

Yale Advancing Ob/Gyn

Developments from Yale Obstetrics, Gynecology
& Reproductive Sciences



Advances in treatment of drug-resistant endometrial cancer

Although Type II disease affects a relatively small portion of your patients, its prognosis can be quite grim. New research suggests this breast cancer drug may have a significant impact on clinical outcomes.



By Alessandro D. Santin, MD

Dr. Santin is Professor, Division of Gynecologic Oncology, Yale University School of Medicine.

Ob/Gyns are usually optimistic when they manage patients with endometrial cancer because, overall, the disease has a good prognosis: five-year survival is about 90%. But there is a dark lining to this silver cloud: Type II endometrial cancer. Up to 15% to 20% have Type II disease and unfortunately the majority of recurrences and deaths take place among these patients.

Type II endometrial cancers are poorly differentiated clear cell and uterine serous tumors that are much more biologically aggressive. And while both Types I and II present clinically in the same way—typically with some spotting or bleeding—during a staging laparotomy more than two-thirds of Type II patients have more advanced disease that has already spread outside the uterus.

Actually, from a histological perspective, Type II endometrial cancer resembles ovarian serous cancer. The *major* difference is that ovarian tumors are chemo-sensitive. Between 80% and 85% initially respond to chemotherapy. (See Gil Mor's story on ovarian cancer stem cells for why this initial salutary response is transitory.) But

when you provide drug therapy to patients with Type II uterine cancer, the response rate is closer to only 20%. So while the tumors look identical, they react very differently to adjuvant therapy.

The good news, however, is that recent research done at Yale and elsewhere strongly suggests that a new chemotherapeutic approach will have a major impact on these resistant tumors. Our laboratory has reported that about 60% of these serous tumors express an oncogene called *c-erbB2*, also called HER2/neu.

This protein is a receptor on the surface of the cancer cell that plays a major role in explaining its biological aggressiveness. The good

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Message from the Chair

Greetings from Charles J. Lockwood, MD, The Anita O'Keeffe Young Professor of Women's Health and Chair of Obstetrics & Gynecology

Welcome to another edition of *Yale Advancing Ob/Gyn*. This issue highlights clinical research in the department that I hope will soon impact your practice. We start with Dr. Alessandro Santin, who describes advances in the treatment of biologically aggressive (Type II) endometrial cancer. His lab has reported that 60% of these cancers overexpress the oncogene HER2/neu, potentially explaining why Herceptin may improve clinical outcomes.

We then describe new fascinating research by Gil Mor, MD, PhD, seeking to explain the high recurrence risk of ovarian cancer. Gil and his group have identified a population of slow-growing, chemotherapy-resistant stem cells in ovarian cancer patients that appear to be responsible for recurrence and metastasis even in patients with stage 1 and 2 tumors. He is currently screening two agents that may suppress them.

Dr. Lubna Pal follows with a presentation of the mounting evidence that vitamin D deficiency increases a woman's risk of cardiovascular disease and pregnancy complications. She argues that evidence is strong enough now to recommend vitamin D supplementation in pregnant women at risk for preeclampsia and gestational diabetes.

Next, the director of our IVF program, Dr. Pasquale Patrizio, discusses the impact of insurance coverage on multiple gestation rates that should be a wake-up call for the 42 states that do not require such insurance coverage.

On the obstetric front, Dr. Ed Funai explains the advantages of obstetrical OR checklists that include reminders to give pre-op antibiotics, count sponges, and provide DVT prophylaxis. Each checklist is blown up into a large poster in each OR and uses sliders that are switched from red to green as safety items are checked off. This device makes people feel more secure and creates an opportunity to "huddle" about what to expect during the case.

Finally, I describe work in our lab testing a radical new treatment for endometriosis. We found that endometriotic endothelial cells uniquely express tissue factor (TF), which we target with a compound called ICON that binds TF and recruits natural killer (NK) cells to destroy the lesion. Because the drug performed so well in an athymic mouse model of human endometriosis and was without systemic or reproductive side effects, we plan Phase I studies in humans. I hope you find these stories interesting and that they will help you care for your patients in the near future.



Charles J. Lockwood, MD

Ovarian stem cells may hold key to metastatic disease

The latest research suggests that slow-growing stem cells with an ovarian cancer “memory” are responsible for recurrence and metastasis.



By Gil G. Mor, MD, PhD

Dr. Mor is Associate Professor in the Department's Reproductive Immunology Unit at Yale University School of Medicine.

Most first-year medical students know that a cancer cell is one that divides very quickly and divides without control. Current chemotherapy is usually effective because it destroys these fast-growing cells. But the challenge for clinicians is that at least 90% of women with advanced ovarian cancer relapse within five years, even with optimal surgical debulking. Why is that?

The work we've done at Yale University suggests that ovarian cancer cells are not as homogeneous as we once thought. When these cells are cultured, we find that most divide very quickly, but others divide quite slowly. In the past, researchers have assumed that the fast-dividing cells were the ones to concentrate on when looking for effective chemotherapeutic agents. But in our lab, we decided to pay more attention to the slow-growing cells, and as a result we discovered that they have all the molecular characteristics of adult stem cells.

A brief review of stem cell physiology will shed some light on why this discovery is so important: If you cut your skin, it's not epithelial cells that heal the wound, but rather adult stem cells in the area of the wound that are able to produce epithelial cells to replace the damaged cells. In simple terms, one reason humans look the same over the years is because these stem cells have a *memory* of what we look like.

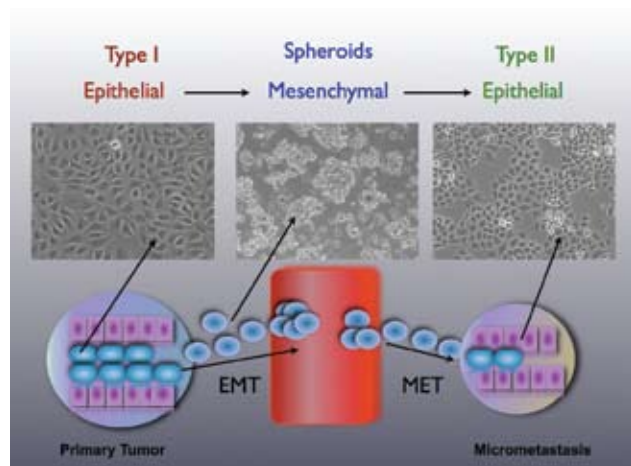
Similarly, we have isolated stem cells that have a memory of cancer cells. So we asked ourselves: What if the cells responsible for cancer recurrence are actually stem cells with a good memory? To use an analogy, these cancer-like stem cells can be compared to an army's generals and the fast-dividing cells to the foot soldiers. Traditional chemotherapy has proven effective in destroying the fast-growing soldiers, but the generals remain behind to train new soldiers.

We believe that these stem cells represent the source of recurrence and metastasis. When we cloned these stem cells in our

lab to understand their molecular characteristics, we discovered that the impetus that causes the stem cells to generate more “soldiers” is tissue damage. In other words, when you kill cancer cells, a message is sent to these stem cells: “You need to repair this organ,” much the same as when normal stem cells receive the message to repair a damaged skin cell. That would explain why chemotherapy can shrink a tumor by 35% only to have it grow back three weeks later.

So determining whether such stem cells are present after surgery is critical to determine the best type of treatment. Our research team has just finished a study in which we found that if a patient, even

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Ovarian cancer stem cells (Type I) undergo a process of transformation into floating cells (spheroids mesenchymal) with the capacity to migrate. These metastatic progenitor cells grow as spheroids and, once attached to a new site, can undergo a “reverse” transformation into epithelial cells, possibly under the influence of the new microenvironment. This leads to the re-creation of the tumor in a different site. EMT: epithelial-mesenchymal transition. MET: mesenchymal-epithelial transition.



Should your patients be taking vitamin D supplements?

Mounting evidence suggests that a deficiency of this prohormone may have profound effects on a woman's risk of cardiovascular disease and preeclampsia.



By Lubna Pal, MD

Dr. Pal is Assistant Professor and Director of the Reproductive Aging and Bone Health Program at Yale Reproductive Endocrinology.

While every schoolgirl has been taught that vitamin D is important for bone health, most women—and many clinicians—have yet to appreciate the complex role this hormone precursor may play in the pathogenesis of prevalent health disorders including heart disease, obesity, diabetes, and a host of other diseases.

Over the years, researchers have analyzed data from the National Health and Nutrition Examination Survey (NHANES) only to find that vitamin D insufficiency is rampant in the otherwise healthy American population in general and in women in particular. Focusing on the patients encountered in my clinical practice, we have identified that two out of three women diagnosed with polycystic ovarian syndrome (PCOS) are lacking in the nutrient! Others have shown an inverse relationship between body mass and serum levels of vitamin D metabolites, and similar trends are seen in our patient population. Thus, while it is apparent that heavier individuals have lower blood levels of vitamin D, the mechanism/s behind this observation remain unclear and, at this point, we can't be sure whether vitamin D is contributing to obesity or the other way around.

We do know that vitamin D is fat soluble, and therefore it is possible that, in heavier people, the vitamin leaves the bloodstream to sequester in the excess adipose tissue, thus accounting for

low serum levels in the obese. Alternatively, lower blood levels of vitamin D in heavier people may be a reflection of a "dilution phenomenon"; i.e., the more "volume" that is available to dilute the nutrient, the lower the blood levels will be. Yet another explanation may lie in lifestyle compromise that goes hand in hand with obesity.

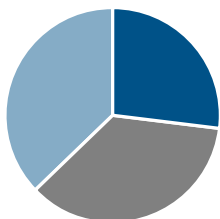
The human animal was designed to replenish its vitamin D stores through regular exposure to sunlight. A predominantly indoor, almost hibernating existence and lack of outdoor physical activity that mankind in general—and we Americans in particular—are adopting likely is contributing to the global pandemic of vitamin D insufficiency.

Increasingly, a lack of vitamin D is being identified as a possible contributor to development of cardiovascular disease (CVD) and diabetes. Several studies have found that lower D levels correlate with higher blood pressure and higher serum levels of insulin, C-reactive protein and homocysteine, all surrogates for CVD. Vitamin D has likewise been shown in cross-sectional and longitudinal studies to improve insulin sensitivity in humans and reduce the risk of type 1 as well as type 2 diabetes.

Emerging data suggest that there are benefits of vitamin D for maternal and child health. Studies have linked maternal vitamin D insufficiency to the risk of developing preeclampsia as well as gestational diabetes. In fact, the evidence is strong enough to recommend vitamin D supplementation in pregnant women identified as at risk for these disorders. The combination of animal studies, *in vitro* data, and epidemiological research and the available information on the safety of this nutrient all point in one direction: that at-risk women would benefit from taking vitamin D supplements. And while double-blind clinical trials to conclusively prove these theories are ongoing and prospective data tying in a "cause and effect" relationship for these observations are sparse to lacking, consider what we are recommending: a safe, cheap intervention in an already deficient population. Potentially, we have a lot to gain and very little to lose.

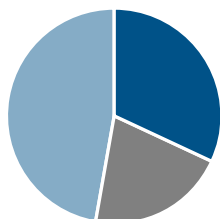
Vitamin D Status in Healthy Infertile Women (n=84)

- Replete (>30ng/ml)
- Insufficient (20-30ng/ml)
- Deficient (<20ng/ml)



Vitamin D Status in the Healthy US Female Population Ages 35-50 Years (n=390): NHANES 1999-2000

- Replete (>30ng/ml)
- Insufficient (20-30ng/ml)
- Deficient (<20ng/ml)



Fixing the inequities in IVF coverage

The expression “Pay now or pay later” takes on a disturbing twist for couples who live in areas of the country that do not mandate insurance coverage.



By Pasquale Patrizio, MD, MBE

Dr. Patrizio is Professor and Director of the Yale Fertility Center and Director of the Reproductive Endocrinology medical practice.

As head of Yale University’s Fertility Center, I have the good fortune to be practicing in one of only eight states that provide insurance coverage for *in vitro* fertilization (IVF). The statistics on multiple births make obvious the lack of foresight on the part of the 42 states that do not require such coverage. To begin with, the rate of IVF multiple births (twins and triplets) is significantly lower in the eight states that provide insurance coverage. And in states without coverage for IVF, the high rate of multiple births ultimately means higher costs for couples, insurance companies, and society at large due to a higher multiple pregnancy rate and attendant premature birth complications including cerebral palsy.

To paraphrase the old Fram oil filter commercials—you can pay now or pay *much more* later. A course of IVF costs on average about \$9,500, not including medications. Thus, it’s not surprising that a couple paying out of pocket would want to make sure the treatment was successful. This is why they often pressure doctors to transfer more embryos than is prudent. If that pressure results in triplets, it is calculated that the patient is likely to deliver at 31.3 weeks, which translates into 41.6 days in the intensive care unit (1) at a cost per child of approximately \$100,000. So three premature infants would cost \$300,000 in insurance—far more than the cost of covering IVF.

Not only is this poor fiscal management; it raises serious ethical issues because the lack of insurance coverage means poorer couples have to suffer from a disease that is being ignored by state governments around the country.

The results of our cost analysis, which were presented at the 2009 American Society for Reproductive Medicine’s meeting in Atlanta, should encourage insurance carriers to provide IVF coverage because it will likely lower their rate of multiple births, promote maternal fetal health, and be cost-effective in the long run. The lack of IVF coverage in the majority of states unfairly deprives many infertile couples of access to effective treatment.

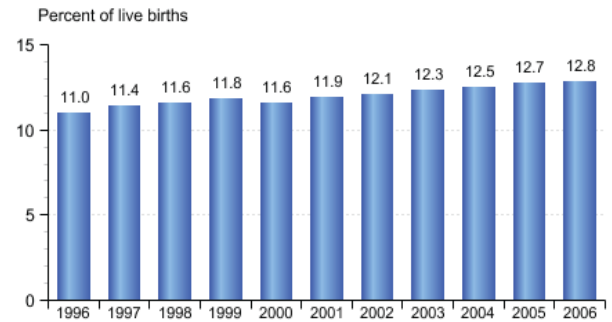
Reference:

- (1) Gilbert WM, Nesbitt TS, Danielsen B. The cost of prematurity: quantification by gestational age and birth weight. *Obstet Gynecol.* 2003 Sep;102(3):488-92.



Preterm birth

US, 1996-2006

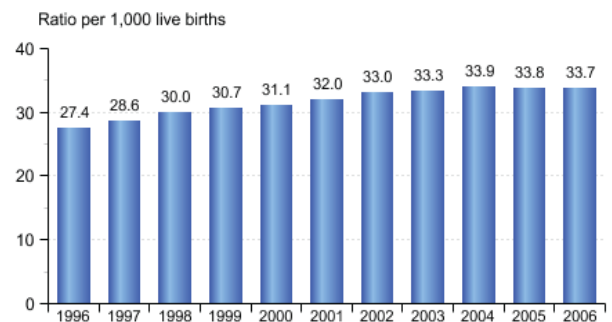


Preterm is less than 37 completed weeks gestation.

Source: National Center for Health Statistics, final natality data. Retrieved November 28, 2009, from www.marchofdimes.com/peristats.

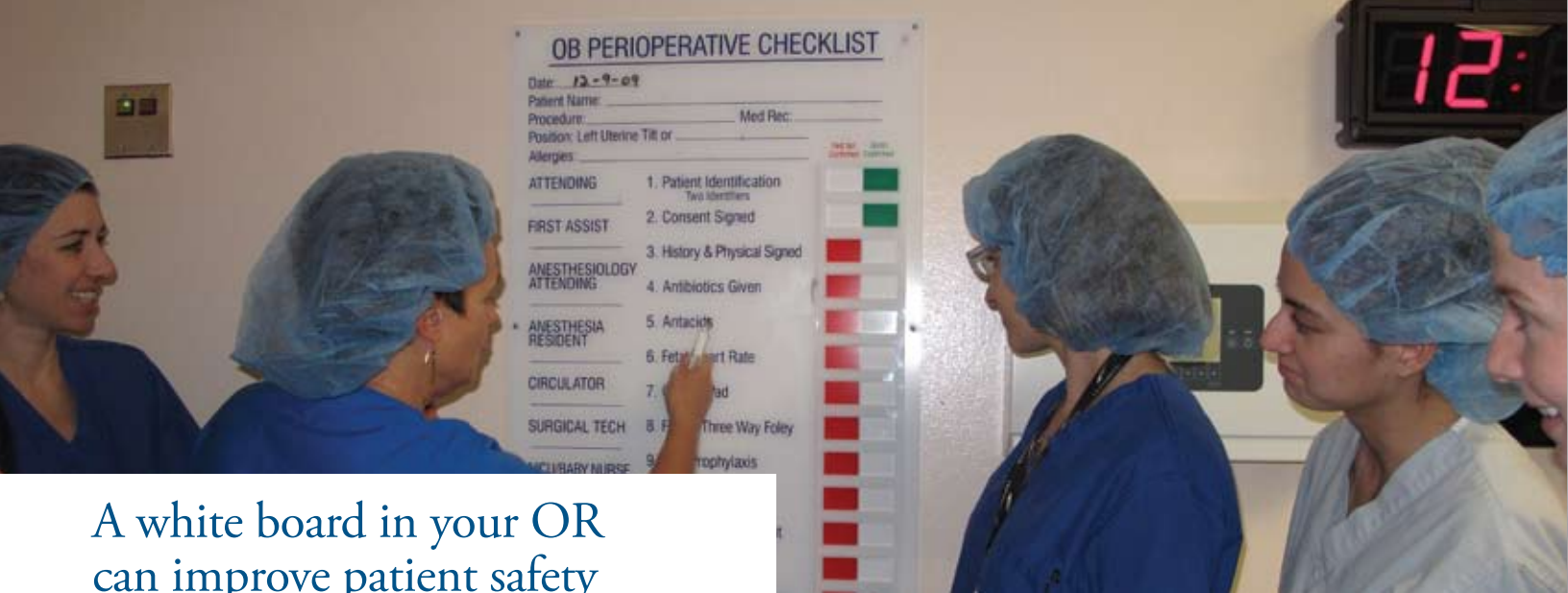
Multiple deliveries

US, 1996-2006



Multiple deliveries include twin, triplet and higher order deliveries.

Source: National Center for Health Statistics, final natality data. Retrieved November 28, 2009, from www.marchofdimes.com/peristats.



A white board in your OR can improve patient safety

An obstetrical checklist that includes reminders to give pre-op antibiotics, count sponges, and provide DVT prophylaxis may seem unnecessary to some veteran clinicians, but mounting evidence suggests it can save lives and offer staffers peace of mind.

Perhaps Ob/Gyns have more than the average interest in patient safety programs because lawsuits against the specialty can generate astronomical settlements. Whatever the reason, this heightened interest has also generated some of the most innovative OR procedures to mitigate these dangers.

OB PERIOPERATIVE CHECKLIST	
Date: 12-9-09	
Patient Name: _____	
Procedure: _____ Med Rec: _____	
Position: Left Uterine Tilt or _____	
Allergies: _____	
ATTENDING	1. Patient Identification Two Identifiers <input type="checkbox"/>
FIRST ASSIST	2. Consent Signed <input type="checkbox"/>
ANESTHESIOLOGY ATTENDING	3. History & Physical Signed <input type="checkbox"/>
ANESTHESIA RESIDENT	4. Antibiotics Given <input type="checkbox"/>
	5. Antacids <input type="checkbox"/>
CIRCULATOR	6. Fetal Heart Rate <input type="checkbox"/>
	7. Ground Pad <input type="checkbox"/>
SURGICAL TECH	8. Foley / Three Way Foley <input type="checkbox"/>
NICU/BABY NURSE	9. DVT Prophylaxis <input type="checkbox"/>
	10. Safety Strap <input type="checkbox"/>
BLOOD AVAILABLE UNITS	11. Initial Surgical Count <input type="checkbox"/>
	12. Time Out <input type="checkbox"/>
	13. Sponges/Laps In Cavity <input type="checkbox"/>

Here at Yale-New Haven Hospital, we have developed white boards for our obstetrical operating rooms. Essentially they are checklists blown up into large posters that are prominently displayed in each OR. They list the names of each staff member so we know who is in the room. In addition to including the patient's name and at least two identifiers, the board requires us to verify several parameters through the use of sliders that are switched from red to green. Among the items on

the checklist are the signed consent, administration of antibiotics and antacids, deep vein thrombosis (DVT) prophylaxis, fetal heart rate, safety strap, and the absolute number of sponges/laps in the cavity.

One feature worth highlighting in more detail is the antibiotic checkbox. We have moved toward giving antibiotics at least 15 minutes prior to incision whereas obstetricians traditionally have waited until the cord was clamped because they didn't want to expose the baby to the antibiotics. Recent data have shown that it does no harm if the baby is exposed to the antibiotic, but giving it before surgery dramatically reduces the risk of surgical site infections in the mother.



By Edmund F. Funai, MD

Dr. Funai is Professor, Associate Chair for Clinical Affairs and Chief of Obstetrics at Yale-New Haven Hospital.

The checkbox for DVT prophylaxis is also worth discussing. About four years ago we adopted a policy requiring every woman having a cesarean delivery to have sequential compression devices placed on her legs. It was a leap of faith at the time. There were impressive data to show that using these devices in GYN surgery helped prevent DVT and pulmonary embolism. However, that was not the case in obstetrics since it was much harder to study enough pregnant patients to generate statistically significant results. But our statistics to date do suggest the policy makes sense. Before we started DVT prophylaxis, we were seeing a DVT or pulmonary embolism every few months, albeit on a very high-risk service that does about 4,500 deliveries a year (we have a patient population that includes many obese patients, a high referral rate of thrombophilic patients, and those with multiple medical co-morbidities). Since we've put this DVT prophylaxis policy in place, we have reduced the occurrence rate to about one per year.

Similarly, having used these boards for around six months, we have found they significantly improve clarity and communication among OR staff. We plan to track the impact of these surgical checklists on the safety culture through our annual safety attitude questionnaire. That survey will include questions on teamwork and collaboration and will evaluate how the white boards affect the overall environment in the OB operating rooms. And while we have yet to collect hard statistics on the value of these checklists, we do have anecdotal data.

We've found that the checklists make people feel a lot more secure in the knowledge that they have completed some of the essential tasks needed to keep patients safe. They also provide a way for people to "huddle"; that is, to talk about what they are about to do, what they expect, and what they think the outcome will be. Simple protocols like these can have a significant impact on patient safety—and on clinicians' peace of mind.



Insights into the biology of endometriosis suggest new treatment options

While we still don't fully grasp the pathology of endometriosis, researchers at Yale University have recently made some important advances in developing a novel treatment.

Endometriosis is a major cause of chronic pelvic pain and infertility. It is also a major source of health care costs due to its refractory nature. We know that endometriosis occurs when endometrial tissue finds its way into the peritoneal cavity, usually through retrograde menstruation. Successful nidation of such implants requires angiogenesis and results in an intense inflammatory reaction. The resultant release of cytokines appears to be responsible for the induction of tissue factor (TF), the primary initiator of clotting, in endothelial cells of endometriotic blood vessels. Moreover, TF itself is a potent inducer of additional inflammatory cytokines. This phenomenon is unique because normally TF is never present in the endothelial lining of blood vessels; in fact, such expression could cause potentially catastrophic clotting. However, we suspect that endometriotic TF is expressed in a cryptic, non-clotting form, given the absence of local thrombosis.

Following our discovery (1), we posited that TF in endometriotic endothelium could present a target for therapy with a unique compound called ICON, a drug that actually attacks endothelial TF. Dr. Alan Garen of the Yale Department of Molecular Biophysics & Biochemistry and Dr. Zhiwei Hu, now of our department, developed the agent that consists of two Factor VIIa molecules that avidly bind TF (2). Normally Factor VIIa causes clotting but Hu et al. mutated the molecules to prevent this kind of reaction. They then



By Charles Lockwood, MD

Dr. Lockwood is Professor and Chair of the Department of Obstetrics, Gynecology and Reproductive Sciences at Yale University School of Medicine.

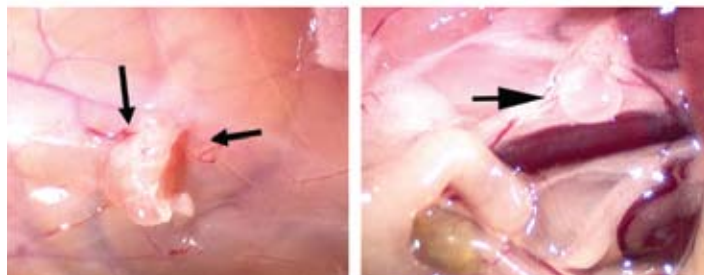
attached the two modified Factor VIIa molecules to the Fc domain of an immunoglobulin molecule, making it an **Immuno-conjugate**, thus the name **ICON**. The drug binds TF with far greater affinity than an antibody, making it *very* specific for TF. When ICON binds to endothelial TF in a lesion, it recruits natural killer (NK) cells to the site, which, in turn, destroy the lesion by eliminating its blood supply.

We've studied the drug in an athymic mouse model of human endometriosis. In this model, human endometriotic tissue is implanted in the peritoneal cavity of "nude" mice. These lesions are allowed to vascularize and develop a typical inflammatory reaction. The endothelial cells from endometriotic blood vessels express TF just as they do in typical endometriosis cases in humans. Treatment with ICON eliminates most lesions entirely and greatly reduces the vascularity and inflammation of residual lesions (3).

There is no apparent toxicity. Because the animals responded so well to this therapy, we hope to take the next step and conduct studies in a primate model. Conformation of safety and efficacy will set the stage for phase I safety studies in humans. If the drug is eventually approved, clinicians will have a powerful new weapon against a disorder that has stymied them for decades.

References:

- 1) Krikun G, Schatz F, Taylor H, Lockwood CJ. Endometriosis and tissue factor. *Ann NY Acad Sci.* 2008; 1127:101-5.
- 2) Hu Z, Sun Y, Garen A. Targeting tumor vasculature endothelial cells and tumor cells for immunotherapy of human melanoma in a mouse xenograft model. *Proc Natl Acad Sci USA.* 1999; 96:8161-6.
- 3) Krikun G, Osteen K, Brunner-Tran K, Hu Z, Schatz F, Konigsberg W, Garen A, Lockwood CJ. A Factor VII/IgG Fc Immunoconjugate Molecule (ICON) Regresses Established Disease in a Mouse Model of Human Endometriosis. *Reproductive Sciences* 2008; 15(2): 82A.



Gross morphological analysis: Representative lesions from placebo (left) or 10 ug ICON treated (right) ATN mice with established human endometriotic lesions displaying obvious differences in gross surface vascularization (n=15).



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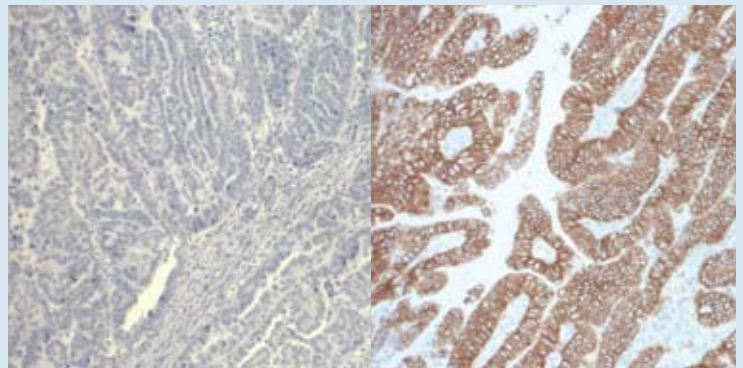
Advances in treatment of drug-resistant endometrial cancer (CONTINUED FROM PAGE 1)

news is that trastuzumab (Herceptin)—which is routinely used in a subset of patients with metastatic breast cancer—is effective against endometrial cancers that express the same gene.

Essentially that means that histochemistry can be done in any pathology department to identify candidates for this form of chemotherapy. Once a hysterectomy is done and the patient is staged, the surgeon would test for HER2/neu gene expression, which is what we do for all breast cancer patients. If the tumor expresses the gene at 2-plus or 3-plus level, this patient could be eligible for trastuzumab therapy. We have provided evidence not only in the laboratory but also in the clinic to support this approach, with a few case reports showing that patients do respond clinically to the therapy when more traditional chemotherapy is not an option. In addition to the case reports, we are designing a prospective study at Yale to evaluate the value of trastuzumab in this patient population.

About 4,000 uterine serous tumors will be diagnosed in the United States this year—a large number when you consider that the majority of these women do so poorly and 75% are going to be diagnosed with the advanced stage of this disease. With these statistics in mind, Ob/Gyns in the trenches should seriously consider testing for

over-expression of the HER2 receptor. It is likely that at least one out of two of these women could benefit from a therapy that is right now *not* being used to treat these patients.



Representative HER2/neu expression by immunohistochemistry (IHC) in USPC tissue blocks. Left: USPC showing negative staining for HER2/neu; Right: USPC showing strong (3+) staining for HER2/neu.

Ovarian stem cells may hold key to metastatic disease (CONTINUED FROM PAGE 3)

though she has localized stages 1 and 2, has a high percentage of the cancer stem cells, the cancer is much more likely to recur.

We are currently screening for therapeutic agents that would target these cancer stem cells. Essentially we are looking for a therapeutic agent that will block this repair process. So far we have two

compounds that we believe will accomplish that goal and suppress cancer stem cells. While the data to date are *in vitro* and animal data, the next stage will be phase 1 clinical trials.