be the whole story. 'There may be an inherent heterogeneity in the ability of the ribozyme to distribute itself in the cell to effect repair of the targets,' says George. 'We are exploring possible explanations for this, including that it may be related to the different phases of the cell cycle.'

Tissue specific

George does not think that ribozyme-mediated mRNA *trans*-splicing can yet be regarded as superior to other gene therapy approaches. However, it has several potential advantages. mRNA is only expressed in tissues where the gene in question is expressed, so targeting it provides a level of tissue specificity in addition to any specificity conferred by the delivery method. It also addresses concerns about gene regulation. The engineered gene does not have to be under the control of the native promoter because the mRNA produced is regulated in the normal physiological way.

The technique could have important advantages against autosomal dominant disorders such as certain dominant muscular dystrophies. 'In these disorders there is often a dominant-negative disease mechanism,' George explains. 'This implies that there is ample wild-type gene being expressed by the normal allele, but there is a negative impact from the mutant allele. So simply increasing the expression of the wild-type gene in the traditional way might not necessarily be effective. The mRNA repair strategy addresses that because you are eliminating the mutant product, while simultaneously increasing the abundance of the wild-type allele.'

Future promise

The aim is to try and treat myotonia congenita in an affected dog. Before that, the researchers will need to increase the transfection efficiency in cultured cells. Testing in dogs will initially involve localized therapy delivered by injection into the muscle, rather than systemic delivery, which George describes as 'very challenging'. Human trials are a long way off.

Jeffrey Chamberlain, Professor of Neurology at the University of Washington School of Medicine (http://www.washington.edu/medical/som) and a leading researcher into gene therapies for muscular dystrophy, says: 'This work is extremely promising in terms of enabling gene therapy for dominantly inherited diseases.' He continues, 'The major challenges for applying it in the clinic will be delivery of the ribozyme shuttles to the appropriate target cells in the body, and identifying whether the ribozymes will persist, or whether they can be delivered repeatedly to maintain health of the patient.'

Reference

1 Rogers, C.S. *et al.* (2002) Functional repair of a mutant chloride channel using a trans-splicing ribozyme. *J. Clin. Invest.* 110, 1783–1789

Gleevec: tailoring to fit

Thomas S. May, freelance writer

Imatinib mesylate, otherwise known as Gleevec, became an 'overnight success' after Phase I clinical trials, which began in 1998, found that the drug caused remission in all of the 31 patients with chronic myelogenous leukemia (CML) who participated in the trial. Not only was Gleevec (STI-571) extremely effective, but it also appeared to cause remarkably few side effects in the test subjects. Phase II and III trials were also promising and the drug was approved for use in CML patients by the US Food and Drug Administration (FDA) in May of 2001.

Unfortunately, however, a majority of CML patients whose disease has advanced to the 'blast crisis' stage eventually relapse and die of leukemia because of 'Gleevec resistance'. However, according to a new study, resistance to Gleevec can be overcome [1].

Overcoming resistance

Gleevec works by inhibiting the activity of the tyrosine kinase Bcr-Abl, which can cause uncontrolled proliferation of white blood cells. About 95% of CML patients have the mutated Bcr-Abl gene, and Gleevec can halt the progress of leukemia in these patients – if they begin treatment early on in the course of their disease.

A team, lead by Brian Druker of Howard Hughes Medical Institute (http://www.hhmi.org), claim that a compound called PD180970 can stop the activity of several mutations found in patients who develop a resistance to Gleevec. 'Our data indicate that PD180970 is active against several Bcr-Abl mutations that are resistant to imatinib and support the notion that developing additional Abl kinase inhibitors would be useful as a treatment strategy for chronic

 1359-6446/03/$ – see front matter PII: S1359-6446(03)02615-1

myelogenous leukemia,' the investigators wrote [1] (see Fig. 1).

Although PD180970 is not soluble enough to be used as a drug, this work offers some hope that it will be possible to develop a drug – or a combination of drugs – to counteract resistance, according to Druker. 'It is important to stress that PD180970 will not enter clinical studies due to its unfavorable solubility,' he said. 'But we've proved a principle, and now need to apply it to other compounds that can be developed into effective drugs,' Druker added.

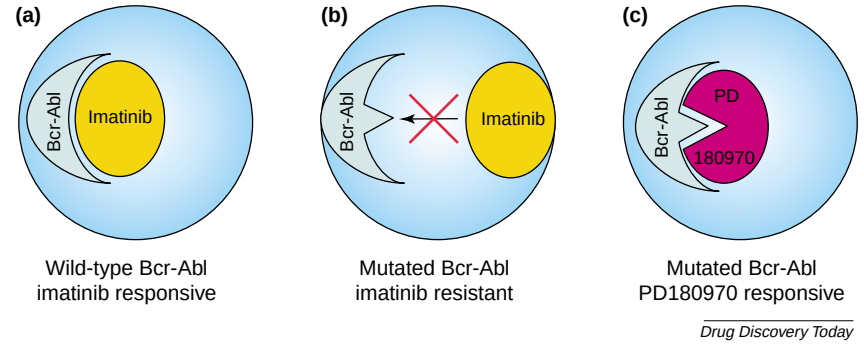

Figure 1. Drug–target interaction in chronic myelogenous leukaemia cells carrying either **(a)** wild-type Bcr-Abl or **(b)** and **(c)** mutated Bcr-Abl. PD180970 binding is not prevented by some Bcr-Abl kinase mutations that induce resistance to imatinib **(c)**. Thus, PD180970 serves as proof of principle that second generation inhibitors might be able to overcome imatinib resistance by kinase domain mutations. Figure courtesy of Paul La Rosée, Oregon Health and Science University (http://www.ohsu.edu)

Custom-tailored treatment

The key to curing more CML patients is to provide customized treatment for each individual, based on the particular molecular mutation that causes their resistance to Gleevec, the researchers say. 'For patients with advanced disease, I would envision a strategy where their leukemia cells would be profiled and the appropriate inhibitor or combination of inhibitors would be selected,' Druker explained.

This approach is similar to the method that has been used to treat HIV (AIDS) patients, Paul La Rosée, Oregon Health and Science University (http://www.ohsu.edu), the study's lead author, points out. Treatment would be tailored to each individual patient, by combining specific inhibitors in an 'inhibitor cocktail' that would be able to combat various Bcr-Abl isoforms, he says. 'The paradigm is to understand the genetic abnormality that drives the growth and survival of cancer, and tailor a treatment to reverse this genetic defect,' comments Druker.

Potential problems

Although it sounds good in theory, some experts argue that there are several potential problems regarding the proposed use of PD180970 or other similar agents. According to Timothy Hughes, an Associate Professor at the Institute of Medical and Veterinary Science in Adelaide, Australia (http://www.imvs.sa.gov.au/), 'the problem with this concept is that, unlike imatinib, which is extremely safe and well tolerated, other ABL kinase inhibitors identified so far, including PD180970, are still quite toxic to normal cells.' Therefore, it may not be possible, or economically feasible, to develop other kinase inhibitors with the safety and tolerance profile of imatinib, Hughes contends.

James Griffin, of the Dana-Farber Cancer Institute (http://www.dfci.harvard.edu/), raises another concern, which relates to the potential usefulness of having multiple kinase inhibitors directed at the Bcr-Abl mutation. According to Griffin, there is some evidence that CML patients who are resistant to Gleevec might also have other mutant clones, and that such patients will rapidly become resistant to other kinase inhibitors as well. 'Also, no one has yet described another Abl inhibitor that blocks all of the imatinib-resistant mutations, or one that blocks some of the most common mutations,' he argued.

The next Gleevec?

Although the compound developed by Druker and colleagues (PD180970) might never become an effective drug for the treatment of Gleevec-resistant CML, research is already under way to find other agents that might be able to successfully treat this disease, even in its most advanced stages.

Charles Sawyers and colleagues at the University of California, Los Angeles (http://www.ucla.edu), have recently tested such a compound [2]. PD166326 'is a prototype of a new generation of anti-Bcr-Abl compounds' that is more effective than Gleevec, the researchers claim. 'Our findings show that tyrosine kinase (TK) drug resistance is a structure-specific phenomenon and can be overcome by TK inhibitors of other structural classes, suggesting new approaches for future anti-cancer drug development,' they concluded.

According to Sawyers, this work, combined with the Druker study, 'makes a very compelling story for rational drug design based on structural biology insights, with obvious clinical implications.'

References

1 La Rosée, P. *et al.* (2002) Activity of the Bcr-Abl kinase inhibitor PD180970 against clinically relevant Bcr-Abl isoforms that cause resistance to imatinib mesylate (Gleevec, STI571). *Cancer Res.* 62, 7149–7153

2 Huron, D.R. *et al.* A novel pyridopyrimidine inhibitor of Abl kinase is a picomolar inhibitor of Bcr-abl driven K562 cells and is effective against STI571-resistant Bcr-abl mutants. *Clin. Cancer Res.* (in press)