PARTNERING FOR CANCER BIOMARKER RESEARCH BIOSPECIMENS WITH THE HOOSIER ONCOLOGY GROUP (HOG)

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ABSTRACT

Poor availability of high quality biospecimens is one of the most frustrating obstacles to cancer biomarker research. To overcome this problem for our NCI-funded Clinical Proteomics Technology Assessment for Cancer (CPTAC) biomarkers center program, the Purdue University/Indiana University team turned to a professional organization to obtain the required human samples for this program. The Hoosier Oncology Group is a not-for-profit organization of practicing oncologists across Indiana and the region with management of oncology patient recruitment for biospecimen procurement and recruitment to clinical trials. HOG provides specific training in best practices for sample collection and processing as well as full collection documentation and materials for reproducible and trouble-free patient sample collection in the doctor's office. HOG manages the primary Institutional Review Board approval and generates approved informed consent documentation. The group activates their existing network of oncologists to implement the sample collection protocol. Relevant de-identified patient clinical information is also collected and supplied with the biospecimens to facilitate interpretation of research results. Because the network of oncologists is not confined to a major metropolitan medical center, patient demographics are diverse and more accurately represent urban and rural populations. The group also engages physician members to obtain control samples from healthy volunteers.

We describe the standardized collection protocol and process employed by HOG for plasma, serum and whole blood sample collection for our program. In addition, we will present results from proteomic profiling of the human plasma samples with liquid chromatography/mass spectrometer based approaches. Our analytical evaluation provides evidence for complex proteomics profiles from these samples, as expected. We will also present data from samples accessed at various time points after collection that indicates continued integrity and utility for HOG-collected plasma samples held at -80ºC.

Sample collection

The following samples have been collected from the breast cancer subjects as of 2-Mar-2008:

• Healthy volunteers	215
• Baseline samples	214
• 3 Month samples	190
• 6 Month samples	158
• 9 Month samples	124
• 12 Month samples	64
• 15 Month sample	42
• 18 Month samples	4

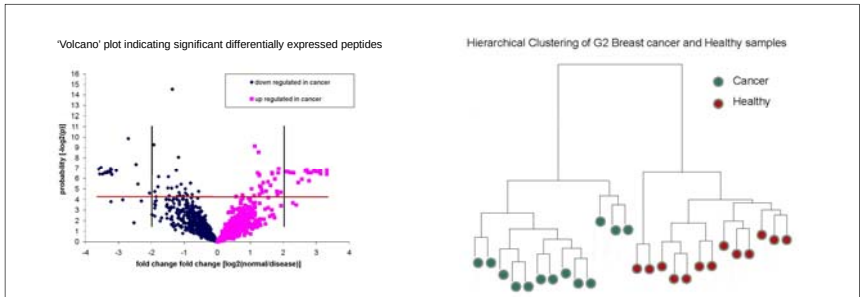
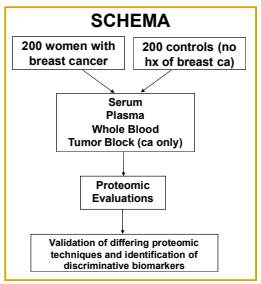


Fig. 3. Statistical analyses of peptide mass spectrometry data from samples obtained by the HOG from G2 breast cancer patients and healthy volunteers indicates that our analytical platform distinguishes groups based on plasma protein content.

A Biological Sample Collection Protocol of Women With and Without Breast Cancer: Hoosier Oncology Group Study BRE06-120 for the Analytical Proteomics Team

SCHEMA



Recruitment

The Hoosier Oncology Group (HOG) has 12 sites which are open for enrollment for the BRE06-120 trial.

- **Cancer patients were recruited from the HOG site's clinical practice.**
- **Healthy volunteers were recruited by the HOG site's from one of the following avenues:**
  - **Patient Referral**
  - **Patient Advocacy Groups**
  - **Site Staff**
  - **ClinTrials.gov**

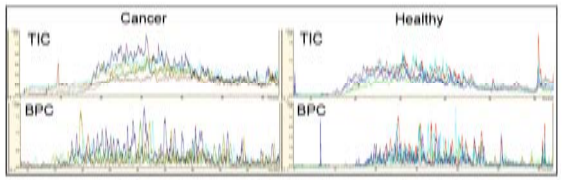


Fig. 1. Five representative total ion (TIC) and base peak chromatographs (BPC) from grade 2 breast cancer and healthy samples collected by the HOG. This proteomic overview indicates that these samples are comparable and similar in protein complexity.

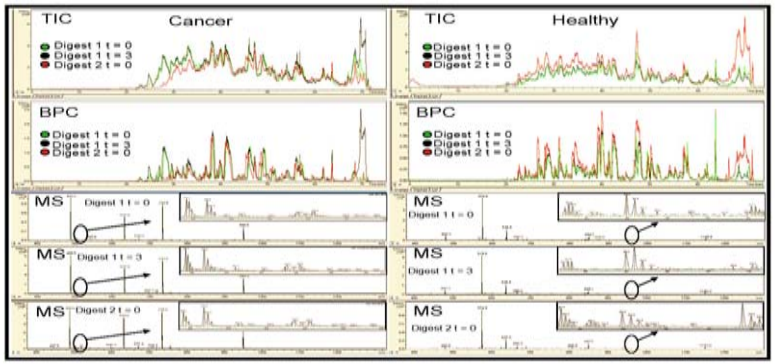


Fig. 4. Reproducibility and technical consistency of HOG-obtained plasma samples on the Purdue proteomics platform. Upper panels compare digestions of the plasma sample, with both the identical digestion run 3 months apart, and with a second digestion of the same sample. Lower panels (MS) provide spectral comparison (intensity for a range of m/z peaks at a specific time point). Inserts enable detail evaluation for comparison of mass spectrometry runs and digestions.

Inclusion Criteria

- ❑ **Breast Cancer Cohort: Informed consent & HIPAA authorization for release of PHI**
  - **Age ≥ 18 years, Female (not pregnant)**
  - **Histologically / cytologically confirmed invasive disease or DCIS**
  - **Preparing to begin a new regimen**
- ❑ **Control Cohort: Informed consent & HIPAA authorization for release of PHI**
  - **Age ≥ 18 years, Females (not pregnant)**
  - **No history of invasive breast cancer or DCIS, No history of malignancy in past 5 years**
  - **Exceptions: basal cell / squamous cell cancer of skin/others with low potential for metastasis**

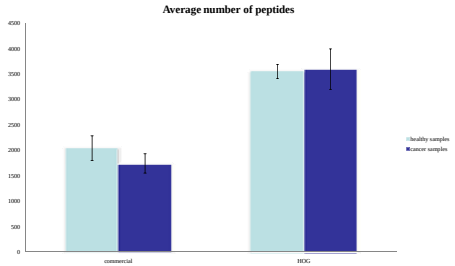


Fig. 2. Comparison of peptide numbers detected by mass spectrometry in human plasma from a commercial vendor and from HOG.

METHODS

Plasma samples from age- and pathology-matched breast cancer patients (10 commercial and 13 collected by HOG) and healthy volunteers (10 commercial and 13 collected by HOG) were trypsin digested and evaluated using chip cube nanochromatography and electrospray ion trap mass spectrometry (Agilent Technologies). The resulting data was analyzed using the Purdue Discovery Pipeline.

CONCLUSIONS

- **Best practices at HOG for collection of clinical trails samples provide excellent biospecimens for proteomic evaluation.**
- **The Agilent integrated chip cube nano-chromotography system enables highly reproducible analysis of human plasma samples on the Agilent XCT ion trap mass spectrometer.**
- **The HOG sample collection partnership and the analytical platform at the Bindley Bioscience Center allow for comparable proteomic analyses over extended periods of time.**

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