C_2D_2 Centre for Chronic Diseases & Disorders

Progression of a prototype therapeutic towards the clinic

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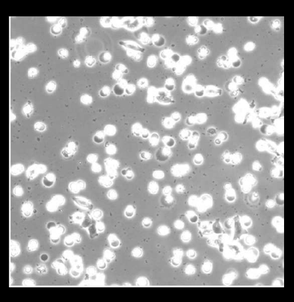
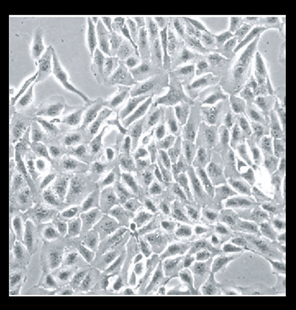
This project combines two separate discoveries and aims to produce a novel therapeutic for the treatment of cancer. The prototype therapeutic is easily manufactured and also readily adaptable for other disease pathologies including diabetes, retinopathy and neurodegeneration.

The proposed therapy involves a process called RNA interference induced by crook siRNA (invention 1) and is targeted against a gene called SIRT1 which is required for cancer cell viability (invention 2).
European and USA patents granted.

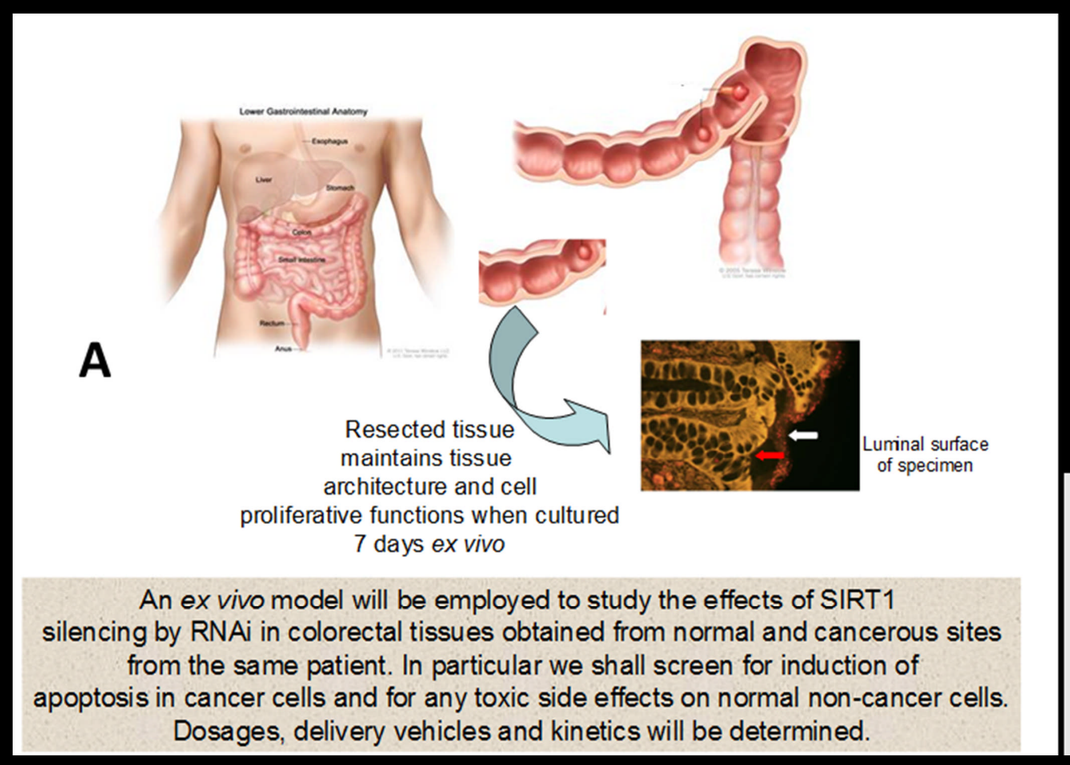
After extensive testing on human cells *in vitro* (see below, left panel) we now wish to progress to an *ex vivo* model of human cancer. A suitable *ex vivo* model, using normal and cancerous tissues from patients with colorectal cancer, is already established.



Human cancer cells killed by a single dose of crook siRNA directed against SIRT1



Normal human cells remain healthy following identical treatment



An *ex vivo* model will be employed to study the effects of SIRT1 silencing by RNAi in colorectal tissues obtained from normal and cancerous sites from the same patient. In particular we shall screen for induction of apoptosis in cancer cells and for any toxic side effects on normal non-cancer cells. Dosages, delivery vehicles and kinetics will be determined.

Ex vivo intestinal crypts are amenable to RNAi. This example shows fluorescent antibody labelling of the β-catenin gene product in two *ex vivo* crypts, following siRNA treatment. Note that depletion of β-catentin is caused by a specific β-catenin siRNA, but not by a scrambled sequence control siRNA .

RNA interference (RNAi): a cellular mechanism for silencing the expression of specific target genes via RNA molecules.

Short interfering RNA (siRNA): short RNA molecules that induce silencing of cognate target genes by RNAi. siRNAs exhibit exquisite selectivity for their target genes and are highly potent. Synthetic siRNAs against genes causal for disease carry huge therapeutic potential. However, siRNAs are rapidly destroyed in the body thus hindering their therapeutic exploitation.

Shepherd's crook siRNA (crook siRNA): siRNA modified by a DNA extension. The DNA forms a crook structure which protects the siRNA from degradation. The RNAi functioning of the siRNA moeity is unaffected and is resistant to degradation and stable over > 16 hours.

Crook siRNA

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