Obesity costs the UK economy nearly £47 billion per year. 40–70% of inter-individual variability in BMI attributable to genetic factors. Although treatment strategies are available, the side effects associated with their use necessitates development of novel therapies. Gene therapy is an emerging strategy for the prevention and treatment of obesity.

- RALA – a 30 amino acid amphipathic peptide vehicle for gene delivery
- FK506 binding protein like – FKBPL is a member of the immunophilin protein family
- Mice lacking one FKBPL allele are highly susceptible to diet-induced obesity
- A peptide mimetic of FKBPL partially reverses weight gain and glucose intolerance
- RALA/FKBPL nanoparticles for treatment of obesity
- FKBPL is protective of obesity in human populations
- Mechanisms involve preventing angiogenesis and inflammation

**VALUE PROPOSITION**

We have had a long-standing interest in the FKBPL gene. Using funding from BBSRC, we developed a heterozygous FKBPL+/− mouse (loss of both alleles was embryonically lethal) to improve our understanding of the role of FKBPL in normal development. FKBPL+/− mice began to develop obesity on a normal diet between 2-6 months (Fig. 1), characterised by higher proportion of body fat than wild-type (WT) littermates (Fig 1B); knockdown of FKBPL does not impact food consumption (Fig. 1C). These data are suggestive of a role for FKBPL in protecting against weight gain, and led us to the theory that FKBPL is a potential **gene therapy for obesity**.

**Figure 1:** Loss of one allele of FKBPL predisposes mice to obesity. A - FKBPL+/− mice weigh more than WT counterparts, B - FKBPL+/− mice have a higher proportion of subcutaneous fat than WT mice. C - Food intake is comparable between WT and FKBPL+/− mice.

RALA is a cationic peptide that we have developed and characterised over the past decade. Its cationic nature allows RALA to interact with anionic nucleic acids to form nanoparticles (Fig. 2). Nanoparticle properties can be optimised by altering the proportion of peptide:DNA (known as the N:P ratio). Nanoparticles with appropriate size (< 100 nm) and charge (≥ 20 mV) can enter cells by endocytosis, and escape the endosome (consequent of a pH-dependent shift in amphipathic conformation) to deliver their cargo. We have shown that cationic RALA nanoparticles have efficacy in numerous models of human malignancies1-3.

**Figure 2:** RALA/FKBPL nanoparticles are formulated by mixing cationic RALA with anionic FKBPL DNA, producing nanoparticles suitable for cellular delivery.

**TECHNOLOGY : RALA/FKBPL GENE THERAPY**

- RALA/FKBPL previously shown to delayed growth of breast cancer xenografts.
- A peptide mimetic of FKBPL inhibits diet-induced weight gain in FKBPL+/− mice, and improves glucose intolerance (Fig. 3).
RALA/FKBPL: TACKLING OBESITY WITH A NEW NANOMEDICINE

Figure 3: A peptide mimetic of FKBPL protects from diet-induced obesity.
A - FKBPL⁺/⁻ mice gain weight more quickly than WT; this weight gain is partially reversed by a peptide mimetic of FKBPL. B - FKBPL⁺/⁻ mice display intolerance to glucose, which is reversed by treatment with the FKBPL peptide.

FKBPL IS PROTECTIVE OF OBESITY IN HUMANS
• A deficiency in serum FKBPL is significantly associated with childhood obesity
• FKBPL levels are significantly decreased in conditioned medium of adipose tissue explants from obese human subjects compared to lean controls
• FKBPL levels increase with interventional approaches such as exercise and bariatric surgery

MECHANISMS
• FKBPL inhibits angiogenesis in adipose tissue possibility disrupting fat expansion
• FKBPL dampens down the immune response

MARKET
• Obesity pharmaceutical market is projected to grow from $407m in 2012 to $8.4b by 2022.
• Foresight report estimates that by 2050;
  ▪ 60% of men, and
  ▪ 50% of women aged 21-60 will have a BMI of 30-40.
• Withdrawal of other products due to adverse side effects creates gap in the market.

INTELLECTUAL PROPERTY
• Use of RALA for the delivery of anionic cargo is protected under patent WO 2014087023 A1.
• Use of FKBPL in oncological/ocular conditions is protected by patent WO 2007141533.
• A patent that describes a role for FKBPL in obesity has been filed (UK patent application no. 1617726.3).

REFERENCES

RALA/FKBPL nanoparticles are -
• stable in biological fluids.
• capable of evoking FKBPL expression.
• less toxic than nanocomplexes formed using commercially available reagents.
• easy and inexpensive to formulate.
• capable of achieving transgene expression in pre-adipocytes (Fig. 5).

Figure 4: The FKBPL gene has been cloned into a minicircle vector. Arabinose treatment cleaves parental plasmid (PP), producing functional minicircle (MC) that is devoid of bacterial plasmid DNA backbone, which is degraded.

Figure 5: RALA/pEGFP-N1 nanoparticles evoke GFP expression in 3T3 F422A pre-adipocytes.