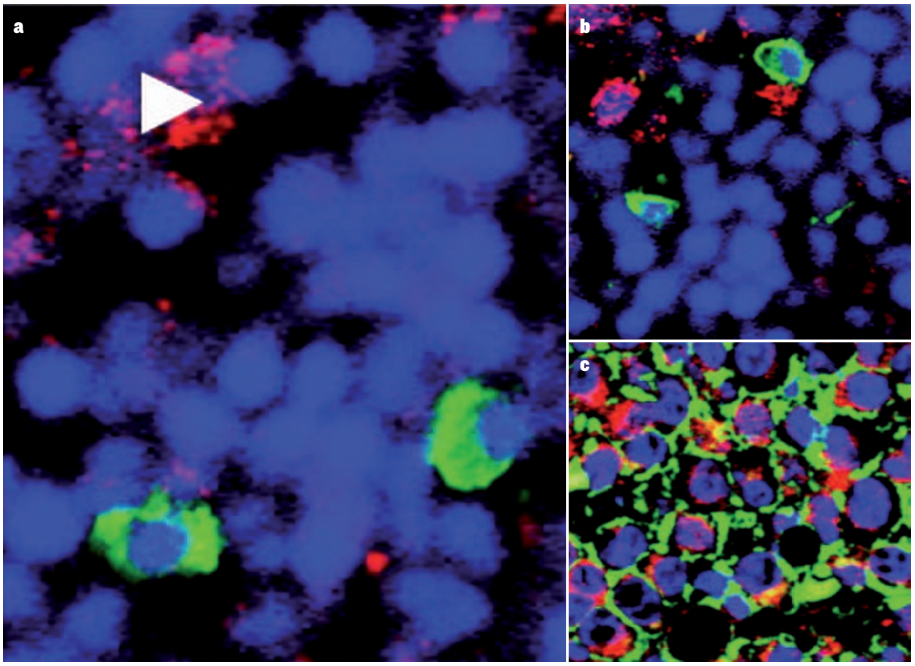

Bone-marrow cells from healthy people (a) and patients with MGUS (b) are clearly different to those from multiple myeloma patients (c) when labelled with two protein markers.

M. CHATTERJEE *ET AL. BLOOD* **109**, 720-728 (2007)/AMERICAN SOCIETY OF HEMATOLOGY

DIAGNOSTICS

The early bird

Identifying the patients most likely to progress from a precancerous condition to multiple myeloma could help doctors catch the disease early and stop it taking hold.

BY LAUREN GRAVITZ

Unlike many other forms of cancer, multiple myeloma does not lend itself to early warnings. The condition that precedes it — an accumulation of precancerous cells called 'monoclonal gammopathy of undetermined significance' (MGUS) — is remarkably common, occurring in 3% of people over 50 years of age, but it converts to cancer at a rate of just 1% a year. Predicting which individuals are most at risk is extremely difficult. But given the rapid progression and high mortality of the disease, researchers are keen to identify them and start treatment as early as possible.

This idea may seem obvious, but it's beset with problems. Myeloma exists on a spectrum, from benign MGUS to asymptomatic 'smouldering' multiple myeloma to the full-blown disease. Treating everyone with MGUS is not a viable option: many doctors worry that they could do more harm than good if they treat people on the healthy end of the spectrum, the vast majority of whom will never develop the disease.

In a 1988 study using the best drugs available, researchers concluded that treatment at the smouldering stage caused unnecessary side effects with no impact on survival time. Since that first trial, however, newer treatments with better prognoses have emerged for multiple myeloma, and many researchers believe that these therapies could work for MGUS as well and should be tested in earlier stages of disease. But, they say, this should only be done if there's a way of stratifying patients based on their risk of progression from MGUS or smouldering myeloma into the symptomatic stages of disease. And only those at the highest risk of converting to full-blown myeloma should be treated.

Studies of early treatment must be done with extreme caution. Unless a patient has smouldering disease and is deemed exceedingly likely to progress rapidly to symptomatic myeloma, the evidence suggests that it might be a mistake to start treatment. According to W. Michael Kuehl, who studies myeloma pathogenesis at the US National Cancer Institute in Bethesda, Maryland, treating MGUS too early could actually induce myeloma. "You might find that if you treat a patient you could get rid of premalignant cells, but they would come back very quickly with malignant clones that had been kept in check by MGUS," he says.

CHAIN REACTION

The definition of multiple myeloma is based on clinical signs such as anaemia or elevated blood calcium levels. This is because when pathologists examine biopsies from MGUS and multiple myeloma patients, the cells are virtually identical. "People have generally said that unless there's end-organ damage we should sit tight and watch," says Vincent Rajkumar, a haematologist and myeloma specialist at the Mayo Clinic in Rochester, Minnesota. "People like me are starting to say we have to go early and treat early if we're going to make a difference."

Currently, patients are diagnosed as having MGUS, smouldering myeloma or multiple myeloma according to three major factors. The first is the amount of protein from identical (monoclonal) antibodies in the blood. The second is the ratio of the number of genetically diverse plasma cells to the number of identical, clonal plasma cells in a bone-marrow biopsy. The third is the presence of clinical symptoms (see '–––How far along', page S35). However, more researchers and clinicians think the disease exists on a spectrum, and that they would be better able to treat and follow-up with their patients if they had a better understanding of where on the disease spectrum each patient lies.

Rajkumar has worked on some of the more promising stratification strategies to emerge in the past few years. One of the more straightforward methods is to measure the tumour burden, or the percentage of clonal plasma cells in the bone marrow. By the time that number reaches 60%, most patients already have symptoms — but if they don't, they will almost certainly develop them within the next two years and would be an ideal group of patients to start early therapy.

Another method the Mayo group found that indicates risk of progression is a blood test that measures levels of immunoglobulin free-light chains. Antibodies, or immunoglobulins, are composed of two heavy and two light protein chains that are made separately and then built up into antibodies inside plasma cells. There are two types of light chain, kappa and lambda. A skewed ratio of free kappa to free lambda light chains indicates a proliferation of clonal plasma cells and thus a higher risk of progression. In a person with MGUS, a kappa-to-lambda light-chain ratio of less than 0.65 or more than 1.65 corresponds to a tripling of the risk of progression. In a patient with smouldering multiple myeloma, a kappa-to-lambda ratio higher than 100 (or less than 0.01), Rajkumar has found, pushes the risk of progression to between 80% and 90%. The researchers are also investigating several other biomarkers. "If we can identify all of these [markers] carefully, we will be able to identify

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STRATIFICATION STRATEGY

There are various ways of assessing the risk of patients with early signs of multiple myeloma progressing to the full-blown disease.

Method	How it works	Potential obstacles
Molecular imaging	Contrast-enhanced positron emission tomography (PET), computed tomography (CT) and magnetic resonance imaging (MRI) scans, in combination with different molecular markers, can pick out signs of progression in the bone marrow, including blood-vessel 'leakiness' and increased blood-vessel growth (angiogenesis) characteristic of cancer.	• Whole-body imaging is expensive and difficult to justify for frequent monitoring. • There are indications that certain contrast agents may increase the risk of disease progression.
Free light-chain ratios	Immunoglobulins are composed of two heavy and two light protein chains. There is evidence that patients with a skewed ratio of kappa-to-lambda free light chains are much more at risk of progressing to full-blown multiple myeloma.	None.
Bone-marrow microenvironment	Some believe that as MGUS develops into myeloma, the tumour cells manipulate their microenvironment to their benefit. Researchers have identified inflammation signatures in the bone-marrow microenvironment — increased transcription factors and other proteins — that they believe are specific to myeloma progression.	• Researchers don't have data from enough patients to ensure they are identifying a consistent signature. • This requires bone-marrow aspiration, rather than a simple blood or urine test.
MicroRNAs	MicroRNAs (miRNAs) are involved in the suppression and silencing of gene transcription, and *in vitro* studies suggest that a combination of up- and downregulated miRNAs that creates a fingerprint specific to myeloma progression. This could be detected in a blood sample.	• So far, there are not enough data from patients to determine whether these signatures would be a reliable indication of risk.
Chromosomal mutations and deletions	Certain deletions (such as chromosome 13), chromosomal translocations (t(11;14)) and mutations (in the *RAS* genes) seem to be correlated with the transition from MGUS to myeloma.	• Each mutation is present in only a relatively small minority of patients.
Flow cytometry	Flow cytometry allows researchers to label and count plasma cells, based on immunological markers on their surface. The more abnormal cells, the higher the probability of converting to malignant disease.	• This requires a bone-marrow biopsy rather than a simple blood or urine test.
Clonal plasma levels	If a patient doesn't already have symptoms, those with a bone-marrow biopsy with more than 60% clonal plasma cells will almost certainly develop disease within the next two years.	• This needs a bone-marrow biopsy rather than a blood or urine test. • Many patients become symptomatic before their clonal plasma cell count reaches 60%.

the people who will progress in the next two years," Rajkumar says.

GO WITH THE FLOW

Another group making good headway in stratifying risk is based at the University Hospital of Salamanca in Spain. Jesús San Miguel, a haematologist with the Salamanca group, found that the percentage of plasma cells that are clonal, as determined by immunological markers on their surface, correlates with the probability of conversion to malignant disease. The cells are collected from bone-marrow biopsies and examined by a process called flow cytometry, in which thousands of cells per second stream past a laser and can be counted and examined for fluorescent markers. "We have shown that in MGUS patients with the benign form of the disease, normal plasma cells coexist with tumour plasma cells," San Miguel says. By contrast, he explains, in a person with symptomatic myeloma, nearly all the plasma cells are phenotypically aberrant or clonal. It is only through the use of flow cytometry that this intuitive result — the more abnormal cells a patient has, the more likely they are to progress — has been confirmed.

After separating out the highest-risk patients as predicted by flow cytometry, San Miguel performed the first long-term trial to see whether early intervention can prolong survival. He tested a combination of two drugs, lenalidomide and dexamethasone, on patients with smouldering myeloma and high counts of abnormal plasma cells. After five years, 98% of the treatment group survived, compared with only 87% of the untreated group. "We are seeing a trend for benefit in overall survival in these high-risk smouldering-myeloma groups," he says.

Researchers are watching both the Mayo and the Salamanca groups closely. "The better stratification you have, the more rational you can be about what to do with treatment," says Kuehl. "But a big part of cancer is just blind luck. We're never going to be able to perfectly predict what happens, but both the Mayo and Spanish stratifications are good steps in the right direction."

Several other techniques for risk stratification are making their way into labs across the United States and Europe, but progress is slow. Genetic approaches hold promise, but if researchers are to tease out high-risk genetic profiles, they must follow MGUS patients from the moment of diagnosis until they convert, which can take a decade or more. This hasn't stopped scientists at the University of Arkansas for Medical Sciences, who are monitoring about 120 MGUS patients as part of a group of trials done by SWOG, formerly the Southwest Oncology Group. "We've been watching these patients and trying to identify features associated with early progression," says John Shaughnessy, a myeloma geneticist at the university. Shaughnessy has the same difficulties as his peers. "It's been an arduous task. It's very difficult to see any signal even after ten years, because it's so rare that these people progress."

EXPRESSION OF HOPE

Most of the patients Shaughnessy is studying also undergo gene-expression studies to determine whether rapidly progressing MGUS has a unique signature of gene activity. Using a 70-gene risk model that Shaughnessy developed to pinpoint the most aggressive myeloma cases, the Arkansas researchers believe they might be able to identify increased risk of MGUS progression. They are also studying a protein made by myeloma tumour cells, called DKK1, which suppresses the differentiation of bone-forming cells. "We can show that if a patient's gene-expression profiling shows that their DKK1 expression is in the highest 25%, they are at increased risk of progression," Shaughnessy says. Half the patients in that group progressed to disease within three to four years, whereas none of those with DKK1 levels in the lowest quartile have done.

Other genes and chromosomal mutations have also yielded encouraging results. Scientists have found that the activity of the *MYC* and *KRAS* genes also seems to be different in MGUS and myeloma. "Let's say you get a patient with MGUS and monitor him closely, and find out that all of a sudden he has *MYC* rearrangements in a lot of cells," says Leif Bergsagel, a cancer geneticist at the Mayo Clinic in Scottsdale, Arizona. In this case, it may be prudent to consider treatment even if no symptoms have emerged. "We don't have the technology to do it now, but as the price of next-generation sequencing comes down, we could quantify even low levels of *MYC* rearrangements," he says. Identifying the genes responsible and their products could ultimately have implications for treatment too if drug companies could identify those most at risk and then target and eradicate just the abnormal cells to stop the full-blown disease taking hold.

For now, researchers are working on a variety of screening tools — from assessing clinical symptoms to blood tests and imaging — to create a fingerprint that could identify who is most likely to progress (see 'Seeing is believing', page S52). "It's a big challenge, but I think finding biomarkers is the easy part," Rajkumar says. "The hard part will be convincing my colleagues that we really can predict who will progress and don't have to wait for symptoms to appear." ■

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