

# Campus

*OSUCCC – James researchers from disparate Ohio State colleges link up to undertake novel interdisciplinary research that can have striking clinical potential*



**GREGORY B. LESINSKI, PhD, MPH,**  
*Tumor Immunologist*

*Clockwise:  
Food Science and Technology Bldg.,  
soy sprouts, College of Pharmacy*

# Connections

BY DARRELL E. WARD

During the 1990s, details began emerging showing how the immune system defends the body against cancer. People with weak immunity due to organ transplants or HIV infection showed a higher risk for certain cancers; animal models and autopsy studies provided further evidence.

Yet, the body's anticancer immune mechanisms often fail – some 1.6 million Americans will likely develop cancer in 2012, and more than 577,000 are expected to die from one of its many forms. The great majority of these deaths will be due to progressive disease as the cancer evolves specific mechanisms to evade the host's immune system and resist immune-based therapy. New strategies are needed to prevent or treat these malignancies, and tumor immunologist Gregory B. Lesinski, PhD, MPH, at The Ohio State University Comprehensive Cancer Center – James Cancer Hospital and Solove Research Institute (OSUCCC – James), believes that boosting the body's anticancer immune response is one of them.

Lesinski, who is a member of the Molecular Carcinogenesis and Chemoprevention Program, runs a basic-science laboratory that teases apart how tumors evade the immune system, or fall prey to it. Together with collaborators in a range of colleges across Ohio State's

land-grant campus, he and his laboratory are working to develop a targeted therapeutic and a whole-food prevention intervention, both designed to enhance the immune response to cancer.

“There's an immune-tumor interaction in all types of cancer, and we see a tremendous opportunity to use the immune system as a therapy against cancer,” says Lesinski, an assistant professor of Internal Medicine. “Our goal is to improve patient care, perhaps through a dietary intervention with a whole food, or by developing a small-molecule inhibitor based on a natural product.”

Lesinski is working on one hand with OSUCCC – James colleagues in the colleges of Pharmacy and Veterinary Medicine to develop small-molecule inhibitors based on the natural product curcumin, which is derived from the spice turmeric, used in many southern Asian recipes. In addition, he is collaborating with colleagues in the colleges of Medicine and of Food, Agricultural and Environmental Sciences to develop a soy bread intervention to reduce inflammation and the risk of cancer recurrence.

The small-molecule inhibitor targets the STAT3 protein, a key regulator of both cancer cell proliferation and dysfunctional immunity in cancer patients. A second-generation version of the

agent is currently in preclinical testing. The fortified soy bread was designed, developed and refined by OSUCCC – James investigators in Ohio State's Department of Food Science and Technology to reduce the risk of prostate cancer recurrence; the bread has been evaluated in a phase II clinical trial.

“It's pretty hard for people at other institutions to do multidisciplinary studies of the quality and level we can do them here because few universities have a National Cancer Institute-designated Comprehensive Cancer Center, a College of Medicine, and such a strong presence in agricultural science through our College of Food, Agricultural and Environmental Sciences,” Lesinski says.

## ANTITUMOR IMMUNITY

Lesinski is a biologist. His lab works with cells and animal models, and analyzes blood samples from patients on clinical trials for various markers of immune response. They use incubators to culture cells, microscopes to examine them, and gel electrophoresis, a method to separate and identify proteins in cell lysates and serum samples.

Lesinski's lab is particularly interested in the immune cells that attack tumors – cytotoxic T cells and natural killer (NK) cells – and in the hormone-like

chemicals called cytokines and chemokines that tumor cells release to inhibit the antitumor immune response. In 2011, Lesinski and his colleagues showed that the release of interleukin-6 (IL-6) and IL-10 by cancer cells promotes inflammation, stimulates the proliferation of suppressor cells that inhibit the anti-tumor response and probably limit immune-based therapies.

Lesinski and others have shown that when the proinflammatory cytokine IL-6 binds with its receptor on cancer cells, it ultimately activates STAT3, a potent transcription factor that regulates a range of genes. “When STAT3 is hyperactivated in cancer cells, it promotes cell survival and growth, immune evasion, angiogenesis and metastasis,” Lesinski explains.

In immune cells, STAT3 activation triggers a phenomenon called cancer-associated immunosuppression. “When STAT3 is activated in immature immune cells, it inhibits their differentiation and leaves them in a more primordial but immune-suppressive state,” he says.

The inhibitor that blocked STAT3 activation in both tumor and immune cells should therefore both knock out the suppressive response while enhancing the antitumor response.

### CURCUMIN COLLABORATION

In 2008, Lesinski met with *in-silico* drug designer Chenglong Li,

PhD, a member of the OSUCCC – James Experimental Therapeutics Program, and with James Fuchs, PhD, assistant professor of Medicinal Chemistry and Pharmacognosy, both in Ohio State’s College of Pharmacy. The two were developing a small-molecule STAT3 inhibitor derived from curcumin.

The molecule showed much potential, but it also presented serious challenges: It inhibited cytokines important for anticancer immune therapies; it had poor solubility and bioavailability; it was rapidly eliminated from the body; and it was nonspecific – it interacted with many different proteins throughout the body.

On the plus side, curcumin inhibited not only STAT3, but also JAK2, a signaling molecule located upstream in the STAT3 pathway, suggesting a derivative molecule might provide double-barreled inhibition. Curcumin was also commonly available and had a relatively simple molecular structure. In 2009, Lesinski led research showing that curcumin induced cell death (apoptosis) in melanoma cells.

Li, an associate professor in Medicinal Chemistry and Biophysics, runs a computational drug-design lab furnished with eight desktop computers. The Ohio Supercomputer Center is nearby when he needs it, and he has a wet lab where he can synthesize molecules. His six graduate students and a postdoctoral researcher are

split between the computer and wet labs.

Lesinski joined the collaboration, and Li believed that, working together, they could develop a curcumin-derived inhibitor that was both potent and highly specific for STAT3.

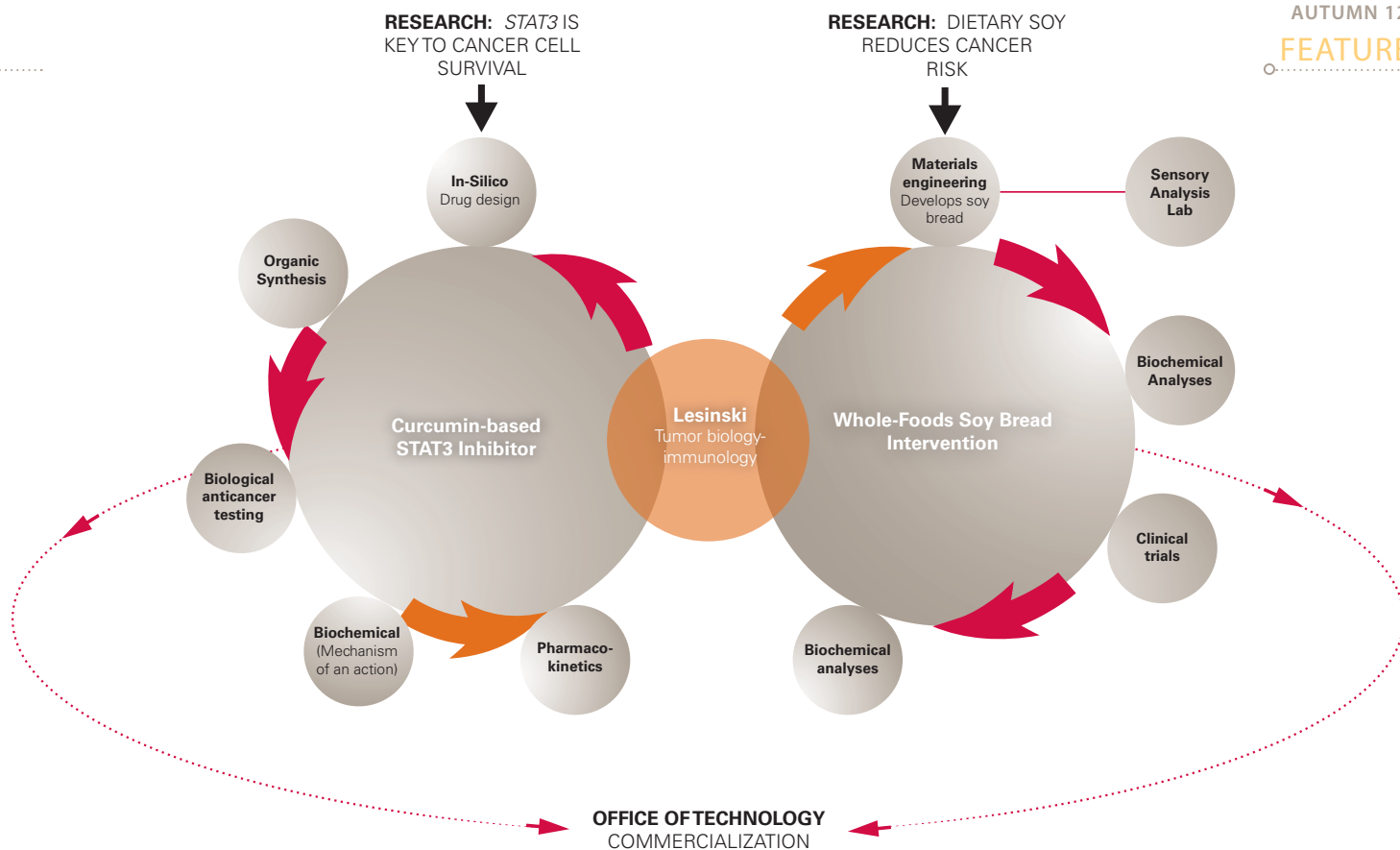
Using *in silico* drug design methods, Li computationally pulled apart the curcumin molecule and eventually constructed a lead molecule called FLLL-32. Fuchs and his lab then used the techniques of synthetic organic chemistry to make the agent, an orange powder.

“As medicinal chemists,” Fuchs says, “we not only have to know how to synthesize a molecule, we must synthesize a bioactive molecule, one that does a job. We blend state-of-the-art organic synthesis with a medicinal approach. We can tackle simple molecules or more challenging structures.”

### A CYCLE OF DEVELOPMENT

Fuchs and his lab synthesized up to 50 grams of FLLL-32 and distributed it to collaborating labs to evaluate its potency against several cancer types, its toxicity and STAT3 specificity, and its mechanism of action:

- Lesinski’s lab tested it in melanoma and renal cell carcinoma, and pancreatic cancer; and in an animal model for its physiological effects, and its effects on cancer and immune biomarkers and on STAT3.
- The lab of Jiayuh Lin, PhD, associate professor of Pediatrics at



Nationwide Children’s Hospital, and a member of the OSUCCC – James Experimental Therapeutics, tested the agent against pancreatic cancer.

- The lab of Cheryl London, DVM, associate professor of Veterinary Biosciences in the College of Veterinary Medicine and a member of the OSUCCC – James Molecular Biology and Cancer Genetics Program, tested it against canine osteosarcoma.

- Pui-Kai “Tom” Li, PhD, associate professor and chair of Medicinal Chemistry and Pharmacognosy, and his lab showed that the agent blocked STAT3 by preventing two subunits from coming together to form a complete STAT3 molecule.

- Mitch Phelps, PhD, who directs the OSUCCC – James Pharmacoanalytical Shared Resource, and his lab evaluated the agent’s pharmacokinetics (i.e., how the body metabolizes the drug).

This schematic indicates the specialties and flow of information involved in the process of designing, producing, testing and refining a targeted anticancer drug (left sphere); and a cancer-preventive soy bread to prevent cancer recurrence (right sphere).

OSUCCC – James tumor immunologist Gregory Lesinski is a collaborator on both projects, which involve diverse specialties, all located on The Ohio State University campus.

The STAT3-inhibitor collaboration includes labs in Ohio State’s colleges of Pharmacy and Medicine that focus on computational drug design, drug synthesis and biological testing (including Lesinski’s lab). The OSUCCC – James Pharmacoanalytical Shared Resource (SR) provided pharmacokinetic analyses.

The soy bread collaboration includes OSUCCC – James researchers in the Department of Food Science and Technology and in the College of Medicine. The bread was developed, taste-tested and evaluated in a clinical trial of men with prostate cancer. The trial included correlative studies of metabolites and immune effects. The OSUCCC – James Nutrient and Phytochemical Analytics SR provided metabolite and pharmacokinetic analyses.

Ohio State’s Office of Technology Commercialization works with researchers to license and help bring agents such as these to market.

*For a slide show of laboratories and facilities involved in these interdisciplinary projects, go to Frontiers online, navigate to this illustration and click in the center of each large sphere.*

Phelps, an assistant professor of Pharmacy with the College of Pharmacy and a member of the OSUCCC – James Experimental

Therapeutics Program, showed that FLLL-32, though better than curcumin itself, still had poor solubility and was rapidly metabolized. “This stalled further development at the *in vitro/in vivo* transition phase for a time,” Phelps says.

Fuchs suggested a solution: Produce a phosphate derivative of the agent. Li and Fuchs altered the

molecular structure and produced the second-generation agent, called FLLL-100P. “This ‘simple’ structural modification ended up taking about a year for us to solve – there were a number of unforeseen difficulties in reactivity that my graduate student, Eric Schwartz, needed to overcome,” Fuchs says.

As before, samples went to Lesinski and other collaborators for biological and chemical testing in a cycle of refinement that would continue until the agent is potentially ready for clinical-trials testing. The change improved the agent’s pharmacokinetic profile and produced a 10-fold increase in serum concentration in an animal model.

“As medicinal chemists,”

Fuchs says, “we not only have to know how to synthesize a molecule, we must synthesize a bioactive molecule, one that does a job.”

CHENGLONG LI, PhD, a member of the OSUCCC – James Experimental Therapeutics Program



**JAMES FUCHS, PhD,** assistant professor of Medicinal Chemistry and Pharmacognosy

Tumeric, the plant and the spice

“Studies in patients can prove whether an intervention truly has an effect,” Lesinski says. “If so, we’ve achieved our main goal; if not, we might tweak the molecule further to improve it...or we might have to start over.”

Either way, he says, “Ohio State is one of the few universities that can do this efficiently, almost like a pharmaceutical company. The College of Pharmacy is just down the street. We can walk there in minutes, discuss a problem and make changes. It doesn’t take conference calls and plane flights; everything is under the cancer center’s umbrella.”

## A WHOLE-FOODS INTERVENTION

Carcinogenesis is often characterized by chronic inflammation, and Lesinski notes that there is great interest in harnessing the anti-inflammatory properties of many natural products to reduce cancer risk. In 2010, OSUCCC – James prostate cancer medical oncologist Steven Clinton, MD, PhD, leader of the OSUCCC – James Molecular Carcinogenesis and Chemoprevention Program, invited Lesinski to join a team focusing on the impact of soy on prostate cancer.

Epidemiologic studies show that in Asian countries where soy is regularly eaten, prostate cancer rates are 10-fold lower than in the United States. In addition, clinical, animal and cell-culture studies have demonstrated soy’s anti-inflammatory effects.

Research, including findings by Lesinski, suggests that phytochemicals in soy called isoflavones have anti-inflammatory properties and may influence specific aspects of the immune response. “We believe that soy might have a role in preventing or reducing inflammation before cancer develops,” Lesinski says.

## A SOY BREAD CHALLENGE

In 2000, food scientist Yael Vodovotz, PhD, arrived at Ohio State from NASA’s Johnson Space Flight Center in Houston, where she developed novel foods for a mission to Mars, including a soy-bread formulation.

Soon after her arrival in the Department of Food Science and Technology and College of Food, Agricultural and Environmental Sciences, a colleague there, Steven Schwartz, PhD, introduced her to Clinton.

Both Clinton and Schwartz were founders of Ohio State’s Center for Advanced Functional Foods Research and Entrepreneurship (CAFFRE), and they approached Vodovotz about collaborating on soy bread chemoprevention studies. This conversation led to preliminary studies and a National Institutes of Health-funded pilot study in men with prostate cancer to test their compliance with a soy-bread product and to study the metabolism of soy phytochemicals.

“We wanted to provide a dose of soy into the diet of American men that would be similar to what

someone in China would consume,” Clinton says. “We felt that bread would be an ideal vehicle for this because bread can be incorporated into any meal. And if we could make a tasty, high-quality bread product, it would be one of the most efficient ways to ensure high compliance for long-term clinical studies.”

Like Fuchs’ medicinal chemist who must not only synthesize a molecule but synthesize a molecule that does a job, Vodovotz had to develop a soy bread that not only tastes good but delivers a specific dose of isoflavones.

Ultimately, the bread would be provided to men with prostate cancer during a four-month clinical trial. The validity of the trial would rely heavily on the sound and uniform formulation of the bread.

Vodovotz, who is also a member of CAFFRE, specializes in the physical, chemical and functional properties of foods and food components. She characterizes the physical and chemical composition of food products; measures mechanical properties that affect food storage, stability and texture; and develops functional foods that target health outcomes.

Her lab is equipped to measure the caloric content of foods and their components; moisture content of foods at nanogram levels; and the compression of foods, an indicator of texture and firmness. “Such measurements enabled us to quantify and maintain the soy bread’s texture, palatability and moisture content as we refined the

*“We have a team of experienced investigators who know how to efficiently recruit and rapidly complete dietary studies at The James. Of course, it would not be possible without our wonderful prostate cancer patients who regularly volunteer for clinical studies.”*



**ELIZABETH GRAINGER, PhD, RD,**  
*a registered dietitian and clinical research specialist in the laboratory of Nutrition and Chemoprevention*

formulation,” says Jennifer Ahn-Jarvis, RN, a graduate research associate and Pelotonia graduate in Vodovotz’s lab.

Vodovotz developed the bread and baked loaves using Ohio State’s Food Science and Technology pilot processing plant. She conducted taste tests at the department’s food sensory lab, which is equipped with 10 cubicles (see the online slide show).

“We tested the soy bread frequently to be sure it was palatable and had flavor and was good to eat,” Ahn-Jarvis says. “Texture affects palatability, and how well subjects will comply with the protocol, which affects the dose of anticancer nutrients we think a participant is getting.”

The bread’s development took several years. Studies were done on [isoflavone content and activity](#), [digestive stability and bioaccessibility](#), and changes in their distribution during soy bread proofing and baking. Other studies looked at [water distribution and molecular changes](#), and physical properties and water state changes in bread with and without almonds.

The soy isoflavone composition was determined by the OSUCCC – James [Nutrient and Phytochemical Analytic Shared Resource](#), headed by director Schwartz and associate director Ken Riedl, PhD.

To boost the absorption of isoflavones from the bread, Vodovotz and her lab developed a formulation that included a small amount of almond powder. Normally, 70-90 percent of soy isoflavones have an attached sugar group. The human digestive system, however, more quickly absorbs isoflavones that lack that sugar group. Almonds contain an enzyme called beta-glucosidase that removes the sugar groups and converts 75 percent of the “sugared” isoflavones in the bread to the “sugarless,” aglycone, form.

“All this work had to be done just to reach the point of clinical testing,” Vodovotz says. “The analyses at the end of the trial will tell us which bread formation we should choose, with or without the almond powder, and provide other ideas for improving the product.

“If all goes well, the final step will be to commercialize the bread,” she says. “The University’s office of Technology Commercialization and Knowledge Transfer would help us with that.”

### THE CLINICAL TRIAL

To evaluate soy bread intervention in patients, Clinton designed a phase II clinical trial for men with progressing metastatic prostate cancer. The study tested the soy bread against the soy-almond

bread in 40 men who were to eat three slices of soy bread per day for eight weeks. This was followed by a “wash out” period, then the men consumed the other formulation for eight weeks.

Elizabeth Grainger, PhD, RD, a clinical research specialist trained as a registered dietitian and translational scientist, coordinated the participants’ clinic visits, educated them about the intervention and any required diet modifications, served as contact person and ensured that all biological samples were obtain.

Ahn-Jarvis, who is a trained registered nurse and is now working on her doctoral degree in Vodovotz’ lab, helped with some of these functions. She also helped direct the production of the four-month supply of soy bread, completed all the quality control analyses for the bread and processed and stored blood and urine samples as they were collected.

“That study, like many that we have done recently with various collaborators, was like a well-oiled machine,” Grainger says. “We have a team of experienced investigators who know how to efficiently recruit and rapidly complete dietary studies at The James. Of course, it would not be possible without our

wonderful prostate cancer patients who regularly volunteer for clinical studies.”

About 1,200 loaves of soy bread were needed for the study. “They needed to be made in a single batch to reduce variability,” Ahn-Jarvis says. “A Columbus-area commercial baker, who was intrigued by the project, greatly supported our efforts and provided that service.”

The researchers monitored participants’ blood and urine samples to study the metabolism of soy phytochemicals, and they examined prostate specific antigen (PSA), an indicator of prostate cancer progression, as well as an array of biomarkers related to the regulation of the immune system and inflammation.

Schwartz and Riedl are food scientists and analytical chemists with expertise in the analysis

and metabolism of diverse phytochemicals that may influence cancer risk such as carotenoids, isothiocyanates and isoflavones. Riedl applies high pressure liquid

chromatograph-mass spectrometry (HPLC-MS) to analyze food components and metabolites in plants and foods, as well as blood and urine samples.

**STEVEN CLINTON, MD, PHD,**  
*leader of the  
OSUCCC – James  
Molecular  
Carcinogenesis  
and Chemo-  
prevention  
Program*

**Yael Vodovotz, PhD,**  
*food scientist*

**STEVEN  
SCHWARTZ,  
PHD**



**CHARACTERISTICS  
MEASURED DURING  
SOY BREAD  
DEVELOPMENT**

- Loaf volume; crust and crumb color;
- Protein and ash content;
- Water mobility; “freezable” water and “unfreezable” water; stiffness at 25 C.



*“It’s part of the beauty of being in a place like Ohio State, where we can interact with faculty in fields totally disparate from our own and find common ground for novel research pursuits in our war on cancer.”*

“Foods are complicated,” Riedl says. “For example, soy contains dozens of interesting compounds, and how foods are processed can alter their patterns and even their structure, which may influence absorption and biological impact.” Lesinski and his lab measured markers of immune response in the white-cell (buffy coat) fraction of the blood samples, work that was funded by a pilot grant from Ohio State’s Food Innovation Center, The Ohio Soybean Council, and the OSUCCC – James Molecular Carcinogenesis and Chemoprevention Program. Some of the findings have been presented at national meetings. For example, they found statistically significant declines in the levels of four proinflammatory proteins and seven proteins related to suppression of the cellular immune response, and a reduction in the number of certain immune suppressive cells.

“Overall, our studies suggest that soy isoflavones and their metabolites can influence immune function and potentially immunotherapy,” Lesinski says. “Some of these might deserve further investigation as lead compounds for clinical use.”

Some of the analyses are still under way. “We are doing thorough and comprehensive immunology to assess how isoflavones in soy and other foods affect markers of inflammation,” Lesinski says.

In addition to measuring cytokines and chemokines in plasma, they’re investigating how isoflavones affect specific aspects of the cells’ biology, including differentiation, response to proinflammatory stimuli, how they respond *in vivo* in a cancer model, and whether dietary enrichment alters the levels of proinflammatory immune cells.

Finally, they are examining individual proteins and signaling pathways inside immune-cell subsets that might be targeted by the whole food, by a crude extract of the food or by individual bioactive fractions or components from within these various foods. “We want to learn what signaling pathways within the immune cells are being targeted by foods or food components to limit the inflammatory processes,” he says.

“If we discover which fractions or compounds in soy or other whole foods are active antitumor agents, we can work with our Ohio State collaborators to develop a small-molecule inhibitor that is fine-tuned to hit an appropriate target.”

The Crops to Clinic Program, which is a collaboration between the OSUCCC – James and Ohio State’s College of Food, Agricultural and Environmental Sciences, is designed to develop novel food products for cancer prevention. Clinton notes that Lesinski’s work expands this effort

into the exciting and dynamic realm of cancer immunology.

“This opens a whole new avenue for looking at how foods affect the cancer process,” Clinton says.

“Our whole-food approach, illustrated by the soy-almond bread research, brings together a high-quality and well-trained immunologist with investigators having expertise in food chemistry, food technology, nutrition, and translational clinical research. The joint effort provides state-of-the-art research in an area where these fields overlap. It really gets down to the great value of team science when you have folks who can interface, communicate and work together so seamlessly,” Clinton continues.

“It’s part of the beauty of being in a place like Ohio State, where we can interact with faculty in fields totally disparate from our own and find common ground for novel research pursuits in our war on cancer.” ■



# NEED TO KNOW

Resources for Professional Development

## ▶▶ FOOD-BASED RESEARCH

### GROWING A CURE

Five Ohio- and Indiana-based farm cooperatives have joined together to support food-based cancer research at the OSUCCC – James and at Ohio State’s College of Food, Agricultural and Environmental Sciences. The five organizations of growers have formed Cooperatives for the Cure, an endowment fund at the OSUCCC – James, in collaboration with the College of Food, Agricultural and Environmental Sciences.

The cooperatives kicked off the endowment with a check for more than \$103,000 presented to the OSUCCC – James in September during the [2012 Farm Science Review](#), Ohio State’s annual showcase of advances in agriculture.

At Ohio State, faculty members, graduate students and researchers in cancer biology work with researchers in plant genetics, horticulture, crop science, food technology and marketing to develop food products that are optimized for clinical trials



of cancer prevention or an adjunct to therapy.

For example, the researchers are using scientific breeding methods to develop crop strains that are high in natural anticancer compounds, such as specific carotenoids in tomatoes, followed by the application of modern food technology to preserve the anticancer activity in novel and tasty foods.

At the OSUCCC – James, many patients and members of the community have opportunities to participate in several Crops to the Clinic™ clinical research studies of novel food products and nutritional strategies to prevent cancer or enhance cancer therapy as well as survivorship.

“We believe that this collective effort will produce discoveries that bring novel food products and nutritional strategies into the mainstream of the war on cancer and reduce one’s risk of specific cancers or perhaps improve the safety and efficacy of therapy,” says Steven Clinton, MD, PhD, director of the OSUCCC – James Prostate and Genitourinary Oncology Clinic and leader of the Molecular Carcinogenesis and Chemoprevention Program. “Our ultimate goal for this collaboration is to contribute to a world free of cancer.”

For more information or to make a tax-deductible contribution, go to <http://growingthecure.org/>. All funds raised go toward food-based cancer-prevention research.

## Events Calendar

### STATE-OF-THE-ART ENDOSCOPIC SKULL BASE SURGERY: A HANDS-ON COURSE

Oct. 25-28, 2012 GREATER COLUMBUS CONVENTION CENTER

**FOCUS:** This course for neurosurgeons, head-and-neck surgeons and other skull-base surgeons covers current indications, limitations and surgical techniques for endoscopic endonasal surgery of the skull base, pituitary fossa, orbit and craniocervical junction, and for the supraorbital keyhole craniotomy approach.

### OHIO STATE’S 2012 SCARLET AND GRAY RECEPTION DURING THE 54TH AMERICAN SOCIETY OF HEMATOLOGY (ASH) ANNUAL MEETING

Saturday, Dec. 8

While attending the 2012 ASH Annual Meeting in Atlanta, please join your Ohio State colleagues at our reception on Saturday at the Omni Hotel at CNN Center (the official headquarters hotel for the 2012 ASH Annual Meeting), International Ballroom, from 7:30-9:30 p.m.

Please RSVP at [go.osu.edu/ASHReception2012](http://go.osu.edu/ASHReception2012). For questions, contact Katie Jones at [Katie.Jones@osumc.edu](mailto:Katie.Jones@osumc.edu) or 614-366-5183.