# Drug-induced islet growth: a novel treatment for diabetes?

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Researchers have identified the active part of a protein that stimulates new insulin-producing cells to form in the pancreas. Known as the islet neogenesis gene associated protein (INGAP), it could represent a novel treatment for diabetes that tackles the cause of the disease rather than just the symptoms.

Diabetes is a major cause of ill-health and premature death, and the incidence of both forms is rising. In type 1 diabetes, autoimmune attack destroys the insulin-producing β cells in the islets of Langerhans in the pancreas and patients rely on insulin injections for survival. Type 2 diabetes begins with insulin resistance but there is often a gradual decline in insulin production, so that many type 2 patients eventually require insulin therapy. There has been some success in reinstating natural insulin production by transplanting donated islet cells, but this approach is hampered by problems with graft rejection and would be limited by the availability of donors.

## Islet neogenesis

A new treatment being developed by GMP Companies (Fort Lauderdale, FL, USA) could stimulate the growth of new, fully functional islet cells to replace those that have been lost. It is based on INGAP, which was discovered by Aaron Vinik of Eastern Virginia Medical School (Norfolk, VA, USA) and Lawrence Rosenberg of McGill University (Montreal, Canada). They found that the synthesis and differentiation of new islet cells from pancreatic ductal cells could be induced in an adult diabetic hamster model by wrapping the pancreas in cellophane tape [1]. Seven weeks after the operation, serum glucose and insulin levels had returned to normal in 50% of the 16 animals treated, compared with 12% of the untreated controls.

They reproduced the same effect by intraperitoneal administration of protein extracts from wrapped pancreas, of which INGAP is a constituent [2]. After isolating INGAP and producing a recombinant version, they cloned the hamster INGAP gene and identified a 15-amino acid peptide that showed the same activity as the whole protein [3]. The researchers later identified a highly homologous human gene (Vinik *et al.,* unpublished). Antibodies to hamster INGAP cross-referenced perfectly to samples of human pancreas in which islet neogenesis was occurring, showing that INGAP is also present in humans.

## Physiological regulation

'The INGAP peptide stimulates the adult proto-differentiated stem cells [in the pancreatic ducts] to differentiate into the complex of cells that make up the islets of Langerhans,' says Vinik. 'They form fully developed neo-islets. We have shown this in hamsters, mice and dogs. In the laboratory these islets behave entirely physiologically – they are turned on in the presence of glucose, and in the absence of glucose they are turned off. This is in contrast to the neogenic islets you see in hyperinsulinaemic syndrome of infancy, which are unbridled and secrete in response to inappropriate stimuli and are not turned off in the absence of glucose.' Further evidence that the new cells behave normally came from studies with non-diabetic animals given large doses of INGAP. Islet mass increased, but hypoglycaemia did not occur (Vinik *et al.*, unpublished).

Vinik is confident that INGAP will not cause harmful over-proliferation of islet cells. 'The new cells reach adult size and then do not grow any further,' he says. 'This means that the resident cells within the pancreas contain restrictive factors that prevent over-growth.' He acknowledges the possibility that, in patients with type 1 diabetes, the new cells could be killed by the same mechanisms that caused the disease originally. INGAP might be most effective several years after onset, when the antibody response to the β cells is waning. Although type 1 patients are genetically predisposed to diabetes, the multiple assaults on the β cells needed to trigger the condition might not occur again. Where autoimmune activity persists, INGAP could potentially be used in combination with immunosuppressant therapy.

GMP Companies has just announced the start of a Phase I/IIa clinical trial of synthetic INGAP peptide in patients with type 1 and type 2 diabetes. This will be the first time INGAP has been tested in humans. It will be given by intramuscular injection. 'At this stage, it is difficult to project the eventual duration of treatment,' says Scott Mohrland, the company's Vice President of Therapeutics. 'The current animal studies suggest that a 4–6-week treatment regimen would be effective.' The trial will assess the safety and tolerability of single and, later, multiple doses. 'In terms of efficacy in later trials, we will be looking for an increase in insulin production using C-peptide as a marker, a decrease in use of injected insulin and improved glycaemic control,' Mohrland adds.

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'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.'

## References


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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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