What if an antigen were expressed in both malignant (ovarian) and benign (endometriosis and fibrotic) tissues yet on the cell surface of normal cells it is absent? What would this mean for developing a therapeutic candidate?

Background
Endometriosis occurs when the endometrium tissues grow outside of the uterus. Moreover, when two deposits of endometriosis touch in the abdomen, they become fused and cause adhesions. Post-operative adhesions can also develop following surgery for endometriosis. Endometriosis and post-operative adhesions are the major cause of many symptoms including pain, excessive bleeding, and infertility, all of which manifest social and psychological effects.

Oxidative Stress Connection among Fibrosis, Adhesion Formation and Cancer
Primary research was focused on investigating the role of oxidative stress and inflammation in the pathophysiology of gynecologic fibrotic disorders. The idea of studying ovarian cancer arose from the need to compare the effects of oxidative stress and inflammation on the pathogenesis of a malignant overgrowth as compared to benign overgrowths, such as postoperative adhesions, fibroids, and endometriosis.

Surprising Discovery—Possibility for highly specific and targeted therapy
Recently, our academic research partner observed that commercially available monoclonal antibody against CD11b induced killing in cells isolated from postoperative adhesion tissues and endometriosis tissues but not in their normal counterparts. The β2 integrin subfamilies are leukocyte-specific receptors that are composed of distinct alpha subunits, CD11a, CD11b, CD11c, and CD11d and a common beta subunit, CD18. CD11b, part of the beta 2 integrin receptor, is only known to be highly expressed in myeloid cells. Surprisingly, we found that CD11b, but not CD18, is highly expressed in fibroblasts isolated from postoperative adhesion tissues and endometriosis tissues as compared to their normal counterparts. More importantly, CD11b monoclonal antibody did not kill normal macrophages which are known to express the highest levels of CD11b, suggesting that it is not CD11b, but rather, a CD11b-like antigen (known by Temple program TTX 334) that mediates cd11b antibody killing of benign and malignant overgrowths.

Furthermore, although the CD11b antibody did not kill normal peritoneal fibroblasts or normal myometrial primary cells in culture, we found that normal peritoneal fibroblasts and myometrium cells both expressed the CD11b-like antigen and this expression was increased with extended time in cell culture. It is well known that phenotypic changes in cells, which may manifest as alterations in the relative levels of expression of various cell surface markers, occur in cells as a function of time in culture. Consistent with this phenomenon, we observed that the increased level of expression of CD11b-like antigen we observed over time in culture correlated with increases in abnormal cell killing by CD11b antibody.

The foregoing results, combined with the fact that CD11b is known to be expressed only by myeloid cells and that the CD11b antibody did not kill macrophages, lent credence to our hypothesis that CD11b antibody effects cell killing by cross reactivity to an antigen(s) that may be similar, and perhaps vicinal to, CD11b on the target cell surface. As such, our present goal is to identify the cytotoxic “antigen(s)” recognized by CD11b antibodies. Moreover, development of a specific antibody to this antigen(s) may provide a new chemical entity as well as novel treatment approach to eliminate benign overgrowths at the cellular level. The outcome of this
research will serve as a foundation for a phase I/II study focusing on humanizing and evaluating antibodies against the discovered target “antigen(s)” and commercialization of a therapeutic product to treat endometriosis.

**Mode of Action of benign gynecologic disorders**

Oxidative stress and inflammation are linked to the pathogenesis of endometriosis and ovarian cancer. Myeloperoxidase (MPO) is a proinflammatory enzyme and a marker for neutrophil activation and oxidative stress. MPO has also been shown to rescue human neutrophils from constitutive apoptosis and prolong their life span, independent of MPO’s catalytic activity, through cell signaling via the adhesion molecule CD11b/CD18. Our scientific team was the first to report the surprising observation that MPO was expressed by ovarian cancer (OC) cells and tissues. They had also shown that OC cells manifest lower apoptosis, which was markedly induced by inhibiting MPO. They found these observations highly provocative and focused efforts on elucidating the mechanism by which MPO might reduce apoptosis in ovarian cancer cells, a pathway which we suspected was linked to inducible Nitric Oxide Synthetase (iNOS).

Our team had previously reported that OC tissues and cells manifest a pro-oxidant state characterized by an increased expression of key pro-oxidant enzymes, specifically iNOS and its product, nitric oxide (NO). MPO, a key oxidant enzyme, utilizes NO produced by iNOS, as a one-electron substrate generating NO$^+$, a labile nitrosating species. Nitrosylation of proteins by the iNOS pathway is a known consequence of oxidative stress. Hypoxic stress induced S-Nitrosylation of apoptosis inducing proteins, such as Caspase-3, suggests a link between the inhibition of apoptosis and MPO/iNOS pathways in dysregulated cell growth. Based on this logic, our team investigated the process of S-nitrosylation of caspase-3, an activator of apoptosis in response to oxidative stress. S-nitrosylation of caspase-3 had been known to inhibit caspase-3 activity, which inhibits apoptosis. They observed a significant increase in S-nitrosylation of caspase-3 induced by oxidative stress, which correlated with a significant decrease in caspase-3 activity in OC cells.

Collectively, these data provide substantial evidence that MPO is a key player in regulating apoptosis in OC cells in response to oxidative stress, which may play a role in maintaining the oncogenic phenotype of OC cells. This work also highlights the apparent cross-talk between the MPO/iNOS pathways in the pathogenesis of ovarian cancer.

**Biomarker for Ovarian Cancer**

Based on their elucidation of the MPO/iNOS link described in the foregoing, our research team was able to establish MPO and free iron as biomarkers for early detection and prognosis of ovarian cancer. We developed compelling evidence that MPO serves as a source of free iron under oxidative stress, where both NO (produced by iNOS) and superoxide are elevated. Iron reacts with hydrogen peroxide (H$_2$O$_2$) and catalyzes the generation of highly reactive hydroxy radical (HO$^*$), thereby increasing oxidative stress, which in turn increases free iron concentrations by the Fenton and Haber–Weiss reaction.

Non confidential

