PREDICTORS OF RESPONSE TO PARP INHIBITORS IN OVARIAN CANCER

Attempts are being made through multiple profiling methods to predict response to cytotoxic or newly-developed biologic agents. However, which method (mutation analysis, amplification, mRNA expression, etc) is the most predictive of a biologic response is not known and difficult to study. A discovered mutation does not necessitate oncogenic addition to that gene, or necessarily predict response to therapy. Complex systems have redundant pathways that may compensate for a single overexpressed family member, as was recently found when attempts to correlate VEGF levels to response to bevacizumab failed in endometrial cancer[1]. Another promising therapeutic scenario for which it would be desirable to have a predictor for response is PARP inhibition (PARPi). The TCGA study in ovarian cancer concluded that about 50% of tumors might respond to PARP inhibitors, based on noted mutation, amplification, overexpression, or hypermethylation of key genes that mediate Homologous Recombination (HR)[2]. Several recent studies have shown that PARP inhibitors have promising activity in patients with recurrent epithelial ovarian cancer, both in the presence and in the absence of germline BRCA1 or BRCA2 mutations (BRCA is a critical component of the HR pathway). A phase II trial of olaparib maintenance therapy in platinum-sensitive BRCA-wt patients recently demonstrated an impressive doubling of the progression-free interval from 4.8 to 8.4 months[3]. However, moving forward into clinical trials, it is not known whether PARPi should be offered to all patients, BRCA1/2 mutated patients, or just those with HR defects (HRD). A "BRCAness" profile has been generated based on BRCA-mutated patients, and confirmed to predict response to platinum-based therapy[4], but this signature remains to be validated regarding patient response to PARPi therapy. Additionally, it is not known whether a patient with one of the TCGA-defined perturbations actually has functional defects in HR, and whether there are other genes not formally in this pathway that might allow response to PARPi. In preclinical models, the most reliable predictor for response to PARP is examining *functional* HRD, as assessed by noting low rad51 foci formation after exposure to radiation (XRT) *in vitro*. While this may represent a gold standard for predicting response to a PARPi, this is a cumbersome assay that requires fresh tissue and is unlikely to be viable as a widely available method. Our **overall goal** is to use this assay and *in vivo* response to PARP inhibitors in a preclinical model, to develop a gene signature that is *clinically feasible* and will allow accurate prediction of response to PARPi.

While examination of cell lines as a means to study a specific pathway or gene manipulation is useful, cell lines are very different from patient tumors. They lack stroma, vasculature, and heterogeneity – limitations that have contributed to failure of many promising therapeutics when taken to clinical trials. Because of their selective growth conditions, ovarian and *lung cancer* cell lines are in many ways more similar to one another than ovarian cancer cell lines and ovarian cancers from patients[5]. This would be especially problematic when trying to find multiple genetic perturbations that may mediate a response to therapy, such as PARPi. In an effort to improve on this model, we have established reliable methods to consistently establish primary xenografts in mice directly from patient samples, with an 80-90% rate of maintaining a "xenoline" from a patient sample (Figure 1A). Growth in subcutaneous (SQ), mammary fat pad (MFP), intraperitoneal (IP) and subrenal capsule (SRC) have shown highest take rates with the SQ route. We have shown that these growing tumors are not simply composed of tumor-initiating cells (less than 10% variability between xenoline and patient tumors in regard to CD44, CD133, and ALDH expression), and retain the heterogeneity and histologic classification of patient tumors. Most importantly, these xenolines retain biologic tumor heterogeneity and respond to combined platinum/taxane therapy similarly to how patients from whom these matched tumors were obtained respond –

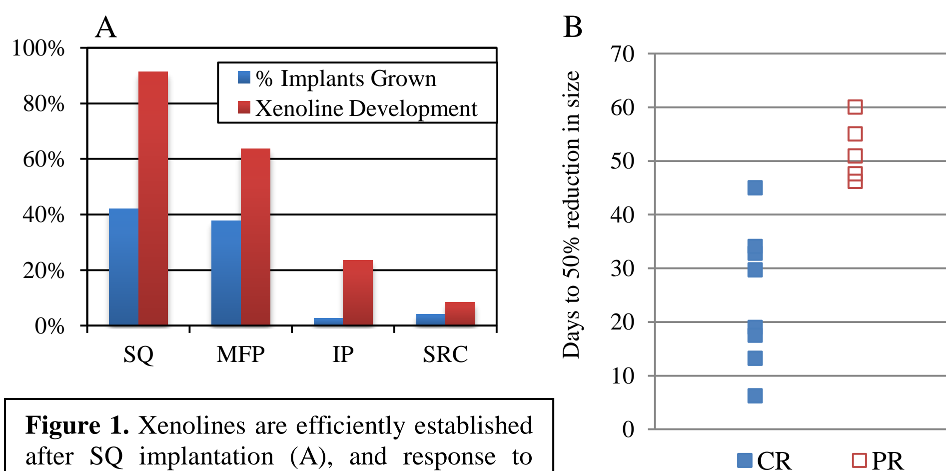

Figure 1. Xenolines are efficiently established after SQ implantation (A), and response to chemo correlates with patient response (B).

xenolines from patients who ultimately had only a partial response (PR) to primary therapy have a much slower tumor reduction (or no response at all) compared to patients who had a complete response (CR) ($p<0.001$, Figure 1B). Xenolines can be expanded to sufficient numbers in just 1-2 generations such that therapies can be tested with sufficient power. Therefore this is a reliable model in which patient tumors can be tested for putative predictive biomarkers, and then response to therapy can assessed with a scientifically rigorous and appropriately controlled method.

We currently have multiple primary xenografts growing in mice that can be expanded as needed to test responses to biologic agents. Working with colleagues in the radiation oncology department, we have profiled these tumors for defects in HR using *in vitro* exposure to XRT and quantifying rad51 foci formation. Defects in HR (and therefore more likely to respond to PARP inhibition) are suggested when a small number of cells develop rad51foci, as in tumor 157 (Figure 2). A spectrum of induction is seen, as noted by tumors 155 and 144 (25% and 74% of cells positive, respectively.)

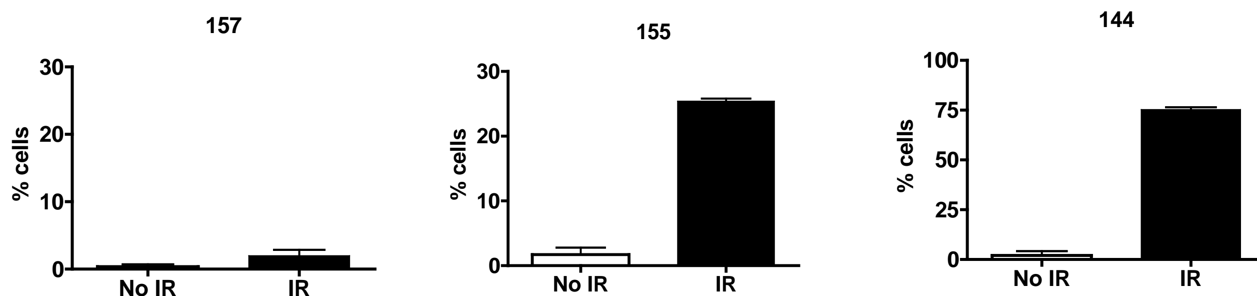

Figure 2. Variability in percent of rad51-positive cells in primary ovarian cancer xenografts after exposure to radiation.

Our groups are uniquely poised to use primary xenografts, rad51 foci formation assessment, and BRCAness signatures to test the **hypotheses** that functional assessment of HR defects can predict response to PARPi therapy, and that this response profile can be used to develop a clinically feasible *PARPi signature* that can be translated to the clinical setting. Reliable stratification of patients to PAPRi therapy in future clinical trials will allow personalization of medicine and hopefully increased response rates. We propose three Specific Aims:

SA1: Determine whether predicted response to PARP inhibition translates to *in vivo* efficacy in ovarian cancer cell line models

Approach: Although cell lines have limitations in translation to patients, they still have a role in examining specific questions in a controlled setting. We have examined several BRCA-wt ovarian cancer cell lines, and seen that rad51 foci formation after XRT predicts a response *in vitro* (data to be presented in expanded proposal), but need to confirm response to PARPi *in vivo*. Cell lines with HR defects *in vitro* will be tested for *in vivo* response to PARPi with an orthotopic mouse model. A line with functional HR intact (and therefore predicted to *not* respond to PARPi) will be tested as a control. Response alone and in combination with carboplatin will be assessed, in both inherently platinum sensitive and resistant lines. Collected tumors will be assessed for biologic endpoints, including induction of survival pathways, proliferation, and activation of DNA repair pathways. Notably, tumors treated with carboplatin alone can be profiled to determine if HRD are present, which would have implications for whether treatment should be pursued in carboplatin–sensitive or –resistant patients.

SA2: Determine the frequency of functional HRD in ovarian cancer patients with an XRT-Rad51 bioassay, as compared to BRCAness profiling and individual gene analysis

Approach: 50 consecutive advanced ovarian cancer specimens will be collected at time of primary debulking surgery and functional homologous recombination defects (HRD) will be determined by Rad51 immunofluorescent analysis after XRT exposure *in vitro* (results of first 18 patients to be presented in expanded proposal, as in Figure 2). Samples will also be profiled with a previously established BRCAness signature[4], and other genes individually identified through the TCGA (BRCA1/2 mutation analysis, PTEN deletion, etc). Correlation between functional defects in HR by the XRT-Rad51 assay and these clinically feasible but unvalidated tests will be determined.

SA3: Determine whether PARPi response predictors correlate with response in patient-derived primary ovarian cancer xenolines, and develop a more accurate, clinically feasible, signature to predict response.

Approach: Primary xenolines will be established in 20 consecutive ovarian cancer patients, and treated with PARPi alone and in combination with chemotherapy. Using response as gold standard, the accuracy of predictive models to correlate with response *in vivo* will be assessed, including the XRT-Rad51 bioassay, the BRCAness profile, and individual gene analysis. Tumors that respond (and those that do not) will then be used to refine a clinically feasible gene signature that can predict response to PARPi.

RESEARCH QUESTION: This research plan will address whether clinically feasible analysis of tumors, such as gene signatures of "BRCAness", correlate with a functional assay of HR repair using patient samples. Additionally, we will answer whether these methods can predict response to PARPi therapy *in vivo*, using a novel heterogeneous patient-derived preclinical model. Finally, while it is predicted that about 50% of tumors have defects in HR based on individual gene assessment, our studies will determine the true incidence of functional defects in HR through a biologic assay. These studies address the **FY12 Areas of Encouragement** regarding novel approaches for improving validity of predictive disease markers and validating molecular targets for therapy. We have chosen to focus on PARP inhibitors, but the model can be easily translated to validate other therapies and approaches that need to be tested preclinically in a more heterogeneous model of ovarian cancer than conventional cell line or single-gene perturbation models of disease.

SYNERGY: The Landen lab has expertise in preclinical models of ovarian cancer, including a patient-derived primary xenograft model that is validated and optimized for use. Additionally protocols are established for performing XRT-based Rad51 assessment of functional HRD. The Konstantinopoulos lab established the BRCAness signature, and has expertise in bioinformatic analysis of tumor profiles. He will be able to define the genetic abnormalities of interest in collected tumors, characterize each regarding the clinically feasible BRCAness signature, and refine it based on the gold standard response to PARPi generated by these proposals.

RESOURCES: There are several resources unique to this team of investigators. Existing protocols are in place for highly efficient establishment of primary xenolines in SCID mice, a model demonstrated to mimic patient heterogeneity and biologic response to chemotherapy (see above). Multiple xenografts are currently growing, and have already been profiled regarding aspects of functional HRD and selected gene expression, with more thorough characterizations underway (separately funded). Team members also have experience modeling gene signatures from high-throughput data sets for characterization of BRCAness, and independent characterization of genes correlating expression with clinical outcomes. Independent bioinformatic teams will also be able to utilize these data sets for their own modeling methods that may predict response to novel agents or strategies. Both of these resources (xenoline development and therapeutic testing, data sets) can be further utilized by other investigators whose aims to predict response to therapy in a model that more accurately mimics patient tumors, as opposed to classical models that use cell lines or single-gene perturbations that are more homogeneous.

IMPACT: PARP inhibitors have shown promise treating BRCA-defective ovarian cancers, and because they depend on defects in homologous recombination for maximal effectiveness, it is hoped that many BRCA-wild type patients will respond as well. This is predicted by observations in the TCGA data set that up to 50% of patients may have defects of some members of the HR pathway, but such abnormalities do not equate to HR defects or response to PARP inhibitors. A functional assay of defective HR, as tested here, may allow more accurate determination of PARP response, which will be confirmed. Because this functional assay is likely not clinically feasible for most clinicians, our data will also allow a more refined gene signature to be developed, using actual patient tumors growing *in vivo* as the gold standard. Validation of this model in these studies will allow it to be used to test other biologic therapies that require a functional pathway for personalizing therapy. As additional biologic therapies and other therapeutic approaches require testing, and more attempts at personalizing medicine in cancer patients are sought, this model will serve as validation that we need to move beyond cell lines and single-gene perturbations for preclinical testing. Having a patient's own tumor growing *in vivo* to test therapeutic efficacy will be a significant step forward in understanding how complex high-throughput data sets can be used to predict responses to therapy in ovarian, and other, cancers.