

“I hope this crusade does not kill women,” Ranieri said, referring to the imputed risks from switching from MIS to open hysterectomies.

Reducing Risk

Isaacson is confident that using a containment bag when morcellating will reduce risk of spreading cancer. He also advocated studying how effective existing containment systems are. But Shriver and others disagree.

“After 20 years, there’s no evidence that bags or any containment devices prevent the outcome we’re trying to prevent. At present, there’s no safe way to morcellate,” Shriver said.

Opponents of a ban suggest a black-box warning for physicians, but Carol Brown, M.D., a gynecologic oncologist at New York’s Memorial Sloan-Kettering Cancer Center, said that wouldn’t be enough to educate patients of risk. Panel members agreed that health care workers must disclose risk and that

informed consent must be sought and given. Some were skeptical that women informed of risks would consent to morcellation.

Another suggestion was to classify women according to risk, but since biomarkers and scanning do not reveal risk, who is at most risk remains unclear. Many patients, such as Amy Reed, are premenopausal and not black and would thus be incorrectly categorized as lower risk. Some panel members criticized the suggestion of establishing a prospective database of complications from morcellation as allowing more deaths to occur when risks are known. A further suggestion was to reclassify morcellators as needing more regulatory oversight.

Need for More Oversight

Noorchashm sees morcellation as part of a bigger problem: lax standards for approval of medical devices.

“It is clear that the 510(k) legislation is deficient in requiring post-market outcomes to be reported back to the FDA, especially when the outcome is mortality or a severe morbidity,” he said. “These are public-health hazards that must be resolved at the level of the specialty and the United States Congress.”

In 2011, at FDA’s request, the Institute of Medicine appointed a committee to study the 35-year-old medical device approval process in light of the aftermarket failure of several devices. The report criticized the process as no longer adequate or flexible enough to deal with the complexity of new devices. The committee concluded that the 510(k) process is not a reliable premarket screen of safety and effectiveness of moderate-risk devices and should be wholly revised.

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Beyond Counting: New Way To Use Circulating Tumor Cells

By Anna Azvolinsky

In 2007, researchers at Boston’s Massachusetts General Hospital (MGH) Cancer Center showed that a microfluidic device, the CTC-Chip, could separate and count rare circulating tumor cells (CTCs) in whole-blood samples (*Nature* 2007;450:1235–9). Last year, the team developed the CTC-iChip, a refined version of its predecessor, and confirmed its utility in catching viable CTCs. This device can profile individual CTCs—including their DNA mutations and RNA and protein expression—at the molecular level (*Sci. Transl. Med.* 2013;5:179ra47; doi:10.1126/scitranslmed.3005616).

Going further, the teams recently showed that CTCs captured with CTC-iChip could be used to establish individual patient-based cell lines and xenograft models (*Science* 2014;345:216–20; doi:10.1126/science.1253533). These cell lines can test for drug sensitivity, which researchers hope

will facilitate individualized cancer treatment options. These findings took place in the MGH laboratories of Daniel Haber, M.D., Ph.D., Shyamala Maheswaran, Ph.D., and Mehmet Toner, Ph.D.

The goal of culturing these cells *ex vivo*, according to Haber, is to enable functional analysis. Genetic testing for mutations often, but not always, is associated with drug response, he said.

“Testing whether a drug really works in killing a cancer cell with a given mutation [would be] helpful. This becomes even more relevant when multiple mutations are present [in CTCs], each of which could be driving the cancer,” Haber said.

CTCs are found in minute quantities in the blood of cancer patients, shed from either the primary or metastatic tumor. Research has statistically correlated CTCs with both patient survival and disease progression for

certain cancers. Because a simple blood draw is appealing to both patient and clinician, researchers and companies have tried to detect and characterize CTCs in lieu of invasively taking a tumor sample.

Still, most research on CTCs is confined to preclinical and academic studies. So far, the U.S. Food and Drug Administration has approved only one CTC detection tool: CellSearch. Made by Johnson & Johnson’s Janssen Diagnostics, CellSearch counts CTCs—those of an epithelial origin that express the surface epithelia cell adhesion molecule—in blood samples from breast, prostate, and colorectal cancer patients.

Agnostic Approach

Unlike CellSearch, the new CTC-iChip does not detect CTC cell surface markers. These biomarkers can vary among cancers and even change in the same cancer patient

as the tumor grows or changes due to treatment. Rather, the technique is tumor biomarker agnostic.

“We just remove all the blood components”—red blood cells and platelets according to their size and white blood cells on the basis of their membrane markers—“which leaves all the heterogeneous populations of CTCs behind,” Maheswaran said. Melanoma cells, for example, aren’t epithelial, and they’re so different from each other that defining them all by one biomarker isn’t possible. “So we are isolating CTCs by simply removing the blood components, which is very desirable.” Another advantage, according to Maheswaran, is that CTCs are captured in solution rather than on a matrix from which releasing viable cells was challenging.

Maheswaran, Haber, Toner, and colleagues created CTC lines from six of 36 estrogen receptor-positive breast cancer patients who had either come off therapy or whose disease had recently progressed after treatment. Cells grew in tissue culture only in samples from patients whose disease had progressed during therapy, but not from patients who responded to treatment. Three of those six cell lines caused tumors when injected into mice.

Screening cell lines for mutations and aberrations, researchers found both mutations in common with a patient’s matched primary tumor sample and new mutations probably acquired during treatment exposure—potential targets for therapy. The team tested these cell lines for sensitivity to single and combination drugs, both standard chemotherapy agents and targeted drugs in development. The analysis was able to reveal the patient’s prior treatment history—whether the patient had been sensitive or resistant to a specific targeted agent and also novel sensitivities to agents acquired through mutations not previously present in the primary tumor.

The analysis revealed that, because the CTCs harbored new mutations not present in the primary tumor, the patient was sensitive to targeted therapy drugs.

“The presence of these new mutations and their implication for new treatment regimens would not have been known without the ability to repeatedly, noninvasively

sample patients’ tumor cells during their therapy,” Haber said.

Using patient-derived CTC lines to identify potential therapies for advanced cancer patients is one way to identify a patient’s next course of treatment.

“There are an increasing number of promising targeted drugs,” many of which are expensive and work only in some patients. “The universe of drug possibilities is expanding rapidly, but these cannot realistically be tested directly in patients,” Haber said.

This new assay may predict a patient’s response on the basis of both molecular and functional analyses.

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“CTC culture analyses are a very promising and potentially powerful way to achieve this goal,” Haber said.

Growing Pains

CTC culturing is still in its infancy, but the MGH team is not the first to show the technique’s potential. At least eight international groups are refining the technique—particularly, improving the success rate for culturing these rare cells from a cancer patient. Katharina Pachmann, M.D., of Friedrich-Schiller University Jena, in Germany, showed that CTCs from breast cancer patients could be grown as spheres from tumors *ex vivo*, a property of metastatic cells (*Ecancermedicalscience* 2013;7:343; doi:10.3332/ecancer.2013.343).

Dario Marchetti, Ph.D., studies biology of breast cancer brain metastasis at the Baylor College of Medicine in Houston. He and his colleagues characterized CTCs with a specific molecular signature that could form brain metastases, but not other types, in an animal model (*Sci. Transl. Med.*

2013;5:180ra48 [see correction on p. 189r5]; doi:10.1126/scitranslmed.3005109).

Marchetti’s laboratory is refining the signature to identify markers necessary for brain metastases. The goal, Marchetti said, is to develop the first diagnostic test to predict risk of primary brain metastases in earlier-stage breast cancer patients and to find ways to prevent secondary metastasis by targeting these cells.



Daniel Haber, M.D., Ph.D.

Although this approach differs from that of the MGH group, both rely on the still relatively rare CTC sample from which to create a cell culture.

“For us, it was three of 38 patient samples from which we could establish cell lines,” Marchetti said. “So there is still a lot of work before we get this right.”

The laboratory of Chwee Teck Lim, Ph.D., at the National University of Singapore appears to be making leaps where others are taking only steps. His laboratory may have cultured more CTCs than any other laboratory has previously reported. According to Lim, the manuscript describing the work is under review for publication.

Many Questions

One issue for culturing these cells is their heterogeneity in circulation.

Marchetti said that no one universal recipe for culturing patient-derived CTCs exists. In fact, each patient’s cells might require slightly different growth conditions. Moreover, CTCs are not detectable in all cancer patients. Whether the heterogeneity of CTCs represents a patient’s primary or metastatic tumor is also not clear. And many more questions remain.

“How much are we potentially changing the cells by culturing them, and which CTCs are the ones that are able to grow in culture? How representative are they of the tumor cells needed to be ablated to treat a patient’s cancer?” said Stefanie S. Jeffrey, M.D. Jeffrey is professor of surgery and chief of surgical oncology research at Stanford University School of Medicine

in Stanford, Calif. Her lab is also in the early stages of growing and characterizing patients' CTCs in a dish.

Jeffrey sees researchers combining CTC assays with circulating tumor DNA (ctDNA) assays to make diagnosis and treatment decisions.

"We can use ctDNA to monitor patients and predict tumor recurrence, but this doesn't tell you which drugs should then be used for treatment. That is where CTCs come in—the ability to culture and directly do drug sensitivity testing, while not currently possible, is an attractive future idea," she said.

"[Drug sensitivity, CTC and ctDNA assays] are a nice union of many fields,"

said Luis Diaz, M.D., associate professor of oncology at Johns Hopkins University in Baltimore. Diaz develops genomic diagnostic and therapeutic approaches for cancer, including ctDNA assays. He is cautiously optimistic about cultured CTCs and would like to see a comparison of CTC cultures with those grown directly from tumor samples.

"The major questions I have are, what do these cells really represent in terms of the tumor, and what fraction of patients will have CTCs from which cultures can be formed?" Diaz said.

According to Diaz, cultured CTCs and CTC-derived xenografts are another version of mouse avatars—xenografts grown from individual tumors for drug screening: "The

jury is still out on whether this will work well in the clinic and the use of these assays will depend on patients' clinical outcomes."

So far, such studies are also costly. But researchers working on culturing CTCs are optimistic.

"We are convinced that CTCs will be used to identify effective therapies for a patient and as a real-time biopsy," Marchetti said.

Jeffrey agreed: "There is a lot we still don't know about this technique, but it's extraordinarily exciting. This is going to be an important tool for precision-based medicine and drug testing."

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Resistant Starch May Reduce Colon Cancer Risk From Red Meat

By Judy Peres

A diet high in red meat increases risk of colon cancer. But eating resistant starch—a carbohydrate that acts like fiber—may reduce that risk, according to a study in August's *Cancer Prevention Research*.

"Red meat and resistant starch have opposite effects on the colorectal cancer-promoting microRNAs," said first author Karen Humphreys, Ph.D., a research associate at the Flinders Centre for Innovation in Cancer at Australia's Flinders University. "This finding supports consumption of resistant starch as a means of reducing the risk."

In a 2011 study, colon cancer risk increased by 29% for every 100g of red or processed meat eaten per day, plateauing around 140g. But no one knew exactly why.

This new study offers insights, said Sonia Kupfer, M.D., director of the Gastrointestinal Cancer Risk and Prevention Clinic at the University of Chicago Medical Center. "It may be that protective factors and risk factors

work through the same pathways." She noted other possible mechanisms: "Heme iron and heterocyclic amines from red meat cooked at high temperature alter gene expression and increase proliferation. Butyrate from microbes decreases proliferation. This paper starts to provide intriguing ways in which we can think about these dietary factors mechanistically altering the colonic epithelium. But there might be other effects as well," she said. "Dietary substances can be complex."

Unlike most carbohydrates, resistant starch passes undigested to the colon. There, gut microbes ferment it, yielding short-chain fatty acids, such as butyrate, which promote colon health. Those short-chain fatty acids, the study suggests, also reduce expression of microRNAs that are associated with severe colon cancer and that increase cell proliferation.

The study involved 23 healthy volunteers, aged 50–75 years. Participants were randomly assigned to either a high-red-meat diet (300g raw per day of lean beef

or lamb) or that same diet plus a resistant-starch supplement called StarPlus (40g per day of butyrylated high-amylose maize starch). After 4 weeks on one diet, participants switched to the other for another 4 weeks. For 4 weeks before each intervention, participants ate normally.

After each phase, researchers took fecal and pinch biopsy samples of rectal mucosa. They measured levels of butyrate and other short-chain fatty acids; proliferation; microRNA expression; and target genes of those microRNAs, including cell cycle inhibitor CDKN1A and proapoptotic genes PTEN and BCL2L11. A high-red-meat diet statistically significantly increased cell proliferation in the mucosa. This increase corresponded with higher expression of oncogenic microRNAs (the miR17-92 cluster and miR21). After 4 weeks on the high-red-meat diet, miR17-92 levels increased by a mean of 30%. Adding resistant starch to the meat diet reversed the increase in the miR17-92 cluster, but not in miR21.