Before deciding to light your first cigarette, imagine knowing your exact chance that this will lead to cancer. Although smoking is the main cause of lung cancer, only 10%–20% of smokers and former smokers actually develop the disease. The reasons for this — and for the changes that lead to many cancers — have eluded researchers for decades.

Lung cancer, as the biggest cause of cancer death worldwide, is a priority of prevention strategies. And to make the biggest impact in cancer prevention, it is vital to target those individuals with the highest risk of developing cancer. This is where biomarkers come in. Individuals identified as being high risk using biomarkers could receive counselling for lifestyle changes (see Breaking the cancer habit, page S16), or they might be eligible for chemoprevention (see First line of defence, page S5). Even before that point, using biomarkers to select people for cancer prevention studies would allow for more powerful trials (see Designing smarter cancer prevention trials, page S20). Even before that point, using biomarkers to select people for cancer prevention studies would allow for more powerful trials (see Designing smarter cancer prevention trials, page S20). Even before that point, using biomarkers to select people for cancer prevention studies would allow for more powerful trials (see Designing smarter cancer prevention trials, page S20). Even before that point, using biomarkers to select people for cancer prevention studies would allow for more powerful trials (see Designing smarter cancer prevention trials, page S20).

The process of carcinogenesis takes years, if not decades. The search is on to discover and validate the often-subtle, microscopic changes in the constituents of the blood, sputum, urine or tissue samples that herald cancer. Prior to activated oncogenic pathways, there is the possibility of identifying sluggish DNA repair mechanisms, changes in gene expression, or detecting the low-level immune response to the presence of a nascent tumour.

This research activity is not without its controversies. There have been numerous false starts, where promising biomarkers did not stand up to rigorous testing. To be useful, a biomarker needs to have sensitivity — that is, the likelihood that it detects disease — of at least 90%. The other key quality is specificity — the probability that a positive signal is a true sign of disease and not an error. That, too, should be 90% or more for a biomarker to be of clinical value. Although no lung cancer biomarkers yet meet that 90/90 standard, there are several promising candidates.

### CHANGING EXPRESSION

Avrum Spira, a pulmonologist at Boston University, has been using bronchoscopy to brush cells from the bronchial airways of healthy smokers and non-smokers, followed by gene expression profiling to compare thousands of genes. He has found that the cells lining smokers’ airways show signs of genomic changes related to inflammation and cell proliferation, even when they appear normal with standard bronchoscopy. By comparing these results to those of smokers with suspected lung cancer, Spira’s group has identified an 80-gene signature that could identify patients with early-stage lung cancer with about 90% sensitivity. This signature, says Spira, “is the proverbial canary in the coalmine”.

However, bronchoscopy is an invasive procedure, so Spira broadened his search to more accessible parts of the airway. He recently found abnormal gene expression in epithelial cells of the nose and mouth that resemble those in the bronchial airway. Analyzing these cells using a simple swab could serve “as a mass screening tool in population-based studies,” he predicts.

Digging into the pathways underlying these precancerous changes, Spira, together with genomicist Andrea Bild from the University of Utah, found phosphatidylinositol 3-kinase (PI3K) signalling pathway, already known to be involved in the development of cancer. By comparing PI3K activity levels in cells from apparently healthy smokers to those of smokers with mild-to-moderate abnormalities, they found that this pathway “might be activated before tumorigenesis,” says Spira, making PI3K a prime biomarker candidate.
In April 2010, an international team of researchers from academia and drug company GlaxoSmithKline reported that dutasteride, a drug already approved for the treatment of benign prostatic hyperplasia, reduced the chances that men considered at high risk for prostate cancer would develop the disease. The four-year trial included more than 8,100 men and met the gold standard for clinical trials: it was randomized, double-blind, and placebo-controlled; it studied parallel groups at multiple medical centres; and it assessed outcomes with biopsies at two years and four years. In the end, men who took dutasteride were 23% less likely to have a positive biopsy for cancer than those on the placebo. GSK submitted this data in its application to the US Food and Drug Administration to market the drug for prostate cancer prevention — this January, the FDA said No. Although it is not unusual for the FDA to reject a drug application supported by apparently positive data, this case illustrates the particular challenges surrounding clinical trials for cancer prevention. When the aim is to decrease the incidence of cancer in large populations, studies on preventive agents require large patient cohorts — sometimes approaching 20,000 participants — and take years or even decades to perform. This combination makes them especially unwieldy compared to tests with therapeutic compounds, which can much more quickly be seen to work, or not, by testing them exclusively in people who have the disease. In cancer prevention drug trials, the usual gold standard barely rates a bronze.

Since preventives are intended for apparently healthy patients, trials require a high confidence that the anticipated anticancer benefit will outweigh any harmful side effects. In the dutasteride trial, statistical analysis showed that the decrease in cancer was driven mainly by a reduction in less serious tumours that might not even require treatment. In addition, men who took the drug were slightly more likely than those on a placebo to develop more aggressive tumours. The FDA’s expert advisory panel concluded that the prevention benefits failed to outweigh this risk. Researchers say two things are needed to decrease the length and size of prevention studies. One is

The PI3K pathway might also be used for chemoprevention. Early trials have shown that the administration of a compound that decreases PI3K activity causes regression of abnormal lesions in the airways of high-risk smokers1.

DNA DAMAGE

As part of daily living, DNA frequently sustains damage. If not repaired, this can lead to mutations that replicate, resulting in abnormal and then cancerous growths. Certain mechanisms usually prevent this from occurring. The enzyme 8-oxoguanine DNA glycosylase (OGG1) repairs DNA by excising damaged bases (see DNA repair duties, page S21). Biochemists Zvi Livneh and Tamar Paz-Elizur, at the Weizmann Institute in Rehovot, Israel, discovered that levels of OGG1 can also be used to predict an individual’s risk of developing lung cancer.

By measuring OGG1 concentration in blood samples, Livneh and Paz-Elizur found that 40% of people with lung cancer had low levels of the enzyme compared to 4% of healthy individuals. Smokers with low OGG1 activity were 5 to 10 times more likely to develop lung cancer than smokers with normal OGG1; when compared to non-smokers with normal OGG1 activity, the risk skyrocketed to 120 times more likely. The same blood test could be broadened to other cancers. For example, smokers with lower OGG1 activity are 70 times more likely to develop head and neck cancer than non-smokers with normal enzyme activity1.

OGG1 is only one of an unknown number of DNA repair enzymes; low levels of any of them could be associated with cancer development. Livneh and Paz-Elizur have expanded their research to include two additional DNA repair enzymes — AAG and APE1 — to cover people with “different risk factors to develop a certain cancer”, says Livneh. A study is underway to access their performance and results are expected in mid-2011.

It is unlikely that any single test, however many markers included, will be sufficient to gauge the risk of cancer development. “We have an additional ongoing study which explores a two-stage protocol for lung cancer prevention,” says Livneh. The first stage involves Livneh and Paz-Elizur’s DNA repair biomarkers plus five biomarkers developed by other groups. These biomarkers measure: alteration in gene expression; levels of DAP kinase, an enzyme involved in programmed cell death; antibodies to mutant p53, a sign that a cell’s tumour suppressor system is damaged; markers of inflammation; and variations in cancer-related genes. “Together these biomarkers are expected to yield a better risk assessment than one type alone”, says Livneh. Individuals identified as high risk will be tested using spiral computed tomography (CT). “For such a high-risk group, spiral CT early detection of lung cancer might be cost-effective and life saving,” adds Livneh.

In the initial stages of cancer, the body is often able to recognize abnormal cell changes and raise a response, producing auto-antibodies. However, this response is limited, and in the later stages of cancer, the immune system becomes compromised and can no longer identify and attack cancer cells. Auto-antibodies are therefore prime candidates for biomarkers of early stage cancer.

By examining auto-antibody formation in presymptomatic individuals who later went on to develop lung cancer, Samir Hanash, at the Fred Hutchinson Cancer Center in Seattle, Washington, has identified three important antigens — annexin-1, 14-3-3 Theta and LAMR-1 — regarded by the immune system as foreign2. So far, specificity of these biomarkers is high but sensitivity lingers around 60%. The challenge for Hanash is to find additional candidate antigens that improve on the performance of this 3-antigen panel.

These figures might be improved by looking for even earlier signs of cancer. Through the Women’s Health Initiative and Physician’s Health Study, Hanash has access to blood samples that were collected up to eight years before a patient was diagnosed with lung cancer. In addition, he is searching for biomarkers of lung cancer in former smokers and in people who never smoked. “It turns out that most of the blood markers we have identified among smokers are also applicable to non-smokers,” says Hanash.

In spite of major investment in biomarker development over the past 15 years, the field of cancer prevention biomarkers looks woefully thin. One of the main reasons, according
to identify high-risk populations to be the preferred subjects for the trials. The second is surrogate endpoints that can provide evidence of whether a preventive drug is working — and do this in just a few years, rather than decades. The key to both is finding better biomarkers — the genes, proteins, and cellular metabolites that can be measured and associated with the development of cancer.

Patterns of these biomarkers that can be uniquely linked with one type of cancer can make it easier to estimate an individual’s cancer risk. Selecting highest-risk patients for studies increases the statistical power of trials with a smaller number of participants. As a second benefit, high-risk cohorts can also shorten trials. If epidemiological studies show, for example, that a known percentage of patients carrying a certain gene will develop cancer within five years, researchers can restrict a prevention trial to those patients and run it for just that duration. Moreover, patients and regulators are likely to be more tolerant of side effects if the targeted users have a high chance of developing cancer without intervention.

The designers of the dutasteride trial did select participants judged to be at higher risk of developing prostate cancer. However, they did so by looking for elevated levels of prostate-specific antigen (PSA) — a protein whose utility as a biomarker for prostate cancer is a matter of debate. If a fully validated biomarker for prostate cancer had existed, GSK might have been able to design a dutasteride trial that required fewer participants and could have yielded a more definitive outcome. In particular, looking at the drug’s effect (or lack thereof) on the biomarker may have clarified whether the increase in detected higher-grade cancers was due to the drug or simply an artefact of the tumours becoming more easily detected owing to dutasteride’s shrinking of the prostate.

Some biomarkers may even function as the surrogate endpoints needed to shorten prevention trials. If, say, a specific group of proteins reliably increases in the blood of patients during the earliest, precancerous stages of disease, doctors could monitor those proteins rather than relying on biopsies to detect malignancy. Molecular biomarkers of potential toxicity, such as the activity of drug-metabolizing enzymes, could also help researchers monitor subjects’ safety and response to drug candidates in clinical trials.

Scott Lippman, an oncologist and cancer prevention researcher at the University of Texas MD Anderson Cancer Center, has proposed fully integrating biomarkers chemoprevention development. After evaluating biomarkers in animal models, researchers would do epidemiologic studies linking the biomarkers to human cancers. They would next model the likelihood that patients with specific biomarkers will develop cancer. Then, in a ‘phase 0’ step between preclinical and phase I clinical trials, researchers could test sub-therapeutic doses to assess a drug’s behaviour in healthy patients without risking harm. Lippman argues that this approach could yield better decisions on whether to undertake a lengthy, and costly phase III trial — and speed the development of preventive agents. Indeed, the fact that GlaxoSmithKline skipped some of these steps might have played a role in the FDA’s decision on dutasteride. The drug inhibits the enzyme that converts testosterone to the more potent 5α-dihydrotestosterone. But neither molecule is yet a validated biomarker for prostate cancer.

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