The Speicher laboratory primarily uses proteomics, lipidomics, metabolomics and related systems biology approaches to study clinically relevant biological problems. In addition to a number of collaborative projects, the laboratory focuses on three main projects in melanoma, ovarian cancer, and diagnosis of early stage pregnancy disorders.

**Project 1: Melanoma Tumor Progression and Resistance to Therapy**

Malignant melanoma is one of the most aggressive forms of cancer and its incidence in the general population continues to increase. Recent dramatic improvements in patient care have occurred using targeted therapies and immunotherapy. However, for most patients benefits are transient and long-term patient survival rates remain low, hence additional therapeutic options are needed.

We are pursuing several related aspects of melanoma progression and therapy resistance. We use systems biology analyses of protein profile changes associated with tumor progression and acquisition of therapy resistance to gain novel insights into key mechanisms of metastatic and therapy-resistant phenotypes. A major focus has been the role of autophagy, which is a common mechanism of therapy resistance that also correlates with melanoma aggressiveness. Clinical trials are currently underway involving combinations of anticancer therapies and hydroxychloroquine, an autophagy inhibitor that binds to PPT1, a palmitoyl thioesterase that removes palmitic acid from protein side chains. However, these efforts are limited by: the lack of knowledge about the downstream pathways affected by PPT1 inhibition; a lack of predictive biomarkers to select patients that are most likely to respond to autophagy-targeting therapies; and the lack of markers to monitor effects of PPT1 inhibition using minimally invasive methods.

Current goals include identification of key pathways affected by PPT1 inhibition and identification of predictive and pharmacodynamic plasma biomarkers to assist in clinical management of patients receiving PPT1 inhibitors. A related goal is to identify new therapeutic targets. Proteome analyses of supernatants from 3-D cultures of melanoma cells with high and low autophagy have led to the identification of a number of promising candidate biomarkers. Several of these biomarkers have been validated in additional cell lines and in a small group of patients in which the biomarkers appear to correlate with tumor autophagy levels. Future efforts will expand the evaluation of candidate biomarkers in patients and pursue an unbiased proteomics approach to identify global changes in posttranslational modifications and protein levels in response to PPT1 inhibition or gene silencing.

**Project 2: Ovarian Cancer**

One goal of this project is to discover and validate novel protein biomarkers of human ovarian cancer that can improve early detection of the disease as well as clinical management after diagnosis; a second goal is to
better understand tumor development and therapy resistance mechanisms in order to identify new therapeutic targets and companion diagnostics for this disease.

Ovarian cancer is the leading cause of death from gynecological cancers in the United States. More than 24,000 women are diagnosed with ovarian cancer every year, and approximately 15,000 die of this disease annually in the U.S. alone. Ovarian cancers are biologically very heterogeneous with common epithelial tumors consisting of serous, endometrioid, mucinous, clear cell, and undifferentiated sub-types. The serous sub-type tumors account for more than half of the epithelial tumors and generally occur in women between 40 and 60 years of age. They are usually highly aggressive and account for the greatest number of ovarian cancer-related deaths.

The five-year survival for invasive epithelial ovarian cancer is about 90% when the disease is confined to the ovaries, but it is only about 30% when the cancer has spread. Even patients with high-grade serous tumors do well if diagnosis occurs early. However, about 75% of ovarian cancers are not diagnosed until after the cancer has spread, primarily because early stage tumors are generally asymptomatic.

There is no effective molecular test for ovarian cancer at present. So far, the best plasma biomarker that detects ovarian cancer prior to symptoms is CA125. However, CA125 is not used as a routine screen for ovarian cancer because it is associated with a large number of false positives and false negatives. The primary utility of CA125 is to monitor efficacy of surgery and ovarian cancer progression after initial diagnosis but only about 60% of patients have tumors that are strongly CA125-positive.

Hence, one goal of this project is to identify new molecular biomarkers for ovarian cancer that would complement CA125 and would form the basis for a minimally invasive blood test for early diagnosis and improved clinical management of the disease after initial diagnosis.

In pursuit of this goal, we identified several hundred candidate ovarian cancer biomarkers using a xenograft model coupled with in-depth proteome analysis of the resulting chimeric (human/mouse) plasma to identify human proteins shed by the tumors into the blood. Multiplexed quantitative mass spectrometry assays using multiple reaction monitoring (MRM) were subsequently used for initial laboratory scale validation of approximately 40 high-priority biomarkers. Approximately half of these biomarkers were significantly elevated in ovarian cancer patients compared with healthy donors or those with benign disease. Some of the most promising biomarkers were further tested using immunohistochemistry of tissue microarrays comprised of patient tumors or normal tissues, and several proteins were identified that react strongly with all ovarian cancer subtypes and, importantly, complement the reactivity of CA125. The utility of these and other biomarkers to detect all cancer subtypes was confirmed by conducting proteome analyses of proteins shed by ovarian cancer tumors (tumor secretomes) using fresh surgical specimens. A current focus of this project is to finalize a panel of the most promising biomarkers to be tested using plasma from independent patient cohorts. Another part of this initiative is to develop high-affinity antibodies for the target proteins and to develop a high throughput multiplexed immunoMS quantitative assay for a panel of the most promising biomarkers. The
biological roles of several of these biomarkers on tumor progression and in response to therapy are also being evaluated.

A second major goal of our ovarian cancer project involves a proteomics and metabolomics-based systems biology approach to the study of tumor progression and therapy resistance taking approaches similar to those described in Project 1.

**Project 3: Biomarkers for Ectopic Pregnancy and Miscarriage**

Ectopic Pregnancy (EP) occurs in about 1-2% of pregnant women and may compromise a woman’s health and future fertility. It is a leading cause of maternal mortality and morbidity accounting for 6% of pregnancy deaths due to a rupture of the fallopian tube with resulting intraperitoneal bleeding. Most patients present before tube rupture with nonspecific symptoms of abdominal pain and/or vaginal bleeding, but these symptoms are neither sufficiently sensitive nor specific, and some women remain asymptomatic. If diagnosed early, EP can be effectively treated with little risk to the patient, but current diagnostic methods (transvaginal ultrasound and serial quantitative serum human chorionic gonadotropin concentrations) are inconclusive in up to 40% of patients. Also, the diagnosis is currently cumbersome requiring multiple office visits, serial blood tests for up to six weeks, multiple ultrasound examinations, and surgical procedures such as uterine curettage and laparoscopy. In addition, up to 25% of pregnancies result in miscarriage or spontaneous abortion.

The goal of this project is to develop more effective, minimally invasive blood tests that can reliably distinguish EP at an early stage from nonviable intrauterine pregnancy (miscarriage/spontaneous abortion or SAB) or from normal intrauterine pregnancy (IUP). The proteomic strategies involve in-depth quantitative comparisons of plasma from patients with EP, SAB and IUP. These approaches have identified a number of novel biomarkers and several specific isoforms of previously known biomarker protein families. These results also confirmed several previously known biomarkers. Follow-up validation of the most promising biomarkers is being pursued in independent patient cohorts to identify a panel of biomarkers with clinical utility. Because robust sandwich ELISA assays are not available for most of the new biomarker candidates, targeted quantitative MS is being utilized to multiplex biomarker validation. An advantage of the targeted MS approach is that it can reliably distinguish between closely related isoforms that may be present in the plasma and that may not be adequately distinguished by existing ELISA.

Future efforts will be directed to producing higher throughput ImmunoMS assays for a panel of the most promising biomarkers based on the targeted MS results. An optimal clinical diagnostic assay is expected to consist of a panel of multiple biomarkers rather than a single protein.