CHARACTERIZATION AND DISTRIBUTION OF OVARIAN PATIENT-DERIVED XENOGRAFTS

Limitations in conventional preclinical studies. One of the cornerstones of preclinical cancer research in the last 30 years has been the of cell lines, both *in vitro* and *in vivo*. Cell lines are available, amenable to manipulation, reproducible, and relatively inexpensive, making them ideal for studying a specific pathway or gene mechanism. But cell lines are very different from patient tumors. Most importantly, they are clonal and lack the heterogeneity of tumors developing in patients. *In vitro* they lack stroma and vasculature; and culturing in plastic induces differentiation and other expression changes. Because of their selective growth conditions, ovarian and *lung* cancer cell lines have been shown to be more similar to one another than ovarian cancer cell lines and ovarian tumors from patients[1]. Similarly, transgenic mouse models that spontaneously develop tumors have limitations. They are derived from an initial insult of just 1-3 genes, and with their short incubation times have less genetic chaos than patient tumors. Thus, this heretofore invaluable resource has underperformed in its ability to determine effective therapeutics, as more than 90% of clinical trials of novel therapies are negative. For example, the mean response rate among phase II trials conducted by the Gynecologic Oncology Group in the 170 queue is 6.1% (range 0-21%, with the best response provided an agent that doesn't even directly target tumor cells, bevacizumab), despite ample preclinical evidence suggesting anti-tumor activity in current models. Clearly an improved model is needed to study the complex biology of a patient tumor, and may be able to better predict response of a therapeutic in patients, making clinical trials more efficient and discoveries in the preclinical setting more likely to work in patients. The **overall goal** of this proposal is to characterize and make available a resource that may be such an improvement, the patient-derived xenograft (PDX).

Advantages of the PDX model. Tumors removed from patients and directly implanted into mice have been given many names over the years, such as xenopatient[2], holograft, or avatar, but recent increases in their use have led to a general agreement that the moniker PDX may win out. Increased use, acceptance, and importance was highlighted by multiple sessions on the model at the 2013 Annual Meeting of the American Association for Cancer Research. While not extensively studied due to the time, expense, expertise, and effort required, PDX models may offer a significant improvement over cell line studies[3]. PDX tumors retain the histology *and* heterogeneity of the cancers from which they originate, instead of allowing only clonal selection of the cells fit to grow in plastic. Their use can allow the conduct of mini-clinical trials, in which the same PDX tumor in multiple mice can then be exposed to multiple treatments than patient trials in which each patient can only randomize to one treatment arm[2]. Obviously small trials in mice are less expensive than large patient-based clinical trials, and several drug companies have begun in-house programs where PDX models are required prior to moving to clinical trials. Development of the PDX may allow development of personalized medicine approaches, whereby a patient's own tumor can be used to determine activity of a drug. Numerous start-up companies are charging patients or researchers thousands of dollars to investigate these (as yet) unproven paradigms. Beyond therapeutics, use of a PDX model can allow investigators to answer questions that are extremely difficult or impossible to study in patients, or in clonal cell lines. These include how tumors change and evolve over time[4]; which of the many genetic abnormalities in a complex tumor is driving growth or response to therapy[5]; and can the most resistant population in a heterogeneous tumor be identified[6]?

Limitations of the PDX model. Use of the PDX has not been widespread because of numerous practical factors. Researchers do not always have accessibility to patient samples, and even if they do they may not be received in a timely fashion to maximize viability. Techniques to allow successful implantation are advanced. It takes weeks to months for an initial xenograft to grow, although subsequent generations generally grow more quickly. High-throughput tests to characterize the tumors and guide biologic investigations are expensive and require a team of analysts to interrogate. Additionally, this model is best suited to studying intrinsic (tumor cell-specific) biologic questions, since the immune system is compromised in the immunodeficient mice in which they must be grown, and the stromal interactions may differ from human tumors as the xenografts are infiltrated with murine stroma. However, if a centralized resource were available where established and fully characterized PDX tumors could be quickly expanded for biologic and therapeutic investigations, it may represent a significant advance in scientific knowledge.

Feasibility of the PDX model optimized. For the past 3 years, we have systematically been studying and refining the best methods for establishing the PDX in SCID mice. We have achieved an 85% success rate in

establishing a PDX after subcutaneous implantation from a patient, which can be expanded for additional studies (Figure 1A). Our initial impetus for establishing such a program was so that we can identify the population in a heterogeneous tumor that mediates chemotherapy resistance. Because most ovarian tumor cells in patients a) are actually sensitive to chemotherapy at presentation, as noted by outstanding initial responses in most cases but b)

Figure 1. "Holograft" PDX tumors are efficiently established after SQ implantation (A), and respond to chemo similarly to patients (B).

have an unacceptably high recurrence rate despite initial remissions, we sought to treat these xenografts with chemotherapy, selecting out the resistant cells for further characterization. We have noted that response to chemotherapy in patients is remarkably similar to that in corresponding PDX, as noted by slow (or absent) responses to chemotherapy by the PDX from patients who have a partial response compared to those with a completer response (Figure 1B). Therefore the PDX line is feasible and biologically similar to the patient-derived sample. There are more questions to be pursued than can be done by one group, for which this model may serve. Thus we propose sharing this resource with the general research community.
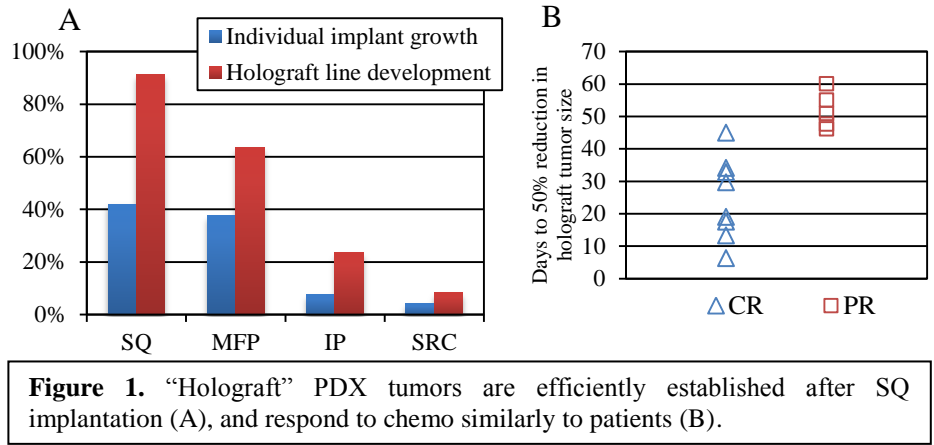
Proposal to share the PDX resource. We currently have 55 PDX tumors growing or stored (and confirmed to grow after thawing). In this proposal, we request funds to support expansion of these samples to be shared with multiple investigators, characterization of the tumors from which they were derived and the murine growth state, and establishment of an infrastructure by which they can be shared. Investigators can the request tumors for their own expansion and novel investigations, circumventing the most time-consuming and expensive phase of these studies: collection, establishment, and characterization of the PDX.

Specific Aim 1: Expand existing tumors in viable formats for sharing. Currently multiple small tumor fragments in each PDX are stored in viability media at early-passage (less than 3). For the resource to be shared with multiple investigators, they will need to be thawed and expanded for additional tumor volume.

Specific Aim 2: Molecular profiling of the cohort. To maximize usefulness to a collaborating investigator, the tumors will be profiled with Deep Exome Sequencing. Both patient tumors and xenografts in mice will be profiled, so that we can fully understand the differences and potential limitations of the model. This profiling will also allow appropriate selection of samples for sharing, such as the case of study of a targeted therapeutic. Established collaborators in the Genetics and Bioinformatics Departments will assist with this profiling.

Specific Aim 3: Establish a process for distribution. As we anticipate a great deal of interest in obtaining PDX samples, a system must be established. A website will be established for submission of requests and sharing of data (all profiling data, and de-identified clinical data will be shared on request). A panel of experts will be selected to review and prioritize proposals. Preference will be given to those programs with the highest likelihood of leading to clinical benefit or understanding of a crucial aspect of ovarian cancer biology. PDX tumors will be provided *without* expectation of authorship, inclusion in patentable discoveries, or possession of intellectual property. After selection, tumors will be provided free (shipping and handling charges only), with the understanding that their discoveries will be shared with other users of this resource.

IMPACT. We know a great deal about primary ovarian cancers as a whole, but very little about which cells in a heterogeneous tumor dictate growth, response and resistance to therapy, and genetic variation. Current models have shown promise in flawed preclinical studies that ultimately fail in the clinic. There is a need to raise the bar of what we consider effective before use in patients by developing a model that more closely resembles the patient tumor. The PDX model holds many advantages, but only by a concerted effort by multiple investigators can we unlock its potential. Unfortunately the model is cumbersome and expensive in its initial stages. Creating a centralized resource that takes care of these initial obstacles, and sharing fully characterized tumors freely to the research community, may lead to numerous new discoveries of clinical benefit in ovarian cancer.