

Markers of dispute



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The gold standard for early detection of prostate cancer, PSA, has recently come under fire for its high rate of false positives. **Virginia Hughes** investigates some of the researchers hunting for better alternatives and asks whether their promises of creating viable—and profitable—biomarker tests will ever be realized.

In 1987, Robert Getzenberg was beginning his doctorate at the Johns Hopkins School of Medicine urology department, the oldest in the country. Founded in 1915, the James Buchanan Brady Urological Institute's historical roots seep from its every corner. Its current location boasts a cozy library showcasing the original chair and desk of the department's founder. Exquisite medical illustrations—including the first to document prostate surgery—line the hallways. Here, under the eye of advisor Donald Coffey, a powerhouse in prostate cancer biology, the energetic young Getzenberg started hunting for distinctive molecular signatures of the disease in rat tumor tissue.

Prostate cancer biomarkers had recently become an enticing line of research. In 1986, a San Diego biotech, Hybritech, Inc., unveiled the first US Food and Drug Administration (FDA)-approved blood test for one such

marker, called prostate specific antigen (PSA)—a protein that is leaked by damaged prostate cells, including cancer cells, into the blood¹.

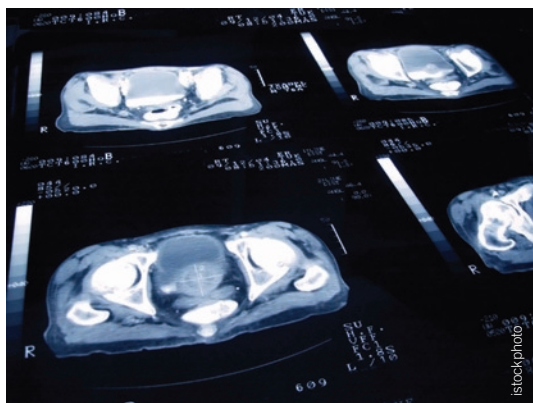
But PSA was far from perfect. As the name implies, the protein is specific to the prostate, not to prostate cancer. Early tests found that most men with common (and benign) prostate inflammation also score high for PSA. So Coffey and Getzenberg were looking for a marker with fewer false positives.

For 50 years, microbiologists have known that the nucleus of a cancer cell looks drastically different from that of a normal cell: instead of forming a smooth circle, it typically has pinches in the membrane that make it look more like a lumpy snowman or a clover. In the 1970s, Coffey discovered the nuclear matrix—the three-dimensional mesh of proteins supporting a cell's DNA—and suggested that this structure plays a part in the life cycle of the cell.

So that's where the duo began their search, in rat models of prostate cancer tissue called 'Dunning tumors'. Using gels that separate proteins on the basis of weight and charge, Coffey and Getzenberg and their colleagues found several proteins, including one called D-2, expressed in the Dunning tissue but not in controls².

In 1994, after finishing a two-year postdoctoral fellowship at Yale University, Getzenberg set up his own lab at the University of Pittsburgh. No one at Johns Hopkins had followed up on his Dunning tumor findings, so, with the blessing of his mentor Coffey, he seized upon the project anew. Within a few years, he says, he had identified D-2's counterpart in human prostate cancer tissue, called early prostate cancer antigen-2 (EPCA-2).

In 2001, Getzenberg found a biotech company in Washington, Tessera Diagnostics, to invest in his biomarker work. Their goal



Warning sign: A scan of an enlarged prostate

was to produce a superior alternative to PSA testing, which by then bragged an annual global market value of about \$300 million. (Beginning the following year, Tessera invested additional money in Getzenberg's lab for investigations of biomarkers for colon and bladder cancer.)

In 2005, the then 39-year-old Getzenberg came back to Johns Hopkins, soon taking over for Coffey, who was still working but had stepped down as director of urology research. He moved into a modern remodel of Coffey's magnificent former office, outfitted with a tank full of a dozen colorful fish and an even larger flat-screen monitor, and continued to work on development of blood assays for EPCA-2.

The research finally came to fruition in 2007, with a paper in *Urology* reporting significant new data. The study involved an analysis of blood samples stored at Johns Hopkins Hospital taken from 330 people: some who were being tested or treated for prostate cancer, some who had benign prostate conditions and some who had been diagnosed with various other cancer types. Getzenberg found elevated EPCA-2 cropped up in 94% of blood samples from men who were having surgery to remove prostate cancer³. The marker was also specific: high EPCA-2 levels appeared in just 8% of the men without prostate cancer. What's more, extremely high levels of EPCA-2 correlated with cancers that had spread beyond the prostate—a clear sign of aggressiveness.

Nevertheless, "we were pretty clear that we felt this was a proof-of-principle study, just to show that this marker could be found in the blood and that it could separate [people who have cancer from those who don't]," Getzenberg recalls from the large porch adjacent to his office.

That tempered message didn't translate to the media, however. Johns Hopkins Medicine

issued a press release, and dozens of popular news outlets picked up the story. Many of the articles quoted Getzenberg saying that large-scale clinical trials for the test could begin within nine months, putting it in doctors' offices in one to three years^{4,5}.

That didn't happen, and things soon turned sour with Getzenberg's investors. On 11 February of this year, the biotech—now named Onconome—filed a lawsuit in Baltimore City Circuit Court against Getzenberg, the University of Pittsburgh and Johns Hopkins, alleging breach of contract and scientific fraud.

On 21 July, the judge dismissed the claims against the University of Pittsburg and Getzenberg, finding that the university was not subject to jurisdiction in Baltimore and that Onconome had agreed to pursue any claims against Getzenberg in Pennsylvania.

On 25 August, a month after obtaining new evidence from laboratory notebooks from Getzenberg's lab, Onconome filed an amended complaint in Baltimore. In early September, it filed a similar case in federal court in Pittsburgh.

The new complaints state that the EPCA-2 test "was no more accurate in distinguishing cancerous tissue from normal tissue than flipping a coin" and that the company's independent scientists could not replicate Getzenberg's results. The company further alleges that Getzenberg's lab technicians did not use blinded samples and that Getzenberg frequently presented "cherry-picked and unrepresentative selections" of his data to its board of directors.

The company claims to have lost \$13 million as a consequence of Getzenberg's alleged misrepresentations. When reached by *Nature Medicine*, Onconome's founder and CEO, Raymond Cairncross, declined to comment on the lawsuit.

Likewise, Getzenberg says that institutional policy

forbids him from commenting on pending litigation. But he eagerly defends the science behind EPCA-2, which he says is being shopped by other companies for commercial licensing.

The Pittsburgh case has not yet completed discovery, and the Baltimore judge has ordered a stay on the case until all parties attempt a mediation, which was scheduled for 4 December as *Nature Medicine* went to press. Whatever happens in court, there's no denying that EPCA-2 development has been slower than the hype had predicted—a disappointment that seems to reflect a broader trend in the prostate cancer biomarker field.

"It's a bit like waiting for the messiah," says Gary Schwartz, scientific director of the Prostate Cancer Center of Excellence at Wake Forest University School of Medicine. "There's been a lot of people who claim to be it, and a lot of hype about stuff, but, in my impression, it just hasn't arrived."

"That's the natural history of biomarker research," adds Shahrokh Shariat, instructor in urologic oncology at Memorial Sloan Kettering Cancer Center in New York. "A good biomarker has to be easier to measure, cheaper, faster and better than what we already have with PSA. And each of those criteria is really hard to reach."



The Hugh H. Young library at the James Buchanan Brady Urological Institute, pictured here, houses the original desk used by the founder of the department.

Anne Gummerson

Gold standard?

The main function of the prostate gland, wedged between the bladder and rectum, is to produce fluid that mixes with sperm to form semen. Prostate cancer survival rates vary considerably. In the US alone, about 27,000 men die from the disease each year.

Autopsy studies of men who die from accidental causes suggest that many are walking around with small, benign prostate tumors that will probably never cause any trouble. By some estimates, prostate tumors can be found in about 30% of men aged 50 and older and in two thirds of men aged 60 or older⁶.

It's perhaps no surprise, then, that this year nearly 200,000 Americans will be diagnosed with prostate cancer. And that's usually thanks to a PSA test.

The FDA first approved the PSA blood test in 1986 for spotting the recurrence of prostate cancer after a prostatectomy. Soon after its initial release, clinicians began routinely using PSA for something that it had not been validated for: early cancer screening. By 1994, the FDA approved it for this purpose, as well.

In the US today, roughly half of men over the age of 50 have had a PSA test in the past two years. Every year, elevated PSA scores lead 1.8 million of them to undergo a prostate biopsy. But only 25–35% of these biopsies actually uncover tumors, many of which, as the autopsy work suggests, will never become life threatening⁷.

This 'overdiagnosis', as it's called, leaves men in quite a predicament: should they treat or remove the tumors, at the risk of unnecessary side effects—including incontinence or impotence—or forgo treatment and risk dying of cancer? The question has spurred a heated debate in the field.

According to the US National Cancer



Robert Getzenberg, pictured in his office

Keith Weller/Johns Hopkins Medicine

Institute, death rates from prostate cancer in the US fell from 39.2 to 23.6 per 100,000 men from 1992 to 2006, respectively—a boon that most urologists attribute to PSA. “It’s still the gold standard and still the test everyone falls back to,” says Alan Partin, chair of the urology department at Johns Hopkins.

“Your perspective depends on which side of the scalpel you happen to be,” counters Barnett Kramer, associate director for disease prevention at the US National Institutes of Health. “You cannot talk about the benefits of screening without talking about the harms of treatment.” In addition to medical risks, he points out that being labeled a cancer patient could limit a man’s

employment or insurance options.

This spring, after years of controversy, the *New England Journal of Medicine* published the first two large, randomized trials of PSA’s efficacy. The first, testing more than 76,000 men, found that PSA screening does not reduce mortality from prostate cancer over an 11-year follow-up⁸.

The second, studying 182,000 European men, showed a modest benefit from screening: there was an absolute reduction of about seven prostate cancer deaths per 10,000 men screened over a nine-year period. In other words, 1,410 men would need to be screened and an additional 48 would need to be treated to prevent one prostate cancer death⁹.

What’s more, lethal cancers tend to grow much more quickly than benign ones. So by the time an aggressive tumor is detected by a screening test, it’s likely to be too late for effective treatment, according to a report on breast and prostate cancer screening in the 21 October issue of the *Journal of the American Medical Association*¹⁰. Upon publication of that report, Otis Brawley, chief medical officer of the American Cancer Society, told the *New York Times* that “the advantages to screening have been exaggerated.”

Kramer worries that, with regard to the surge of new, alternative prostate cancer biomarkers, history is likely to repeat itself.

“The dissemination of PSA was premature, and there’s that same danger for any new biomarker that comes along,” says Kramer.

Prostate cancer biomarker candidates

Biomarker	Blood/Serum	Urine	Expressed Prostatic Secretions/Semen	Tissue
PSA, PSA Isoforms	+			
PSCA	+			+
AMACR	+	+	+	+
GSTP-1 Methylation		+	+	+
PCA3/uPM3		+	+	+
EPCA	+			+
HK2	+			
Autoantibody signatures	+			
Hepsin				+

(*J. Urol.* **178**, 2252–2259; 2007)

“In any given month you’ll see another report of another marker. But none of them come from strong enough study design that they’re ready for prime-time.” Many experts say that EPCA-2, for instance, doesn’t live up to the hype it’s generated.

As soon as he read the 2007 paper in *Urology*, biochemist Eleftherios Diamandis says that he “was 100% sure that the assay would have never worked for measuring any protein, let alone the EPCA-2.”

He argues that Getzenberg’s team, according to their published methods, used 100,000 times too much serum to coat the standard assay plate. This made it impossible to for minute quantities of specific proteins, such as EPCA-2, to bind the plate. “What they thought was binding to the plate would have never bound because of other proteins competing for [the space],” says Diamandis, whose lab at the University of Toronto is

also searching for biomarkers of aggressive cancers.

Diamandis spelled out these objections in a letter that he submitted to the journal *Clinical Biochemistry*, which published it in December 2007¹¹. “I’m totally convinced that my critique is valid,” he says.

“People say these aren’t the greatest assays,” Getzenberg says. But “we’re not assay development people; we are biomarker discovery people.” His lab’s job is to identify the biomarkers detectable in the blood of key patient groups, he says. After that, they “hand them off” to a company to develop more rigorous assays for the clinic.

“You tell me: how could somebody discover a biomarker without measuring it?” Diamandis says, incredulously. When asked about the merit of the lawsuit, he says, “I don’t think it’s fraud; I think it’s incompetence.”

Diamandis adds that over the past few years, many companies have invested in biomarker research, and many have come away disappointed.

On 14 September, Onconome submitted to the Baltimore court system a partial transcript from a videotaped deposition from one of Getzenberg’s former senior lab members. Eddy Leman, who had run the EPCA-2 assay on the samples published in the 2007 *Urology* paper, admitted that he was the only person in Getzenberg’s lab to ever get the EPCA-2 assay to work, and that it only worked for him once. He also acknowledged that he forgot to document that particular run in his lab binder.

That’s contrary to the published study, which states that the researchers ran the assay three times on a pilot data set of 30 samples and that the variation across the three runs did not exceed 10%.

Prostate clues in the genome

Genetic association studies, involving tens of thousands of men with prostate cancer, have identified about 30 gene variants that are mildly associated with the disease (*Nat. Genet.* **41**, 1116–1121; 2009).

But whereas these discoveries may eventually help point to biochemical pathways underlying cancer, they don’t do much for predicting risk. That’s because most of the variants are common, present in at least 5% of the population. Carrying one only slightly increases an individual’s risk.

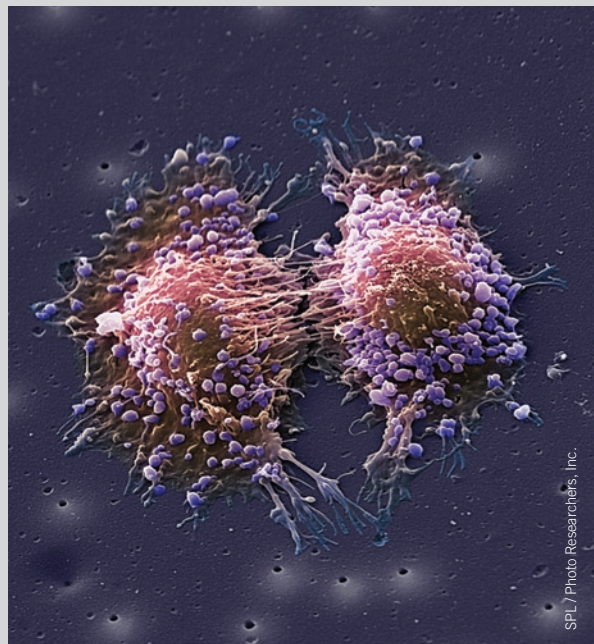
In 2005, Arul Chinnaiyan found a genetic signature that could be more useful. Analyzing genes that are overexpressed in prostate cancer tissue, he found that more than half of tumors harbor an abnormal chromosomal fusion between the *TMPRSS2* gene and that encoding a specific transcription factor, ERG (*Science* **310**, 644–648; 2005).

“It’s our belief that certainly this fusion is an initiating event in prostate cancer,” says Chinnaiyan, director of the University of Michigan’s Center for Translational Pathology. “Our goal is to develop it into some sort of relatively economical screening test.”

But the fusion marker and the variants pinpointed in the genetic association studies suffer from one of the same problems as PSA: they reveal men who have, or are likely to develop, any kind of prostate cancer, rather than only the aggressive forms.

At Johns Hopkins, Bill Isaacs’ group is now screening men who have aggressive tumors and comparing their genetic makeup to men who have nonthreatening cancers. He says he’s found the first common risk variant that confers risk of the aggressive kind but has not yet published the data.

Meanwhile, in October, engineers and biochemists from the University of Toronto unveiled a crude prototype of a \$10, handheld silicon chip device that screens urine samples



Deadly division: Two prostate cancer cells

for *TMPRSS2* fusions in 30 to 60 minutes (*ACS Nano*. **3**, 3207–3213; 2009). The device could be adapted to screen for multiple genetic or protein biomarkers, says lead investigator Ted Sargent. “The entire premise of our approach is that there will not be a silver bullet.”

Virginia Hughes, New York

“The fact that the assay only worked once, out of years of work, and did not work countless other times is also proof that the assay was always and ever after a sham,” the complaint states.

Getzenberg says that, like most principal investigators, he doesn't work directly on experiments in his lab, and he himself has never run an EPCA-2 assay. When asked how many times the EPCA-2 assay worked for his lab technicians, he said, “it's hard to put a number on that, because there's times where we had six to nine months where it would run every day. And then you'd change reagents or something and then we'd have to calibrate things.”

Although the search is unquestionably painstaking, Diamandis says he's optimistic about finding a viable PSA alternative. “Otherwise I would have closed my lab.”

Biomarker cocktails

Just about everybody agrees that the future of prostate cancer screening will include panels of genetic, blood and tissue markers.

Identifying which ones and which combinations are the best predictors is one of the major goals of the Early Detection Research Network, sponsored by the US National Cancer Institute. “The conventional way of looking at things was trying to identify just a single biomarker, but I think the future really will be panels of markers,” says one of the project's principal investigators, Arul Chinnaiyan, director of the University of Michigan's Center for Translational Pathology.

Reflecting the difficulty of biomarker development, the prostate cancer-specific blood test that's furthest along, PCA-3, was

discovered almost two decades ago, also in the Johns Hopkins urology department.

Just as Getzenberg and Coffey were discovering the D-2 rat protein, another Hopkins scientist, Bill Isaacs, was looking for the genetic underpinnings of tumors.

At the time, Johns Hopkins was one of the only places doing radical prostatectomies as a treatment for prostate cancer, giving Hopkins researchers unprecedented access to tumor tissue. (Today, in fact, about a dozen of freezers in the Institute hold prostate or seminal vesicle samples from 9,000 men, according to Isaacs.)

Back in 1992, one of Isaacs's post-docs, Marion Bussemakers, compared the RNA sequences of tumor tissue versus normal tissue.

“Sure enough, there was this one major band that was highly expressed in tumor specimens, and you could barely detect it in normals,” Isaacs recalls.

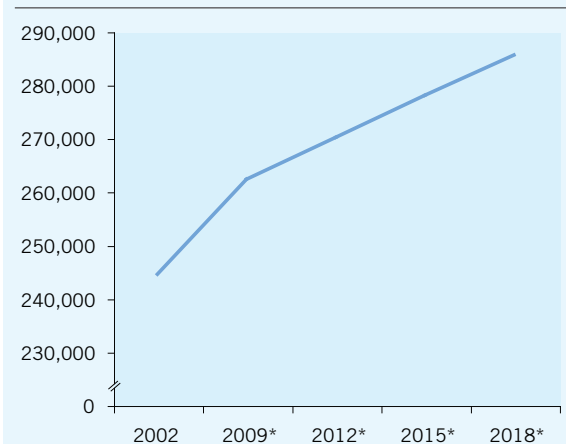
PCA-3 could be a useful secondary test for men who have high PSA scores but negative biopsies, “where you want to have an extra level of confidence that the man does not have cancer,” Isaacs says. PCA-3 is not unproblematic; it's not clear whether a patient would need a prostate massage to expel enough prostate fluid into the urine. Still, the test is commonly used in Europe as a secondary screen. A 500-subject clinical trial to assess the test's efficacy is now underway at ten US institutions, including Johns Hopkins.

Last year, Shariat, of Memorial Sloan Kettering, identified nine blood markers that, when assessed together using a specific algorithm, predict the likelihood that a man who's had prostate cancer removed will have a recurring disease. The biomarkers, related to various biological functions, including inflammation, strength of the immune system and cancer cell growth, provided a prediction that looks 15% more accurate than estimates based on standard clinical risk factors¹².

Ideally, when merged with family history and other standard measures, this kind of statistical model could be part of a patient's medical record and inform the discussions they have with their doctor, Shariat says.

Now, in the second year of a five-year clinical trial, Shariat has tested his panel in

Estimated new cases of prostate cancer in the United States, 2002–2018



Datamonitor/Pipeline Insight: Prostate Cancer, published 14 August 2009; * = forecast

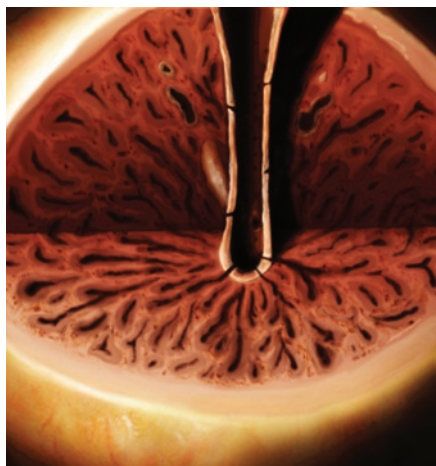
more than 1,000 subjects at four independent institutions. Seven of the nine markers are holding up in all populations, he says.

Shariat is very cautious about hyping his data, citing the past disappointments of other biomarkers. “All of these biomarkers need to be validated, just like drugs are, using a structured, phased approach,” he says. “They cannot yet be used for clinical decision making.”

As for Getzenberg, he says he has preliminary results from a ‘sandwich assay’ of EPCA-2—a more sophisticated type of test that uses two antibodies to catch the same protein—that identifies aggressive cancers. He plans to publish that data in the next six months. Is his test leading the biomarker pack? “It's just too early to tell. My feeling is that a lot still has to be done.”

Virginia Hughes is a freelance journalist based in Brooklyn, New York.

1. Stamey, T.A. *et al. N. Engl. J. Med.* **317**, 909–916 (1987).
2. Getzenberg, R.H. *et al. Cancer Res.* **51**, 6514–6520 (1991).
3. Leman, E.S. *et al. Urology* **69**, 714–720 (2007).
4. Brown, D. *The Washington Post* 26 April 2007, A3.
5. Underwood, A. *Newsweek* <<http://www.newsweek.com/id/35171>> (26 April 2007).
6. Kramer, B.S. *et al. Ann. Intern. Med.* **119**, 914–923 (1993).
7. Smith, D.S. *et al. Cancer* **80**, 1852–1856 (1997).
8. Andriole, G.L. *et al. N. Engl. J. Med.* **360**, 1310–1319 (2009).
9. Schröder, F.H. *et al. N. Engl. J. Med.* **360**, 1320–1328 (2009).
10. Esserman, L. *et al. J. Am. Med. Assoc.* **302**, 1685–1692 (2009).
11. Diamandis, E.P. *Clin. Biochem.* **40**, 1437–1439 (2007).
12. Shariat, S.F. *et al. Clin. Cancer Res.* **14**, 3785–3791 (2008).



Where trouble begins: The prostate gland sends out signals of cancer