'This approach may be one way of addressing the shortage of donor organs, one of the main problems with islet transplantation,' said Mairi Benson, Information Science Manager at Diabetes UK (London, UK). 'However, the risks associated with immunosuppression, which would be needed to prevent the new β cells from being destroyed, would remain a problem in type 1 diabetes. The research is still at an early stage but we shall be monitoring developments as trials start in humans.'

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AGENT makes cyanide a useful killer

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A fusion protein incorporating an antibody that is specific for a tumour antigen and a plant-derived enzyme capable of splitting the sugar linamirin to release cyanide, could provide a novel targeted anticancer treatment. The system – known as AGENT (Antibody Guided Enzyme Nitrile Therapy; Fig. 1) – was used to target tumour cells *in vitro*, where it selectively killed tumour cells at doses that left non-cancerous cells completely unscathed [1]. 'With further development, we hope that this system will form the basis of an anticancer therapy for ovarian and breast cancer,' says senior author Mahendra Deonarain (Imperial College, London, UK).

Exploiting a plant enzyme

Plants such as the African potato and the hydrangea contain an enzyme, linamarase, which is capable of generating cyanide when animals or insects cause damage to the plant, thereby deterring further attacks. Deonarain and colleagues have exploited this reaction by attaching linamarase to a cancer-seeking antibody, which is specific for carcinoembryonic antigen (CEA), a protein that is found only in certain cancers. The linked protein is harmless until linamarin is introduced, whereby the sugar-splitting reaction releases small amounts of cyanide at sites where the antibody has bound.

Overcoming fusion problems

Since the early 1960s, groups have been experimenting with chemical conjugates of antibodies and enzymes with promising results, but to fulfil the requirements of a clinical trial the complex must be in the form of a fusion protein: this has posed technical difficulties, mainly because of problems in finding an appropriate vector to express the fusion proteins in sufficient quantities. 'It took a few years to engineer and express a gene that encodes a composite of the scFv antibody (reactive against CEA) and linamarase, a plant-derived β-galactosidase,' explains Deonarain. Various bacteria and yeasts were used as hosts without success until the team tried a 'supersecretory' mutant strain of *Saccharomyces cerevisiae*. Using this strain, they eventually managed to express the fusion protein in sufficient quantities.

Assessing the novel system

Christina Kousparou and Deonarain then isolated and purified the recombinant protein, showed that it bound specifically to CEA and confirmed that it had enzymic activity [1]. The ability of the fusion protein to kill cells *in vitro* was then assessed under different conditions. 'The system definitely had cytotoxic activity; the best results were obtained with a 3–4-h incubation time with the fusion protein at a concentration of 100 $\mu g\ ml^{-1}$, followed by prolonged incubation with linamarin – 24–48 h,' says Deonarain. One cell type, LS174T (a colorectal cancer cell line) showed a reduction of viable cells by 50% when exposed to a 0.4 mM solution of linamarin. Controls confirmed that neither the fusion protein nor the sugar alone were toxic to the cells and other experiments demonstrated that the cells were dying because of necrotic cell death, rather than apoptosis.

After promising results *in vitro*, some *in vivo* tumour uptake studies were commenced in mice, but the plant enzyme caused problems. 'Although the plant enzyme is good because it is completely different to any mammalian enzyme, AGENT cannot be activated by normal cell metabolism. However, it is heavily glycosylated giving it a tendency to aggregate in the liver and spleen,' explains

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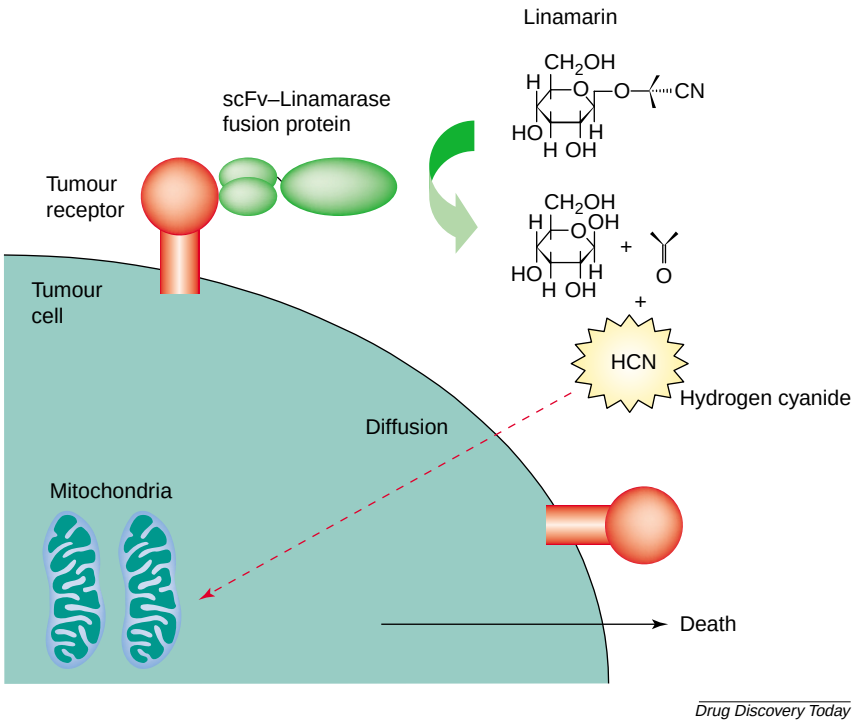

Figure 1. In the AGENT system, an antibody–enzyme fusion protein specifically localizes to tumour cells by recognising a tumour-associated antigen. This is followed by the administration of the prodrug linamarin, a naturally occurring sugar, which is hydrolyzed into cytotoxic cyanide. This kills cells by diffusing through the cell membrane and inhibiting mitochondrial respiration. A few non-targeted cells close by can also be killed by the generated cyanide. Figure kindly supplied by Mahendra Deonarain (Imperial College, London, UK).

Deonarain. Therefore, *in vivo* application of linamarin was not pursued. Deonarain and his team have, however, since identified a human enzyme from the same family that can be genetically redesigned to perform the sugar-splitting reaction that produces cyanide. They are currently developing an expression system and hope to have a product for *in vitro* and animal testing by the summer of 2002.

Howard McLeod (Washington University School of Medicine, St Louis, MO, USA) comments that AGENT represents the latest variation on Antibody Directed Enzyme Prodrug Therapy (ADEPT). 'Any one of several targeting approaches would be possible; in this study, conjugation with a CEA antibody was used, but the linamarase (or the human equivalent) could also be the warhead of a VDEPT (virally directed), GDEPT (gene directed) or ADEPT strategy,' he says. However, McLeod stresses that the concept is 'interesting, but early days.' He warns that the most important information missing from this study relates to safety. 'There is no data on normal tissue toxicity in the mice or whether detectable regional or systemic levels of cyanide could be found,' he notes.

Clinical trial prospects

The team already has the gene for the enzyme from another laboratory and the cyanide delivery system patent is owned by Antisoma Research Laboratories (London, UK) who funded Deonarain's research. 'Although Antisoma is small, they have a good infrastructure already set up for clinical trials in this area; a large clinical network will be available to us if all goes well in the next few years,' reports Deonarain.

Deonarain also points out that another antitumour antibody produced by Antisoma, one that is specific for an antigen present in the neovasculature of tumours but that is absent in normal blood vessels, could also be used in the AGENT system. Newly growing tumour blood vessels are bathed in blood and are respiring actively. These cells would make much better targets for the AGENT system than cells at the centre of a large tumour that are usually respiring anaerobically. 'By destroying blood vessels, we hope to strangle the tumours to death,' he adds.

'It is ironic that such a notorious killer could turn into a potential life-saver. We are all very excited by this research and are committed to making it work in the clinic one day. As is true for this type of research, there are many hurdles at this early stage but preclinical studies could begin within two years and pilot clinical trials within four years', concludes Deonarain.

Reference

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