Promising mouse model results

Efficacy is the primary objective of current studies; experiments in mice using the high-fat diet model of hypercholesterolaemia and the genetically modified ApoE knockout model have shown promising results. According to Bennett, in both models, mice that were obese and that had significantly elevated cholesterol levels showed a reduction in overall cholesterol and LDL cholesterol after 3–4 weeks of treatment with ISIS147764. 'The response was dose-related, and was accompanied by a more modest decrease in the plasma levels of trigycerides,' he reports.

Statin-based drugs work more quickly, but ISIS147764 could prove suitable for subgroups of patients who are unresponsive to statins, or for whom statin therapy is contra-indicated. 'From a clinical standpoint, the initial results with ISIS147764 are very exciting,' comments Richard Honkanen (University of South Alabama College of Medicine; http://southmed.usouthal.edu), whose group is also investigating the potential of antisense technology in cardiovascular disease [2]. He points out that ISIS147764 represents the first drug designed to directly treat patients that overproduce ApoB-100. 'Statin therapy, which targets HMG-CoA reductase and lowers LDL primarily by enhancing LDL clearance, is clearly beneficial, but the cardiovascular event reduction is limited to ~30%,' he explains. Because ISIS147764 attacks the problem from the other end – preventing the formation of LDLs via inhibiting the expression of ApoB-100 – Honakanen believes that antisense strategies could bring in a new era for the medical management of heart disease. Bennett agrees, adding that a recent publication suggesting that many more people might benefit from cholesterol-lowering therapy than previously thought [2] adds new impetus to developing antisense drugs that complement statin therapies.

Improving antisense product formulation

The next phase of development will test the new drug in another genetically modified mouse model, and also in a rabbit model of hypercholesterolaemia. 'Antisense therapies are species specific, but the time to identify a rabbit or human ApoB-100 antisense molecule is likely to be less than a month,' confirms Bennett. Several other antisense products are already in clinical trials, and the technology is such that refinements of earlier products will aid the development of those still at the preclinical stage, speeding up this process considerably. 'First-generation products are given by intravenous injection, and are shorter acting, whereas second-generation products have a longer duration of action and can be given subcutaneously, making it possible for patients to give themselves the injections,' says Bennett. The short-term goal is for a preparation that needs to be injected only once every two weeks, but in the longer-term, Isis is also working towards an oral formulation.

Looking ahead to clinical trials

'Our detailed development strategy for ISIS147764 has not yet been worked out; we hope that the next phase will show equally promising results and we will work towards Phase I clinical trials as soon as possible,' confirms Bennett. Although the preclinical development of antisense drugs is much quicker than candidates identified using other methods, the timing from the start of Phase I trials will be more comparable, taking about four years to progress to Phase III trials. 'Phase III trials for an antisense product for hypercholesterolaemia would require a large number of patients, in multiple centres, and we will probably try and set up a partnership with another company to make that possible,' says Bennett.

Reference

1 Bennett, F. (2002) Inhibition of murine APOB B100 by antisense oligonucleotides in a murine model of hyperlipidaemia. *Cardiovasc. Drugs Ther.* 16 (Suppl.) (in press)
2 Golden, T. *et al.* (2002) Use of antisense oligonucleotides: advantages, controls, and cardiovascular tissue. *Microcirculation* 9, 51–64
3 Heart Protection Study Collaborative Group (2002) MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 360, 7–22

Gene therapy progress for HIV

Julie Clayton, freelance writer

The HIV virus could assist in its own downfall if two new gene therapy strategies succeed. Both approaches are due to enter Phase I safety trials in late 2002, using new vectors and protocols that their proponents hope will overcome many of the obstacles of the past 10 years. Researchers led by Carl June, a molecular immunologist at the Abramson Family Cancer Research Institute (http://www.abramsoninstitute.org), reported their progress at the *7th European*

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Conference on Experimental AIDS Research in Genoa [1].

The basis for HIV gene therapy

Gene therapy for HIV has developed from previous work that has attempted to simply bolster the immune defences of HIV-infected patients by using their own T cells that are removed and then propagated *ex vivo* before reinfusion back into the same individuals. This approach initially failed because of the poor survival of the reinfused cells, which lacked the growth factors to resist apoptosis or avoid senescence.

Recently developed cell culture techniques are now enabling researchers to stimulate T cells to mimic the growth signals normally provided by dendritic cells *in vivo*, by using antibodies against the CD28 and CD3 surface T-cell receptors. These manipulations significantly enhance the survival of the reinfused cells in patients, as reported recently in a Phase I safety trial [2]. Gene therapy goes a step further, by introducing foreign genes into the T cells before reinfusion that encode products able to boost T-cell defences against HIV infection.

Phase I trials

In a recent Phase I study by June and colleagues [3], the T cells of HIV-infected patients were manipulated to express a hybrid protein consisting of the external portion of the cell surface receptor CD4, to which HIV normally binds before entry, and the internal portion of the T-cell antigen receptor. Upon HIV binding, the fusion protein triggers T-cell activation, so that regardless of the natural antigen specificity of the transfected T cells, they should become active against the HIV virus.

The results of the trial showed good long-term survival of the manipulated T cells in patients, comprising 1% of the circulating T-cell pool for at least one year post-treatment; however, there was no significant reduction of the amount of HIV. To address this, a Phase II follow-up trial is now under way, testing the efficacy of including T-cell-specific growth factor interleukin-2 in daily infusions, to see whether this enhances the elimination of HIV.

A new approach

As an alternative approach, June's collaborators at ViRxSys (http://www.virxsys.com) have developed a new vector consisting of a gutted-out version of HIV itself. Stripped of structural genes that normally complete the formation of mature virions, the RNA vector cannot replicate. The key gene-therapy element is a portion of antisense RNA that binds to and blocks the HIV gene that encodes the coat protein, glycoprotein 120.

In vitro tests of HIV-infected cell lines and freshly isolated blood cells show that the vector can be transfected into cells with up to 90% efficiency and inhibits replication of a variety of HIV isolates, according to June. In accordance with guidelines from the National Institutes of Health (NIH; http://www.nih.gov) [4], June's team must first complete extensive safety tests before proceeding with clinical trials, in particular to ensure that the vector does not recombine with wildtype HIV once inside patients. These tests include use of the severe combined immunodeficient (SCID-hu) mouse of the Balb/c strain, which contains an engrafted human immune system.

The initial Phase I trial of HIV as a gene therapy vector will involve the sequential treatment of five patients whose HIV infection is resistant to protease inhibitor drugs. After a month of monitoring to ensure that no recombination with wild-type HIV has occurred, the researchers could proceed to treating the next patient. They will then measure the patients' viral load by PCR analysis. Ultimately, June plans to introduce the vector into other cell types as well: 'We think in the long run we can make a transgenic human immune system,' he said.

This work was welcomed by George Lewis, an immunologist at the Institute of Human Virology at the University of Maryland Biotechnology Institute (http://www.umbi.umd.edu): 'I'm encouraged by what I saw: they're doing it right, they're doing it carefully, and getting interesting results.'

References

1 June, C.H. (2002) Use of lentivirus vectors to engineer T cells for immunotherapy of HIV infection. *7th European Conference on Experimental AIDS Research*, 8–12 June 2002, Genoa, (Abstract PS19)

2 Levine, B.L. *et al.* (2002) Adoptive transfer of costimulated CD4+ T cells induces expansion of peripheral T cells and decreased CCR5 expression in HIV infection. *Nat. Med.* 8, 47–53

3 Deeks, S.G. *et al.* (2002) A Phase II randomized study of HIV-specific T-cell therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy. *Mol. Ther.* 5, 788–797

4 Posakoff, G.M. (2001) Lentiviral vectors approach the clinic but fall back: National Institutes of Health Recombinant DNA Advisory Committee review of a first clinical protocol for use of a lentiviral vector. *Mol. Ther.* 4, 282–283

Corrigendum

Please note a correction to the News article entitled *Glyxins to treat neurological disorders*, by Martina Habeck, published in *Drug Discovery Today* Volume 7, No. 13, pp. 690–691.

In the third column of the text on p. 690 and in the figure legend on p. 691, it incorrectly states that the tetrapeptide NT-13 is made up of the amino acids proline-threonine-threonine-proline. The correct amino acid sequence is threonine-proline-proline-threonine.

The author would like to apologize for this inaccuracy and for any confusion that this might have caused.

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