Sarioglu, PhD, a biomedical engineer at Georgia Institute of Technology in Atlanta. Unlike other methods that target single CTCs with the hope of capturing clusters, the new device, Sarioglu says, is specifically designed to catch clumps of CTCs.

Sarioglu and molecular biologist Nicola Aceto, PhD, of Massachusetts General Hospital (MGH) in Boston, are co-first authors of a paper describing Cluster-Chip (Nat Methods 2015;12:685–91). They initiated the research while working with Mehmet Toner, PhD, and Daniel Haber, MD, PhD, at MGH.

Measuring about 7.50 cm × 3.75 cm, Cluster-Chip is a glass slide that traps CTC clusters as blood passes through a matrix of triangular pillars with 12-μm gaps between vertices. Although CTCs are slightly larger (about 15 μm wide), single cells are squishy and easily squeeze through the matrix. However, CTC clusters catch on the points of the triangular pillars and remain trapped on the slide.

To measure the device’s capture efficiency, the researchers spiked healthy human blood samples with clusters of fluorescently labeled MDA-MB-231 human breast cancer cells. Cluster-Chip captured 99% of clusters containing at least four tumor cells, 70% of three-cell clusters and 41% of two-cell clusters. For optimal performance, the researchers used a flow rate about 10 times slower than normal blood circulation and processed samples at 4°C instead of room temperature.

Compared with other methods, Sarioglu says, Cluster-Chip may give a truer estimate of how common CTC clusters are. When used on blood samples from 60 patients with metastatic breast or prostate cancer or with melanoma, CTC clusters were found in 30% to 40% of patients. Earlier experiments with antibody-based microfluidic tools developed by the same researchers detected CTC clusters in only 5% to 10% of patient samples.

Chwee Teck Lim, PhD, of the National University of Singapore, says devices like Cluster-Chip provide a “means of capturing circulating tumor microemboli.” Isolating CTC clusters can give insight into their role and function, including how they might differ from single CTCs. For example, “Do CTC clusters contain specific subpopulations of CTCs that make them more likely to metastasize?” Lim asks. “We do not know yet.”

**AIM2 Blocks Colon Cancer in Three Ways**

Two recent papers explain how the inflammasome protein AIM2 protects against colon cancer, suggesting strategies to prevent and treat the disease.

AIM2 detects double-stranded DNA from bacteria and viruses and forms part of the inflammasome, an infection-fighting protein complex. Previous research also suggests that AIM2 is a tumor suppressor because its expression is turned down or off in many melanomas, colorectal cancers, and prostate tumors. However, researchers don’t know precisely how AIM2 affects tumorigenesis, so two independent teams investigated its role.

In one study, Justin Wilson, PhD, of the University of North Carolina in Chapel Hill, and colleagues dosed mice with two compounds that induce colon tumors. Mice lacking AIM2 developed more precancerous intestinal polyps and colon tumors than did controls. The researchers also studied mice that carry a mutation in the gene adenomatous polyposis coli. In humans, this gene is mutated in many sporadic colon cancers and a hereditary form of the disease. Mice with the mutation spontaneously developed colorectal and intestinal tumors, and the team determined that losing AIM2 increased their tumor load.

The researchers found that colon tissue from the AIM2-lacking mice showed increased levels of activated AKT. A promoter of cell survival and proliferation, AKT is often overactive in tumors. As Wilson and colleagues reported in *Nature Medicine*, AIM2 teams up with the protein DNA-PK to block AKT (Nat Med 2015 June 24 [Epub ahead of print]).

In the other study, Thirumala-Devi Kanneganti, PhD, of St. Jude Children’s Research Hospital in Memphis, TN, and colleagues discovered that AIM2 also curbs tumor growth by curtailing proliferation of stem cells from the colon epithelium (Cell 2015;162:45–58). In culture, these cells divided more rapidly if they were missing AIM2. The team also saw increased intestinal stem cell proliferation and faster tumor growth in mice that lacked AIM2.

The protein might also influence tumor growth by controlling the composition of the intestinal microbiota. AIM2’s absence altered the abundance of several bacterial species—including two species linked to colon tumors—the researchers reported.

Both studies agree that AIM2’s ability to suppress tumor growth is independent of its function as an inflammasome protein. Both suggest that AIM2 inhibits AKT, and both point to avenues for therapy or prevention.

Wilson and colleagues gave the same tumor-inducing compounds they’d used before to AIM2-lacking mice. One group of animals also received an AKT blocker, and those mice developed fewer polyps and tumors than did mice that received a placebo.

“If you can inhibit AKT, you might be able to treat tumors” in which AIM2 is scarce or absent, says Jenny P.Y. Ting, PhD, senior author of the *Nature Medicine* paper. No AKT inhibitors are approved for treating cancer, but several are in development.

Kanneganti’s team evaluated whether changes in the animals’ intestinal microbiota reduced tumor formation. They housed AIM2-lacking mice with normal animals. The rodents slowly acquired...
the bacteria of their cage-mates, which reduced tumor formation in the AIM2-deficient mice. “Mice lacking AIM2 have a distinct microbiota ecology,” says lead author Si Ming Man, PhD, raising the possibility that modifying gut microbiota might prevent colon cancer.

Experts praised the work of both teams. “The two papers are very significant and will give us a basis to explore the role of the human AIM2 protein in development of epithelial cancers,” says Divaker Choubey, PhD, of Ohio’s University of Cincinnati College of Medicine.

That both groups demonstrated that the role of AIM2 in preventing colon cancer is important, says Naeha Subramanian, PhD, of the Institute for Systems Biology in Seattle, WA. “Two groups published at the same time, and their conclusions are concordant.”

New Insight into Mucinous Ovarian Cancer

Historically, ovarian cancer has been treated as one disease. However, molecular studies indicate four main tumor subtypes that show distinct treatment responses: serous, clear-cell, endometrioid, and mucinous ovarian carcinoma.

To find germline variants that might flag individual susceptibility—and new therapeutic targets—for these tumor types, researchers turned to genome-wide association studies (GWAS). One recent study describes three new genetic loci specifically associated with the risk for mucinous ovarian carcinoma (MOC; Nat Genet 2015;47:888–97).

“One you find genetic loci that affect the risk, it’s like giving clues to Sherlock Holmes for where to look next, and what aspects of the biology to study,” says Andrew Berchuck, MD, director of gynecologic oncology at Duke University School of Medicine in Durham, NC, and senior author of the study. “These variants could shed some light on the pathogenesis of this disease.”

Many clinical trials currently focus on therapeutically targeting germline mutations, such as BRCA1 and BRCA2, known high-risk factors for high-grade serous ovarian cancer (HGSOC), the most common subtype. However, only 3% of women diagnosed with ovarian cancer have primary, invasive MOC, leaving a dearth of data to analyze.

In this study, researchers compared genotypes from 1,644 women with MOC to those of 21,693 unaffected controls and discovered three new risk loci. One variant, near the HOXD9 gene, is in a region of the genome that predisposes women to the HGSOC subtype; the other two lie near the INFL3 and PAX8 genes, both implicated in the development of colorectal cancer.

With this information, researchers can follow dysfunctional cell pathways that may point toward the cancer’s origins. Previous studies have shown that HOXD9 belongs to a family of transcription factors that control cell differentiation. The precise functions of INFL3 and PAX8 aren’t known, but data suggest that their expression may help maintain a malignant state.

“This study is really a starting point to try to understand the biology of mucinous ovarian cancer, particularly if they can identify the biological relevance for these genes,” says Elizabeth Swisher, MD, a professor of obstetrics and gynecology at the University of Washington and medical director of the Breast and Ovarian Cancer Prevention Program at the Seattle Cancer Care Alliance.

One caveat with GWAS: Each locus may have many single-nucleotide variants that could be responsible for the risk association, notes Berchuck. Although researchers can test the correlation between each variant and the level of gene expression, such laboratory-based simulations aren’t definitive evidence of causality. In the future, gene-editing methods may allow researchers to mimic the variants, to more accurately assess the biologic effects.

“This is just the start,” says Simon Gayther, PhD, a professor of preventive medicine at the University of Southern California’s Keck School of Medicine in Los Angeles and corresponding author of the study. “The more we do these studies focusing on MOC, the more it will become clear that this cancer has its own biology, its own genetic susceptibility, its own prevention approaches, and therapies that need to be developed.”

For more news on cancer research, visit Cancer Discovery online at http://CDnews.aacrjournals.org.

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• Thanks to a fundraising frenzy, the Oregon Health and Science University’s Knight Cancer Institute will receive $1 billion for cancer research. In 2013, Phil Knight, the CEO and co-founder of Nike, pledged to give $500 million to the center on the condition that it raise $500 million more within 2 years.

• After being canceled last year due to design flaws and trouble recruiting participants, the NIH National Children’s Study (NCS) may be resurrected, although in a different form. Appropriations committees in both the U.S. House and Senate approved spending bills for the 2016 fiscal year that include $165 million to form the National Children’s Study Alternative.

• Proton Partners International Limited began work to build the United Kingdom’s first proton beam cancer treatment center, which will be located in Newport. The company plans to build two other centers in the UK—one in Northumberland and the other in London.

• The American Society of Human Genetics issued a position statement saying that genetic testing should be limited to a single-gene analysis or targeted gene panels in children and adolescents (Am J Hum Genet 2015;97:6–21). It also says that testing for adult-onset conditions should be avoided unless a childhood treatment exists, and that secondary findings should be disclosed only when there is “clear clinical utility.”

• The U.S. Court of Appeals for the Federal Circuit ruled that Sandoz must wait 6 months to market Zarxio, its version of the biologic drug Neupogen (filgrastim; Amgen), following FDA approval. That means that an injunction imposed on the marketing of Zarxio will remain in effect until September 2.

• The European Commission approved the PD-1 inhibitor pembrolizumab (Keytruda; Merck) for patients with advanced melanoma. It also approved the PD-1 inhibitor nivolumab (Opdivo; Bristol-Myers Squibb) for patients with locally advanced or metastatic squamous non–small cell lung cancer who have already received chemotherapy.
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