High hopes, and a lot of money, have been pinned on the first protease inhibitors to reach clinical trials. This is hardly surprising; proteases influence so many important cellular systems that inhibitors could target many intractable killers — heart disease, cancer, stroke, Alzheimer’s disease and many more.

At first, protease inhibitors lived up to this promise. HIV protease inhibitors (FIG. 1) — in combination with reverse transcriptase inhibitors — revolutionized treatment for people with HIV. HIV-1 protease performs an essential step in the life cycle of the virus. In HIV, proteins are formed in one long strand, which must be snipped by HIV-1 protease into the proper pieces to form the mature virus. Blocking HIV-1 protease causes HIV to make copies of itself that cannot infect new cells.

In addition, angiotensin-converting enzyme (ACE) inhibitors have become standard treatment in many patients with cardiovascular disease, such as heart attacks and heart failure. By halting the metalloproteinase ACE in its tracks, vasoconstriction is prevented, lowering blood pressure, which is one of the biggest risk factors for these conditions.

Other protease inhibitors in development also began to cause great excitement. Matrix metalloproteinase inhibitors (MMPIs) could stop many types of cancers from spreading throughout the body; and caspase inhibitors could stop cells damaged by a whole range of traumas and disease from going on to kill themselves via apoptosis. But several years down the line, results have failed to live up to expectations, with drug companies being accused of rushing drugs into clinical trials before their effects were fully understood.

Matrix metalloproteinase inhibitors
The trials and tribulations that MMPIs have endured serve as a good example of the potential pitfalls of protease inhibitors. For many years, the conventional wisdom has been that MMPs are crucial to the spread of cancer, breaking down the connective tissue between cells and the linings of blood vessels, and thereby allowing tumour cells to escape from their original location and seed secondary tumours elsewhere. So, researchers rushed to develop synthetic MMPIs, which block the activity of MMPs by binding to the zinc atom in their active sites. Early animal studies using broad spectrum inhibitors were compelling, with one study showing that the use of the inhibitor batimastat on mice which had been injected with ‘human cancer cells’ could lead to mice living six or seven times as long as animals that received a placebo.

More’s not the merrier
But as the first human trials were rushing ahead, vital new information about the true role of MMPs was being uncovered, and the full story was much more complex than anyone had imagined. When the trials started, only three MMPs had been identified, but before long, there were 24 known MMPs associated with different cell types.

Further studies showed that MMPs are involved in a number of important normal processes, such as cell-surface-receptor cleavage and release, and cytokine and chemokine activation and inactivation. So, inhibiting MMPs could interfere with normal tissue function, causing unknown side effects and possibly inhibiting useful host defence processes that control cancer growth. What once looked simple is now recognized to be a very complex, interdependent system, with the activity of many MMPs being regulated at many different levels, and each MMP being produced and activated in multiple steps and then controlled by endogenous inhibitors (FIG. 2).

With hindsight, this lack of complete knowledge can explain the lack of success of early human trials. For example, one early study involving the MMPI tanomastat to treat small-cell lung cancer was inadvertently targeting MMP-2, an MMP now thought to have no role in this particular tumour type. Early Phase I trials revealed that prolonged treatment with MMPIs produced musculoskeletal pain and inflammation. These effects were reversible, but led to lim-
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**Hitting the right target**

More information on the structures of all MMPs is needed to find new selective binding sites that target specific MMPs at different stages of tumour development. Until recently, most research into selective inhibitors has concentrated solely on the active site, but now there is also work looking into blocking other, unprimed subsites either to the ‘left side’ or ‘right side’ of the active site. In different MMPs the active site is differently arranged, allowing for the possibility of selective inhibition. For example, in MMP-1 the active site is physically shallow and cannot accommodate inhibitors with bulky side chains, whereas the active site in MMP-2 has a ‘deep pocket’. On the basis of this pocket, MMPs have been designed and appear to inhibit MMP-2 and -9, but not MMP-1, -7 and -11.

The search for more effective inhibitors has also involved natural products. Green tea catechins inhibit MMP production and activity, and are now in clinical trials. Neovastat extracted from shark cartilage is in Phase III trials for the treatment of advanced non-small-cell lung cancer, metastatic renal cell carcinoma and multiple myeloma.

Increasing knowledge of how MMPs are regulated, transcribed and activated is revealing new regulatory points at which MMP production could be targeted. MMP-based gene therapy and gene targeting strategies are also being pursued. However, there is always the caveat that knocking out one MMP might have unknown consequences on others. MMPs are entwined in a complex cascade with themselves and other proteases, and these proteases could try to compensate if an MMP is blocked.

**Single or multiple targets?**

Despite the clear need for more work on selective inhibitors, there is still a widely held view that broad-spectrum MMPIs will be needed to treat cancer at particular stages. The selective inhibition of single targets in early stages of disease might be desirable, but at intermediate stages, more widespread targeting of MMPs might be necessary, even at the cost of inducing side effects. Of course, even at best, MMPIs will not kill tumours, but they might weaken them, making them more prone to attack by conventional treatment.

Six MMPIs are presently in advanced stages of clinical development: marimastat in pancreatic cancer (Phase III trials); BMS-275291 in advanced non-small-cell lung cancer (Phase III); prinomastat for several tumours and earlier stages of disease (Phase II); metastatin in Kaposi’s sarcoma patients (Phase II); neovastat in renal-cell carcinoma (Phase III); and MM1270 for advanced malignancies (Phase I). Recently published results from the marimastat trials are some of the most encouraging to date. Survival for patients with gastric carcinoma improved significantly, and marimastat also slowed the progress of pancreatic cancer.

**Caspase inhibitors**

The other protease inhibitors in the front line of clinical research, but which have also failed to live up to their

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Figure 1 | HIV proteases. The development of HIV proteases revolutionized HIV treatment, but also provides a classic model for how to develop drugs based on the structure of a protein. The figure shows how molecules can be designed that fit the space around the structure of the active site of the protease. Here, the crystal structure of HIV protease is shown with the inhibitor ampravir bound. From this, GlaxoSmithKline developed the HIV protease inhibitor which sells under the trademark name of Agenerase. The protease is represented with ribbons and Agenerase is shown as a space-filling model, in which carbon is represented in light grey, oxygen in red, nitrogen in blue and sulfur in yellow. Reproduced from Blundell, T. L., Jhoti, H. & Abell, C. High-throughput crystallography for lead discovery in drug design. Nature Rev. Drug Discov. 1, 45–54 (2002) © Macmillan Magazines Ltd.
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Early promise, are the caspase inhibitors. Caspases equip a cell to destroy itself via a process called apoptosis. These proteases lie dormant in a complex and poised system that can sometimes ‘misfire’, usually following injury but also in chronic disorders. So, caspase inhibitors could potentially not only induce death in unwanted tumour cells, but also prevent cells damaged by lack of oxygen or other degenerative disease from killing themselves. The implications for the treatment of stroke, Alzheimer’s disease, Parkinson’s disease, spinal injury and many other conditions are obviously enormous, and the rush from animal studies into clinical trials has been fast.

The first preclinical animal studies used relatively non-specific caspase inhibitors, but even so, the results were encouraging. Clear benefits were seen in studies of liver, cardiac and cerebral ischaemic injury. In other disease models, animal studies showed caspase inhibitors decreased apoptotic cell death in traumatic brain injury and in Parkinson’s disease, and even in infectious diseases like bacterial meningitis and sepsis. But once again, the results in clinical trials indicate that these animal studies can’t mimic the full picture in humans.

New strategies are now focusing on the need for a better understanding of the entire cascade that leads to apoptosis, along with the selection of better targets and more selective inhibitors. Twelve caspases have been identified, each with different roles in the apoptotic cascade. Initially, the majority of work centered on caspase-3, which has been identified as a major player in apoptosis in many different cell types. But now, many other
players further up the cascade are being targeted. Although some trials have already been halted due to lack of efficacy, others are still in progress for the treatment of many conditions, including cerebral stroke, cardiac ischaemia, sepsis, acute liver damage and the neurodegenerative disease amyotrophic lateral sclerosis (ALS).

Still hopeful
Despite the setbacks, protease inhibitors continue to create excitement. Bortezomib (Velcade; Millennium Pharmaceuticals) became the first proteasome inhibitor to be approved earlier this year for multiple myeloma, a blood-borne cancer. The finding that blocking the cell’s major protein-degrading apparatus can successfully treat cancer has led to a raft of similar treatments coming through the drug development pipeline. Also, strategies that target the proteases β- and γ-secretase are generating great interest as potential treatments for blocking the production of amyloid-β, the peptide that forms the plaques in brains that are a hallmark of Alzheimer’s disease. High hopes continue to be pinned on protease inhibitors, and learning from previous failures will help increase the chances of success.