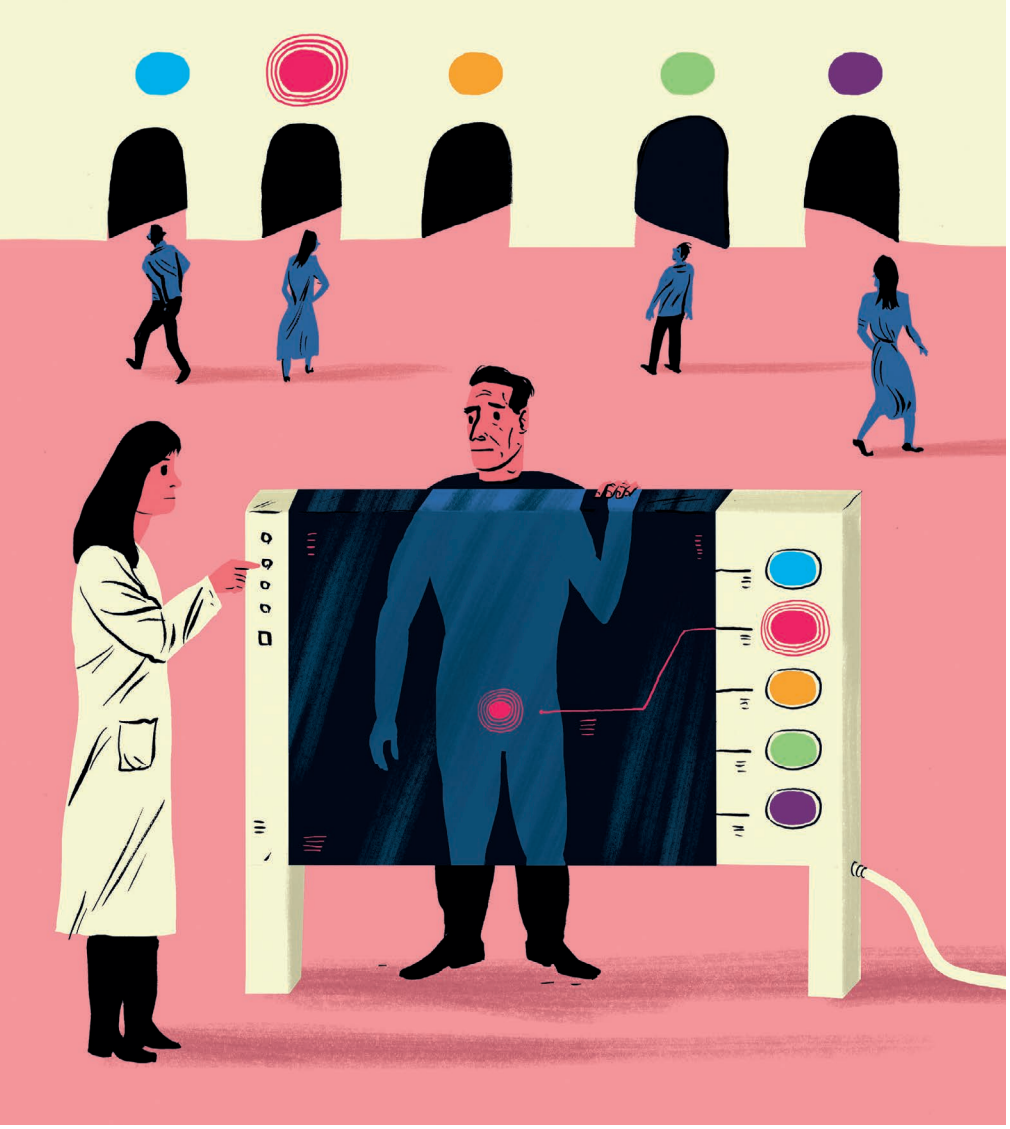

PAUL BLOW

GENETICS

A clearer view

Researchers delving into the details of bladder cancer are finding a rich trove of genetic information.

BY JEANNE ERDMANN

Inspiration can strike at any time. For Dan Theodorescu, it came during his half-hour commute home from the University of Virginia School of Medicine in Charlottesville, where he divided his time between surgery to remove cancerous bladders and drug-discovery research. It was 2009, and he was mulling over a genetic conundrum in bladder cancer. He wanted to stop a mutated gene called *RAS* from giving rise to a signalling protein involved in tumour growth. Many researchers had tried and failed to target this wayward gene. While pondering what to do next, a famous quote came to mind: "Insanity is doing the same thing over and over again and expecting different results." So he decided to change tack.

Rather than block *RAS* directly, Theodorescu wondered if he could inhibit a vital protein instead. In particular, he wanted to handcuff a downstream henchman of the *RAS* gene known as Ral. Signalling proteins such as Ral usually shift between active and inactive states. Given the difficulty of drugging Ras, he thought, might it be possible to lock Ral into its inactive state?

Theodorescu's team discovered that, in its inactive form, the Ral protein is 'open', exposing a pocket. After five years, the researchers found a small molecule dubbed BQU57 that can wedge itself into the pocket to prevent Ral from closing and becoming active — rather like using a stick to prop open the mouth of an alligator to stop it biting. Now BQU57 has been licensed for further development by NantBioScience, a subsidiary of biotech company NantWorks, based in Culver City, California.

Inspired by this breakthrough, Theodorescu gave up patient care to devote more time to his research. There are now many more genetic conundrums to investigate. Researchers have a growing treasure trove of genetic data on bladder cancer, some of which threaten to overturn a cherished idea about one of the supposed causes of bladder cancer. Genetics has also allowed bladder cancer to be reclassified from two categories into five distinct subtypes, each with different characteristics and weak spots. All these advances bode well for drug development and for improved diagnosis and prognosis.

PREDICTING PROGRESSION

Among the groups studying the genetics of bladder cancer are two large international teams: Uromol (named for urology and molecular biology), which is based at Aarhus University Hospital in Denmark, and The Cancer Genome Atlas (TCGA), based at institutions in Texas and Boston. Each team tackled a different type of cancer, based on the traditional classification of whether or not a tumour has grown into the muscle wall of the bladder. Uromol worked on the more common, earlier form, non-muscle-invasive bladder cancer, whereas TCGA is looking at muscle-invasive bladder cancer, which has a lower survival rate (see page S34).

The Uromol team sought to identify people whose non-invasive tumours might return after treatment, becoming invasive or even metastatic. Bladder cancer has a high risk of recurrence, so people whose non-invasive cancer has been treated need to be monitored for many years, undergoing cystoscopy every few months — an invasive and expensive procedure (see page S48). Finding genetic features that could predict which tumours will progress would allow doctors to focus the most intensive treatment and surveillance on the most high-risk patients, explains Lars Dyrskjøt, a molecular biologist at Aarhus University Hospital and lead author on the Uromol study.

The Uromol team looked for predictive genetic footprints in the transcriptome of the cancer, which contains all of a cell's RNA and can tell researchers which genes are turned on or off (only active genes are transcribed into RNA). The team analysed[1] 460 tissue samples from people with bladder cancer provided by 10 institutions across Europe, and they included 16 invasive bladder-cancer tumours for comparison. They found three subgroups with distinct basal and luminal features, as proposed by other groups, each with different clinical outcomes in early-stage bladder cancer. These features sort bladder cancer into genetic categories that can help predict whether the cancer will return.

The researchers also identified mutations that are linked to tumour progression[1]. One type in particular seemed to correlate with the poorest outcome: mutations in the so-called

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APOBEC genes, which code for enzymes that modify RNA or DNA molecules. The APOBEC enzymes help to destroy viruses, but mutated forms of APOBEC genes can go rogue and derange multiple genes. This effect could lead to cancer and cause it to be aggressive.

In work published this year[2], the Uromol results were used to create and validate a risk-assessment score based on a panel of 12 genes. More than 1,200 people with non-invasive bladder cancer were followed for four years. The researchers found that the 12-gene score was better than existing tools at predicting which tumours would progress to the muscle-invasive form. "The next step would be to perform a clinical intervention study where we change surveillance and treatment of patients based on our molecular methods," says Dyrskjøt. The team is seeking funding to perform the study.

SORTED INTO SUBTYPES

The second major research group, TCGA, is a large US effort, led by the National Cancer Institute and the National Human Genome Research Institute, that involves thousands of researchers across the country. The project has already mapped genomic changes in 33 cancer types, including breast, skin and lung cancers. The TCGA researchers, who study muscle-invasive bladder cancer, have looked at tumours that were already identified as fast-growing and invasive. In 2014, the team published[3] the first results from 131 tumours, which helped to refine the two major subgroups recognized at the time for muscle-invasive bladder cancers: the luminal and basal–squamous subgroups. The second set of TCGA data[4], from October this year, included an additional 281 tumours, bringing the total number to 412.

The work by Uromol, TCGA and other labs has provided a clearer view of the genetic landscape of early- and late-stage bladder cancer. There are five subtypes for the muscle-invasive form: luminal, luminal–papillary, luminal–infiltrated, basal–squamous, and neuronal, each of which is genetically distinct and might require different therapeutic approaches. "We think basal–squamous and luminal–infiltrated are more likely to respond to immune checkpoint therapies," says David Kwiatkowski, who studies cancer genetics at Brigham and Women's Hospital in Boston, Massachusetts, and is an author on the TCGA study[3].

These categories can help researchers to understand bladder cancer. For example, the basal–squamous type is more common in women than in men. Basal–squamous and the three luminal subtypes mirror the molecular features of breast cancers with the same names. And the neuronal subtype, which shares genetic features with rare neuroendocrine cancers, has the poorest survival rate .

Bladder cancer has the third-highest mutation rate of any cancer, behind only lung cancer and melanoma. Mutations in those cancers are largely caused by external carcinogens: tobacco smoke and sunlight, respectively. The TCGA team has confirmed Uromol research showing that most bladder-cancer mutations occur in the APOBEC genes. It is not yet clear why APOBEC mutations are so common in bladder cancer, but studies of the mutations have yielded one startling implication. "The APOBEC enzyme causes mutations early during the development of bladder cancer, and independent of cigarette smoke or other known exposures," Kwiatkowski says. "This is pretty amazing." He adds that TCGA's findings threaten to overturn the idea that smoking is a cause of bladder cancer.

Dan Theodorescu (left) and colleagues see a wealth of genetic data that can be used to tackle cancer.

"We are putting this out as back-of-the-napkin thinking about the future."

The surprises do not end there. The TCGA researchers found[4] a subset of bladder-cancer patients — those with the greatest number of APOBEC mutations — who had an extremely high five-year survival rate of about 75%. Other patients with fewer APOBEC mutations fared less well, Kwiatkowski says. This seems to contradict the earlier Uromol findings — a discrepancy that will need further investigation. But the TCGA data[4] are still in the early stages of analysis. "We are putting this out as back-of-the-napkin thinking about the future, and how these subtypes might need to be incorporated into current clinical-trials testing," says Seth Lerner, a urologist at Baylor College of Medicine in Houston, Texas, and a TCGA author.

MAPPING VULNERABILITY

The TCGA data are now available for wider investigation. "Other researchers will continue to analyse the data statistically in different ways for years to come," says John N. Weinstein, a bioinformatics specialist at the MD Anderson Cancer Center in Houston and another TCGA author. Theodorescu is also excited about the possibilities. "For people like myself, who do hypothesis-driven research, it's like an encyclopaedia," he says.

This detailed knowledge of bladder-cancer genetics may help to pinpoint the specific vulnerabilities of cancer cells in different people. "There's this strange paradox that cancer cells seem so robust in the patient as they grow and take over," says Jesse Boehm, associate director of the cancer programme at the Broad Institute in Boston. But the path to cancer brings along genetic baggage that can be exploited, he adds. Over the past decade, Broad Institute researchers have identified[5] more than 760 genes that cancer needs to grow and survive. Their genetic map might take ten years to finish, but it will list every genetic vulnerability that can be exploited. "The goal of cancer precision medicine is to take the patient's tumour and decode the genetics, so the clinician can make a decision based on that information," says Boehm.

Theodorescu often thinks about his decision to abandon surgery for research. "It's a different kind of satisfaction," he says. "There's the satisfaction of helping the human in front of you, seeing the sparkle in their eyes when you tell them you may be able to cure them, versus seeing an experiment that cures cancer in mice." But he knows that those cured mice could be just the first step on a much longer, but ultimately more rewarding, journey. ■

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