THE CANCER HUNTER

Nickolas Papadopoulos' early fascination with molecular genetics fueled a career-long adventure mapping cancer genomes, unearthing cancer genes and devising tests for the minimally invasive detection of cancer.

Nickolas Papadopoulos was about as surprised as he was relieved to hear the voice on the other end of the line when he answered his lab phone one afternoon in the summer of 1992. The voice belonged to Bert Vogelstein, a man he had never met before and to whom he had sent his one and only application for a postdoctoral position. If he didn't get it, Papadopoulos was ready to fly home to his native Greece. He had labored and fussed over his letter to the up-and-coming cancer geneticist, but the fact was he had no experience, let alone publications, in the field. Fortunately, Vogelstein had ignored that deficiency and was now inviting him over for an interview. Just come over, he said. No need to prepare a presentation.

The interview, touching on everything from Papadopoulos' musical skills to technical queries to the big questions of cancer research, went well. When it was over, Vogelstein asked a graduate student to take Papadopoulos out to lunch while, it turned out, he prepared another surprise. "When I came back from lunch, Bert had gathered a few people in a little room outside his office that we called the kitchen—a refrigerator, small tables and a whiteboard," Papadopoulos recalls. "He said, 'OK, now you're going to present your work to us.' " Vogelstein brushed off his protests, saying it wouldn't be a problem if he knew his stuff. After the presentation and a little Q&A, he had someone show Papadopoulos around the lab. When he returned, he found Vogelstein hammering out a letter on his electric typewriter. The job would start in a few months, it said; Papadopoulos had to promise he wouldn't even interview elsewhere.

Papadopoulos signed, and so joined a scientific adventure that has transformed the fields of cancer genetics and diagnostics. Working with a who's-who of scientists led by Vogelstein and Kenneth Kinzler—co-directors of the Ludwig Center at Johns Hopkins—Papadopoulos has had a hand in the discovery of several novel cancer genes and the mapping of scores of cancer genomes. He and his colleagues

NICKOLAS PAPADOPOULOS

LUDWIG JOHNS HOPKINS

Photo by Flynn Larsen

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have in recent years also scoured that vast repository of genetic information to develop increasingly precise and minimally invasive DNA tests—or liquid biopsies—for cancers. A study Papadopoulos led with Vogelstein, Kinzler and Hopkins colleagues reported in 2018 in *Science Translational Medicine* the initial, retrospective evaluation of such a test for the early detection of ovarian and endometrial cancers, which are typically detected only in their advanced stages, when a cure is usually impossible. Another paper similarly reported in *Science* a single blood test that screens for eight common types of cancer. The malignancies detected by these liquid biopsies, which require further clinical development, account for more than 60% of cancer deaths in the U.S.

Chasing fascinations

Papadopoulos was born and raised in the historic city of Thessaloniki, in Greece, where his mother was a homemaker and his father a salesman for the Dutch multinational Philips. "He was traveling all over Greece making sure every house had a TV," says Papadopoulos. His mother, who had helped make ends meet as a seamstress during the Second World War and felt lucky to have attended school, was adamant that her children complete college. Not that Papadopoulos needed much encouragement. He had always wanted to be a biologist.

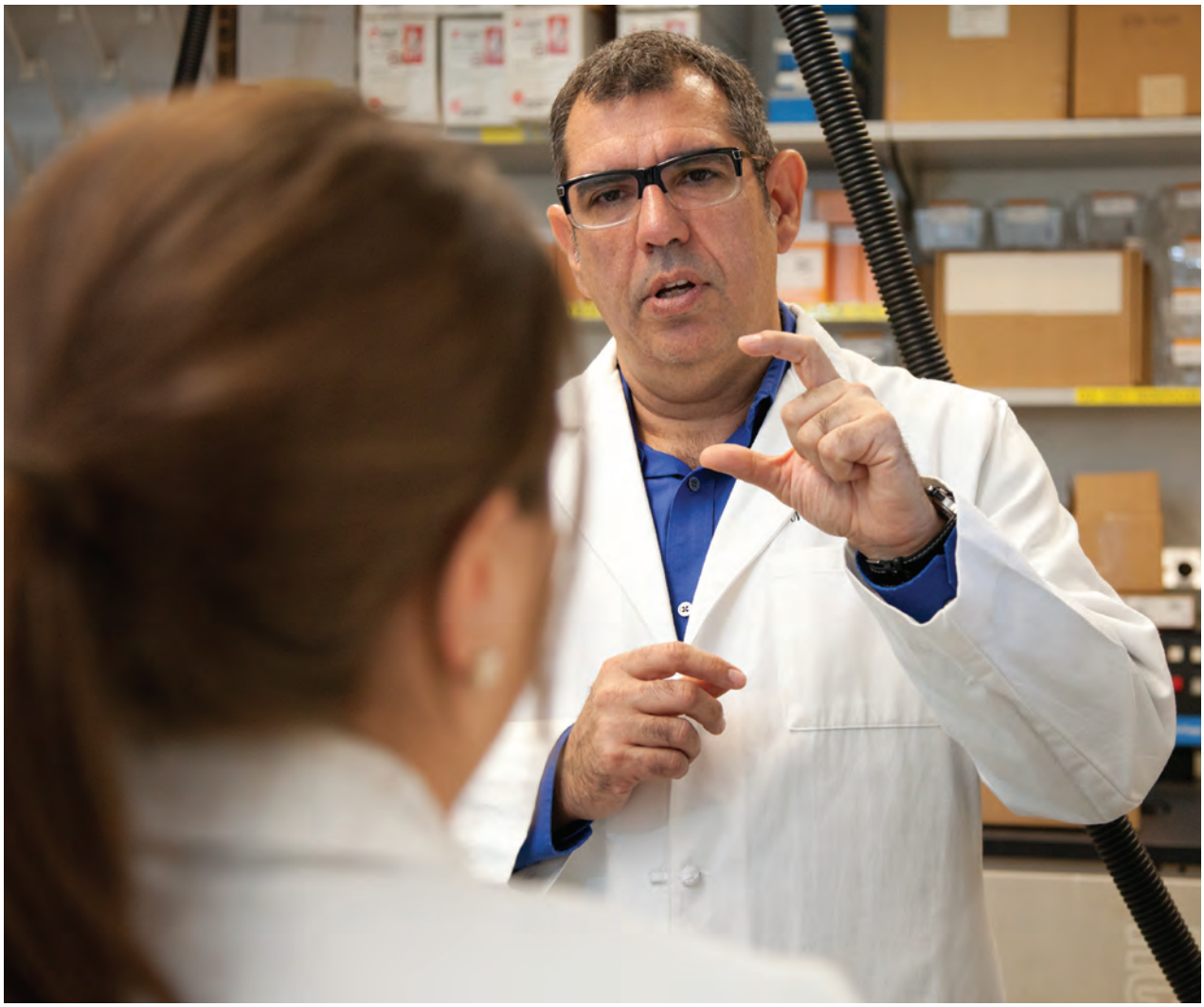
After getting his bachelor's degree from the University of Thessaloniki, Papadopoulos considered going to the UK for his postgraduate education. But his brother fell seriously ill, and Papadopoulos accompanied him and their mother to the U.S. for his treatment at a hospital in Houston. He used his spare time to take a course in English at the University of Houston and then applied to a master's program at the school. During his first year, his advisor—with whom he was investigating how muscle innervation influences the expression of myosin, a constituent of muscle fibers—moved to the University of Texas, Houston. He asked Papadopoulos to stay on as a PhD candidate in a joint program of UT Houston and the MD Anderson Cancer Center.

Papadopoulos took full advantage of the move, taking courses in molecular genetics and cancer biology. "I started having second thoughts about what I wanted to be when I grow up," he says. It was in one of those cancer biology courses at MD Anderson that Papadopoulos first heard of Bert Vogelstein and his work exploring mutations in colon cancer.

In 1992, Papadopoulos moved with his advisor to Baltimore, to a National Institutes of Health lab in the Francis Scott Key Medical Center, where he wrapped up his doctoral studies and sent his letter to Vogelstein.

Running start

Starting at Vogelstein's lab in late 1992, Papadopoulos entered a race to find the



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genes responsible for an inherited propensity for cancer known as Lynch syndrome. In December 1993, the researchers reported in *Science* that a gene encoding an enzyme involved in the repair of mismatched DNA bases drives HNPCC, a colorectal cancer associated with Lynch syndrome. Over the next few months, Papadopoulos became a primary contributor to the discovery of other mismatch repair (MMR) genes, reporting the discoveries in *Science, Nature* and *Cell*. The discoveries led not only to the identification of a novel family of human DNA repair enzymes, but also to the development of clinical tests for Lynch syndrome.

After his postdoc, Papadopoulos was hired by Columbia University in 1997, where he opened his own lab. But in 2000 a biotech named GMP Genetics recruited him as its chief scientific officer. Papadopoulos wore many hats at the startup, learning translational research on the fly even as he tended to such mundane matters as laboratory floorplans and equipment procurement. After five years at the company, he'd had enough.

Following a stint as a consultant, Papadopoulos got in touch with Vogelstein to discuss how he could get back into academia: Aside from the intellectual adventure of academic research, he missed training young scientists, something he

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says he still finds to be among the most rewarding aspects of his daily work—not least due to the consistently high caliber of trainees in the Hopkins group.

"I sent Bert my proposals and he said, well, that's what we want to do too," Papadopoulos recalls. "Why don't you come here, and we can do it together."

Exomic landscapes

Papadopoulos rejoined the group just as the Ludwig Center at Johns Hopkins opened its doors in 2006. The group was then busy mapping the full spectrum of expressed genes—or exomes—in various cancers, working with a company to get the DNA sequencing done. Papadopoulos applied his industry experience to set up a next-generation sequencing facility at the Center. Over the next two years, the Ludwig Johns Hopkins team published the first maps of the breast, colon and pancreatic cancer exomes, as well as that of the brain cancer glioblastoma. The maps contained a trove of new information on the mechanisms of carcinogenesis and clues to the development of diagnostics and therapies. Subsequently, the Ludwig team would map 88 of the first 100 cancer exomes, exposing many novel oncogenes.

Papadopoulos, a key part of those efforts, hypothesized that the exomes of relatively rare cancers would reveal novel mechanisms of malignancy, and led the mapping of two of them: a type of pancreatic neuroendocrine tumor and ovarian clear cell carcinoma. He was correct. Papadopoulos and his colleagues reported in *Science* in 2010 one of the first examples of mutations to a chromatin remodeling protein—which manipulates the stuff of chromosomes to make genes available for expression—in cancer. The pancreatic tumor's exome, described in *Science* the following year, revealed mutations in three genes that are predictive of patient survival. One of the mutations also explained a known aberration in the chromosomes of these cancer cells and, it turned out, those of a brain cancer—for which it is today used as a diagnostic marker.

Fishing for cancer

A big part of what Papadopoulos and everybody else at Ludwig Johns Hopkins wanted to do was design DNA tests that could be routinely used to detect tumors early, monitor responses to cancer therapy and catch relapses swiftly. To do that, the team needed a way to find in body fluids the vanishingly rare fragments of DNA shed by tumors. "Now that we had these genomic

Photo by Flynn Larsen

landscapes of cancers, we felt we had enough information to detect tumor DNA in body fluids if we could develop technologies sensitive enough to detect the mutations," says Papadopoulos.

While he was away, Kinzler and Vogelstein had developed just such a technology, digital PCR, that permitted the capture, expansion and detection of DNA shed by colon tumors. (The process has long since been automated, and the work itself led to the development of the first home test for colon cancer.) "We kept maturing the technology to the point that we developed something we called the safe sequencing system," says Papadopoulos. Co-developed by Kinzler, Vogelstein, Papadopoulos and a former student at the Hopkins Center, Isaac Kinde, the method (SafeSeq-S) harnesses massively parallel sequencing to fish out between one and five genuinely mutated DNA sequences among 10,000 normal ones.

The team first assessed whether the system could detect ovarian and uterine tumor DNA in fluid from a Papanicolaou (Pap) smear, a routine cytology test for cervical cancer developed in the 1950s by another Greek immigrant. "We thought we don't need to add burden on patients," says Papadopoulos. "If women are willing to get a Pap smear, why not extend that test to cover some other tumor types, especially ovarian cancer,

Photo by Flynn Larsen

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where right now we have no approved screening test?"

The researchers reported in two papers in *Science Translational Medicine* in 2013 and 2014 that their methods could detect all uterine tumors and 41% of ovarian tumors in Pap smears, as well as tumor DNA shed into the blood by a variety of common cancers. A biotech named PapGene was established in Baltimore to develop liquid biopsies based on the technology. In 2019, after receiving a large infusion of additional venture funding, the company emerged with the name Thrive Earlier Detection.

Toward early detection

With the feasibility of the tests established, Papadopoulos and his colleagues began improving the method's sensitivity and accuracy with support in part from the cancer prevention initiative launched by Ludwig and the Conrad N. Hilton Foundation. The test they developed for endometrial

and ovarian cancers, PapSEEK, looks for aneuploidy—abnormal numbers of chromosomes typical to malignant cells—and mutations in 18 genes. It was evaluated in 658 cancer patients and 1,002 healthy women.

PapSEEK, they reported in *Science Translational Medicine* in 2018, was nearly 99% specific for cancer, which is essential to avoiding false positives. It also picked up 81% of endometrial cancers and 33% of ovarian cancers. When fluid was collected with a brush that extends deeper into the cervical canal, PapSEEK detected tumor DNA in 93% of endometrial cancer patients and 45% of those with ovarian cancer. Sensitivity was further boosted to 63% for ovarian cancer when both blood and pap fluid were tested.

To further develop the blood-based test for a more general cancer screening, Papadopoulos and his colleagues had to figure out ways to dramatically improve both its sensitivity and specificity—the latter being critical to avoiding potentially traumatizing false positives. To improve sensitivity, the researchers added to their DNA screen a panel of proteins that are known to be elevated in various cancers. The proteins would also help locate the source of the mutated DNA circulating in the blood, which picks up DNA from everywhere.

The test they came up with, CancerSEEK, looked for eight proteins along with 16 mutated gene sequences and was evaluated in a multi-institutional study on hundreds of controls and patients with nonmetastatic cancers of the ovary, lung, liver, pancreas, stomach, esophagus, colorectum and breast. Papadopoulos and his colleagues reported in *Science* in 2018 that the specificity of the test exceeded 99%, while its median sensitivity was 70%—ranging from almost all ovarian cancers (98%) down to a third of breast cancers. "The sensitivity of the test is not where we want it to be, but we are working on ways to increase it," Papadopoulos says. They are, for example, adding DNA probes for aneuploidy and other chromosomal aberrations associated with malignancies.

Photo by Monty Rakusen

The team has also devised a liquid biopsy for urothelial and bladder cancers based on the analysis of urine samples. They reported in *eLife* in 2018 that the test, UroSEEK, detected 75% of urothelial cancers and—when combined with cytology, an existing method of surveillance—95% of bladder cancers. All three of the tests are being developed further by Thrive, which Papadopoulos says is in a better position to put them through clinical trials. He and his colleagues, meanwhile, continue to innovate. That is, after all, something they do rather well.