

BC Cancer Agency Canary Biomarker Assay Development Proposal

The aim:

To assist Dr. Nicole Urban in the evaluation of 20+ candidate biomarkers for the early detection of ovarian cancer. The BCCA team will perform ELISAs using POCRC sera, produce antigen and antibody reagents, and assist in the development and validation of new assays.

The need:

Research-grade, Luminex bead-based sandwich antibody assays are required to evaluate potential ovarian cancer serum markers in small quantities of stored serum. Bead-based assays will be used to run mid-large scale studies using POCRC, PLCO and WHI sera to identify a panel of markers with maximal sensitivity and specificity for detecting ovarian cancer 1+ years prior to symptoms, earlier than CA125. To date, Canary researchers have identified 20+ potential markers that require immediate evaluation. Additionally, ongoing discovery efforts are expected to yield a steady pipeline of new markers for future evaluation. Highly sensitive and specific paired antibodies are required to develop immunoassays for these markers.

The opportunity:

The BC Cancer Agency, in close collaboration with Nicole Urban's group at the Fred Hutchinson Cancer Research Center, can assist this project by:

- (a) evaluating those markers for which there are existing ELISA assays (commercial or academic) using sera provided by the POCRC;
- (b) producing antigens and antibodies to those markers for which reagents are unavailable;
- (c) assisting in the development of bead-based assays to detect markers in serum.

Current status:

As shown in detail in the accompanying table (Current Status of Canary Biomarkers), the 20+ potential markers identified by Canary researchers are at different stages of development and evaluation:

- (1) Dr. Nicole Urban's group has fully developed and validated bead-based assays for 3 markers: CA125, HE4, and Mesothelin. These are ready for large-scale studies. The development of bead-based assay for other six or more other markers is in progress. Sensitive and specific assays for some markers could not be established with currently available reagents, suggesting that new antibodies may be required for some of these markers.
- (2) For seven Canary markers (HK7, HK8, MUC 1, IGF2, BMP7, TACSTD1 and CHI3L1) and more than 20 additional markers (e.g. HK5, HK6, HK10, HK11, Prolactin, Leptin, Osteopontin, B7-H4, DcR3, SPONDIN-2, ReG-IV), ELISA assays have been developed by commercial or academic laboratories. Most of these ELISA kits have not yet been systemically evaluated with ovarian cancer serum samples, so the performance of the kit and the corresponding marker in this context is unknown. Nevertheless, because commercial kits can be purchased and evaluated immediately, this will be given top priority.

- (3) For six Canary markers (IFI27, SPONDIN-1, CDH6, PAX8, PRAME and GALNT3) and three additional markers (Ku80, CA72.4 and ESE1), no ELISA assays are available but single or paired antibodies are available from academic or commercial sources. The performance of these antibodies in ELISA and bead-based assays can be determined immediately. Moreover, these antibodies can be used to assess marker expression across a panel of tumor tissues by immunoblotting.

The strategy:

- (1) Evaluation of biomarkers using existing ELISAs. For those markers for which a commercial ELISA kit is available, the kit will be purchased and used to evaluate POCRC sera from ovarian cancer patients and controls. For those markers for which commercial ELISA kits are not available, we will attempt to obtain ELISAs from academic labs where possible. ELISA results will be provided to Nicole Urban for statistical analysis.
- (2) Prioritization of markers for which no ELISA is available. Those markers for which no ELISA is available will be carefully prioritized for further development. If two or more antibodies are available for a given marker, we will test these in pairwise combinations for their utility in an ELISA format. Failing that, antibodies will be used in immunoblotting to evaluate the expression of a given marker across a panel of POCRC ovarian tumors. If no antibodies are available for a given marker, we will perform quantitative PCR analysis of mRNA expression across the same panel of tumors. Results will be discussed with Dr. Urban's group and other Canary researchers. Those markers with the most promising protein/mRNA expression patterns in serum or tumor tissue will be prioritized for further assay development.
- (3) Production of recombinant antigens. For many markers, recombinant antigen is required for screening yeast antibody libraries, immunizing mice, screening resultant antibodies, or serving as calibration standards for assay development. As needed, we will purchase or clone full-length cDNAs and express these as epitope-tagged proteins in multiple expression systems, including mammalian cells, yeast, insect cells, or *E. coli* using standard methods.
- (3) Production of monoclonal antibodies. Standard methods will be used to produce both monoclonal and polyclonal antibodies for selected markers in mice or rabbits. For immunization, we will attempt to use antigen in a native form to increase the likelihood that the resulting antibodies will recognize the protein in patient serum. Antibodies will be tested in ELISA and bead-based sandwich assays to find antibody pairs that detect the corresponding protein in patient serum with high sensitivity and specificity.

Management and communication plan

Dr. Nicole Urban will oversee all work at the FHCRC, and Dr. Brad Nelson will oversee all work at the BC Cancer Agency. The team will hold monthly teleconferences between the BCCA and FHCRC. We will also meet in person at least quarterly, either at Canary science team meetings, or specially scheduled meetings. Written progress reports will be submitted to the Canary Fund as requested.