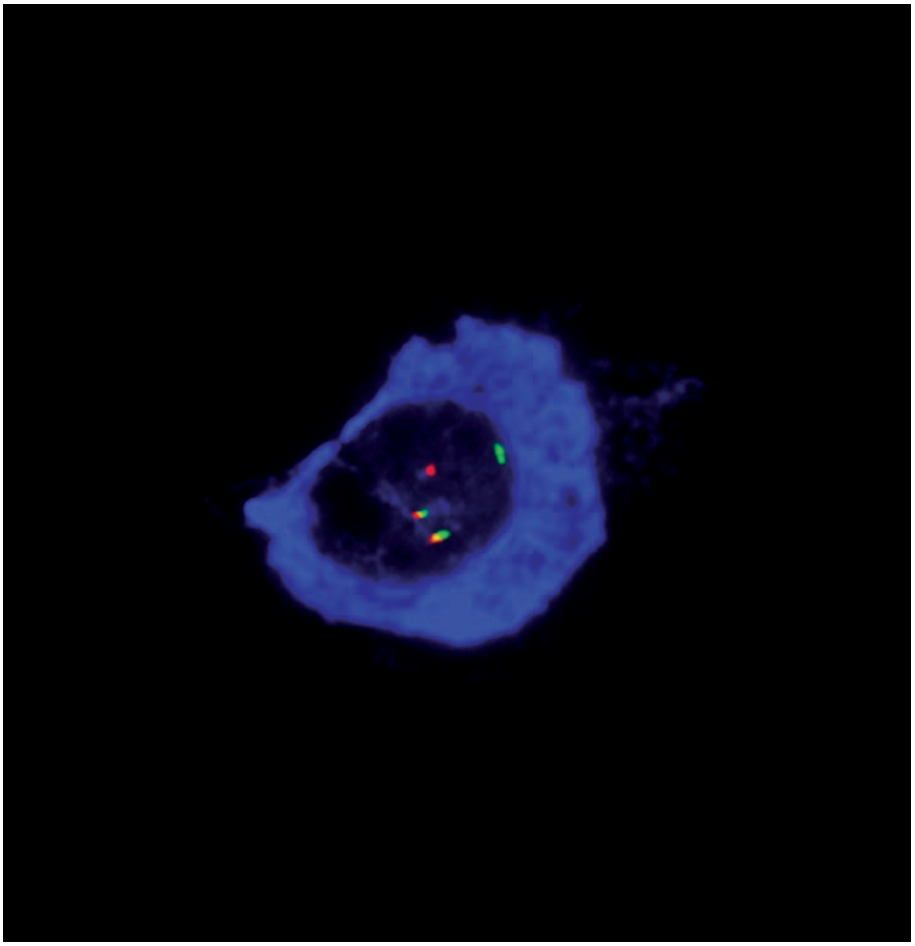


A common aberration is a switching of parts chromosomes 14 and 11 (shown here in red and green)

RAFAEL FONSECA

GENETICS

Profiling a shape-shifter

Unlocking the genetic secrets of multiple myeloma could reveal new ways to attack this killer disease.

BY COURTNEY HUMPHRIES

In the chain of events leading to multiple myeloma, two factors seem to dictate the disease process: the internal genetic alterations within cancerous plasma cells, and their external environment in the bone marrow. Nikhil Munshi, who studies myeloma at the Dana-Farber Cancer Institute in Boston, Massachusetts, says that although the cells' environment is important, "most of the tumour behaviour is predetermined by the genetic make-up". Multiple myeloma is more prevalent in African Americans, for example, and sometimes seems to be inherited in families, but even so, most of its genetic aberrations arise independently.

Understanding the nature of these genetic abnormalities has been the focus of decades of research. Tools that characterize the cancer cells at the molecular level have been useful for classifying patients, determining the severity of the disease, and guiding treatment strategies.

But multiple myeloma poses a daunting challenge for scientists. It takes many forms, and patients who have the disease can experience very different symptoms, disease courses and responses to treatments. Indeed, the variability extends to the tumours themselves. Genomics has led to fresh discoveries — and more complexity. "The more sophisticated the genomic technology becomes, the more heterogeneity we've identified," says Kenneth Anderson, head of haematologic neoplasias at the Dana-Farber Cancer Institute in Boston, Massachusetts.

LOST IN TRANSLATION

Multiple myeloma stands apart from other cancers in always being preceded by an identifiable premalignant state, called 'monoclonal gammopathy of undetermined significance' (MGUS). This window into the early stages of the disease provides an opportunity for scientists to study patients who are at risk of developing myeloma and to tease apart the genetic differences between the benign condition and the disease. These studies, along with research on patients at different stages of disease, have shown that myeloma unfolds in a stepwise progression as the genetic abnormalities accumulate.

In myeloma, antibody-producing B cells often acquire extra copies of chromosomes and genetic translocations across chromosomes. Most of the translocations involve chromosome 14, at the immunoglobulin switch region, which normally allows B cells to control which antibody, or immunoglobulin, they produce. As a result, immunoglobulin production is increased.

Leif Bergsagel, a haematological oncologist at the Mayo Clinic in Scottsdale, Arizona, explains that these early 'primary events' all activate cyclin-dependent kinases, which control the cell cycle. The aberrant activation of these kinases, he says, is "a unifying event in the pathogenesis of the disease". Further genetic abnormalities unfold as the disease progresses, including mutations that activate the *RAS* oncogene, switch on the transcription factor NF-κB, dysregulate the transcription factor Myc, or inactivate the tumour-suppressor protein p53. One of the most notable characteristics of these mutations is that none of them are common to all patients — or even most patients. This variation has made it possible to stratify patients into at least six different disease subgroups, and to separate them according to low, high or intermediate risk. More recently, gene expression profiling has also been used to identify high-risk patients, who constitute about 15% of newly diagnosed patients.

Getting a clear picture of the disease's various forms should yield better ways to classify patients, choose treatments and develop drugs. "There's a growing conviction in the cancer community that the path to better therapies for multiple myeloma is to identify the root causes of the disease," however multifactorial they are, says Todd Golub, a genomic-medicine specialist at Dana-Farber and director of the cancer programme at the Broad Institute in Cambridge, Massachusetts. "Historically, we've been doing that in a very *ad hoc* fashion."

IN SEQUENCE

Golub is one of the leaders of an ambitious effort to sequence the myeloma genome. In a paper published in *Nature* in March 2011, Golub and a consortium of researchers published the most

complete genomic analysis of myeloma to date[1]. They reported sequencing 38 cancer genomes — 23 by whole-genome sequencing and 16 by whole-exome sequencing, with one patient analysed by both. One surprising discovery was that 42% of patients had mutations in genes involved in RNA processing or protein translation. This finding suggests that flaws in protein regulation may play a major role in the disease. Golub says this is a fundamentally new idea that "was not on anyone's radar".

The analysis also confirmed previous findings, including those concerning activation of NF-κB. "Now we have a much richer view of what's going on," says Golub. Sequencing identified mutations in 11 different components of the NF-κB pathway, showing that several different mutations can lead to the gene's inactivation, and that an assortment of different factors can have the same effect.

This is a theme that Golub thinks could be important in making sense of the disease's diversity. "Individually, these mutations are rare," he says, "but collectively, as a set of mutations and a set of genes that activate a particular pathway, they're quite common." Essentially, he says, many diverse mutations funnel into a few common pathways. It may be that targeting these pathways could be a useful therapeutic strategy, rather than trying to home in on each individual mutation.

The researchers also made one discovery that could have a more immediate clinical benefit. One patient harboured a BRAF kinase mutation that was previously unseen in myeloma but known to be involved in other cancers, especially melanoma. Sequencing a further 161 patients showed that 7 had BRAF mutations. The findings indicate that a small number of myeloma patients might benefit from the BRAF inhibitors already developed to treat melanoma.

The Multiple Myeloma Research Foundation (MMRF) in Norwalk, Connecticut, which funded Golub's study, is now leading an effort to profile more patients for BRAF mutations in the hope of launching a clinical trial to test the melanoma drugs — an endeavour that the MMRF's chief scientific officer Louise Perkins admits is "an expensive and risky bet". The approach may prove fruitful for the few myeloma patients who could benefit from drugs targeted to their specific mutations but originally developed for other cancers.

The MMRF study represents the most comprehensive set of cancer genomes yet published. It brought together several academic institutions, including the Broad Institute and a long-time competitor, the Translational Genomics Research Institute based in Phoenix, Arizona. This effort was just a first step: the MMRF hopes to sequence at least 250 myeloma genomes in the near future.

MOVING TARGET

The ability to isolate multiple myeloma tumours from the blood and bone marrow has made the disease more amenable to genetic profiling than many solid tumours. But significant challenges to understanding the disease remain, particularly from the cancer's own chaotic genome. In addition to the inherent complexity in the myeloma genome, there is evidence that the cancer genome evolves over time in the same patient. "It's a moving target," says Munshi. These changes may be linked to the disease becoming more severe or developing drug resistance, and may eventually prove useful in monitoring the disease and treatment. Munshi's group has found evidence that the myeloma genome is unstable, and is investigating whether targeting this fundamental instability could be a promising therapeutic strategy.

"In addition to the complexity in the myeloma genome, there is evidence that the genome changes over time in the same patient."

As well as these genetic changes, researchers have recently found that microRNA expression correlates with different subtypes of disease and survival, providing another potential molecular tool to analyse and target disease mechanisms.

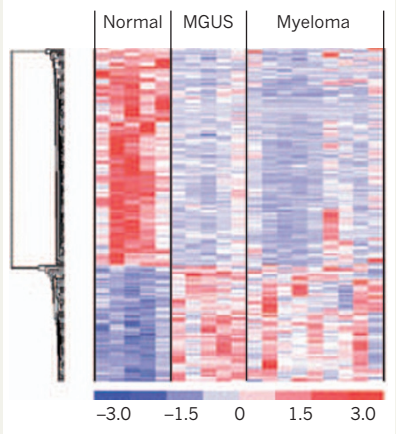
The goal now, says Anderson, is to combine these methods of molecular analysis to provide a clearer picture of the genome's heterogeneity. Because the cancer's genome changes with progression and treatment, it is important to study large groups of patients to find reproducible patterns in the way their treatments affect the cancer genome. Anderson is optimistic that such studies will make it possible to find clinically useful signatures of disease type and progression, which will aid the ultimate goal: "to get the right treatments to the right patients at the right time". ■

Courtney Humphries *is a science writer based in Boston, Massachusetts.*

1. Chapman, M. A. *et al. Nature* **471,** 467–472 (2011).

TRACKING PROGRESSION

The pattern of gene expression clearly changes as MGUS and myeloma develop.



AMERICAN ASSOCIATION FOR CANCER RESEARCH

RISK ASSESSMENT

Looking for clues

Multiple myeloma is not a uniform disease. Ideally, doctors would like to treat each patient according to the distinct molecular profile of their cancer. Cytogenetic analysis makes it possible to group patients into several subclasses based on their particular genetic abnormalities. Several of these abnormalities are associated with either a higher or a lower risk: a deletion in chromosome 17 found in about 15% of patients, for instance, is associated with a short survival time and can be used to identify high-risk patients. But there is no definitive way to classify each myeloma into a molecular subtype that correlates with clinical outcome.

Over the past decade, several groups have used gene expression profiling to group patients. A team at the University of Arkansas for Medical Sciences (UAMS) in Little Rock has developed a model for predicting prognosis based on the expression profiles of 70 genes. John Shaughnessy, director of the Lambert Laboratory of Myeloma Genetics at UAMS, says the profiles show that myeloma is, genetically, "not one disease but eight different molecularly definable groups". About 20% of newly diagnosed patients, he says, have signatures indicating a high risk of the cancer returning after treatment. Shaughnessy started a company, Signal Genetics, which profiles patients using these 70 genes to aid doctors' decision-making.

As a sign of how heterogeneous the cancer is, other groups have developed their own metrics. The Intergroupe Francophone du Myelome in France, for instance, has come up with a 15-gene model. Few of the genes identified in the two models overlap. Part of the problem is that different groups of patients are given slightly different treatments; the models tend to lose predictive power when applied to other sets of patients.

So far, these techniques have identified the patients with the worst prognosis. "It tells you they're not going to do well — it doesn't tell you how they would do better," says Leif Bergsagel of the Mayo Clinic in Arizona. Identifying patients who are unlikely to benefit from current medication could help them avoid unnecessary treatment and identify candidates for new treatments. But no genetic event has been found to be linked with cure or even long-term survival — there is, as yet, no sign of success. **C.H.**