom have an extensive background in translational cancer research with particular expertise in immunotherapy. The Executive Committee will be responsible for decisions regarding resource allocation and ensuring quality standards for work done within the Mouse Hospital. In addition, the activities within the Mouse Hospital will be overseen 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. Operations are
overseen by Cynthia Clendenin, VMD, who serves as scientific director and oversees well-trained professional staff that administers drug and collects clinical data.

Since its inception over five years ago, the Mouse Hospital has served several different “clients.” This has included support for the research activities of investigators at the University of Pennsylvania, academic collaborators at other institutions, and Industry collaborators. Such relationships will be 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. Additional services (e.g. orthotopic transplantation models, ultrasound-guided intratumoral treatments, etc.) are routinely being developed within the mouse hospital and can be deployed for certain projects.

The Mouse Hospital paradigm has been successful in several preclinical trials performed here at Penn and in other centers elsewhere. Successful examples have included treatments with γ-secretase inhibitor (GSI) [8], CD40 agonist antibodies [9], and other agents with an emphasis on immunomodulatory drugs. Because many agents administered in the mouse hospital are also undergoing evaluation through human clinical trials, mouse hospital affords the opportunity for “co-clinical trials” that seek to replicate the clinical effects observed in humans while simultaneously extracting additional mechanistic information from the mouse studies.

**Types of Studies Conducted:**

**Randomized controlled studies:**

These studies follow a standard paradigm ranging from 2-8 treatment arms. The control arm typically involves standard-of-care chemotherapy for pancreatic cancer (gemcitabine + nab-paclitaxel) while experimental arms can include drug of interest alone and in combination with gem/nP. Animals are enrolled when tumors reach a given size as detected by ultrasound (typically 50-150 mm3) and tumor size is monitored over time.
Trials can be conducted as “fixed endpoint” studies – in which animals are sacrificed after a given treatment interval (e.g. 2 weeks) to assess the biological impact of treatment (i.e. effects on tumor cells phenotype, tumor microenvironment etc.). Alternatively, trials can be survival studies, in which the endpoint is morbidity or death, to assess the impact of therapy on animal lifespan. Cohorts of 10 or greater mice per treatment arm are employed to achieve power to detect clinically meaningful differences in survival. Samples are collected for both types of studies for further cellular and molecular analyses.

**Rapid multi-arm combination testing:**

In this iteration, multiple drug combinations are tested in parallel to identify combinations leading to tumor regression. The advantage of this paradigm is that fewer mice are used per condition (5-6 mice/group) allowing for more hypotheses to be tested per study. This approach is possible because regressions are never seen under standard therapy conditions, making any drug combination leading a tumor to regress a significant finding. Combinations giving a signal can then be studied in greater detail using the randomized paradigm above. The figure below gives a representative example of such a combination study, where conditions 3 and 4 show benefit over condition 1 (standard chemo).
Parties interested in utilization of the Mouse Hospital should contact Drs. Clendenin, Stanger, Beatty, or Vonderheide. A detailed project description can be assembled for consideration following discussions with Dr. Clendenin and the project leader.

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References


