RALA: A NOVEL GENE DELIVERY PLATFORM

• Novel peptide based gene delivery vehicle
• Proven capability for delivering nucleic acid, as well as small molecule cargo
• *In vivo* proof-of-concept for several onco-therapeutic payloads
• Low cost of goods and ease of manufacturing

**THE OPPORTUNITY**

RALA is a novel thirty amino acid amphipathic peptide, with proven ability to rapidly condense nucleic acids to form stable nanoparticles that are capable of entering cells and transporting their cargo to the nucleus with high transfection efficiency and low associated toxicity. The RALA peptide has been evaluated in several *in vivo* proof-of-concept studies, and is best exemplified by the therapeutic delivery of the inducible nitric oxide synthase (iNOS) gene in highly aggressive models of metastatic breast and prostate cancer. Low cost of goods and ease of manufacturing offer an advantage over other gene delivery platforms.

Cancer Research UK is currently seeking partners for licensing and/or collaborative development of the RALA peptide, and welcomes interest in exploring alternative applications and payloads.

**THE TECHNOLOGY**

**RALA peptide**

Through a series of biophysical, *in vitro* and *in vivo* experiments, the Inventors have demonstrated several properties of the RALA peptide that make it an attractive delivery vehicle, suitable for pharmaceutical development and large scale production ([1, 2]):

- Highly efficient at condensing nucleic acids (DNA, RNA, siRNA, miRNA) as well as some small molecules into nanoparticle form (<100 nm)
- Cellular delivery: RALA nanoparticles are capable of entering cells, disrupting endosomes and transporting their cargo to the nucleus
- High transfection efficiency with low associated toxicity
- Low immunogenicity: no vector neutralisation or anti-RALA/DNA antibodies detected following repeated delivery of nanoparticles in immune competent mice
- Targeted delivery: cell-specific delivery achievable by placing nucleic acid payload expression under control of a cell-specific promoter, and/or incorporating a cell-targeting motif within the RALA peptide
- *In vivo* delivery: *in vivo* proof-of-concept studies demonstrate efficacy for delivery of siRNA, miRNA, DNA and bisphosphonate molecules
- Highly stable: Nanoparticles remain in complex for over a month at room temperature, and more than 6 hours when incubated in serum; nanoparticle efficacy is not compromised following lyophilisation
- Ease of manufacturing and low cost of goods: rapid self-assembly (*Figure 1*) of RALA nanoparticles (<30 mins) which can be formulated into isotonic solution

**Exemplification of the RALA peptide: RALA/hOC-iNOS**

Nitric oxide (NO) is a key second messenger in most normal tissues, where it is generated at low concentrations, predominantly by the catalytic action of two constitutively expressed isoforms of nitric oxide synthase. The effects of NO in tumours are bimodal, such that intermediate levels optimise tumour growth and interventions to either raise or lower concentrations can inhibit tumour growth.

*Figure 1:* RALA nanoparticles are formulated simply by mixing RALA and plasmid DNA for 30 min at room temperature. RALA nanoparticles assemble spontaneously as a result of electrostatic interaction between the anionic DNA and cationic peptide. RALA/plasmid nanoparticles are ~60 nm in diameter, with a charge of ~20 mV.

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The controlled generation of high levels of iNOS has several advantages including damaging DNA, reducing the efficiency of DNA repair proteins, inhibiting HIF-1 transcription in hypoxic cells, and inhibiting the activity of anti-apoptotic factor NFκB [3, 4].

The Inventors have placed the iNOS gene under the control of the tumour specific human osteocalcin (hOC) promoter. Tumours that have metastasised to bone display high expression of RUNX2 (the main transcription factor involved in activating the hOC promoter) [5, 6] making it possible to transcriptionally target the iNOS therapy to sites of high RUNX2 expression.

In mice bearing MDA-MB-231-Luc2 breast metastases, the RALA peptide efficiently delivered hOC-iNOS gene therapy, evoking tumour suppression; mice tolerated repeat doses of RALA/hOC-iNOS nanoparticles, and such treatment reduced tumour burden and prolonged survival (Figure 2). Elevated blood nitrite levels, considered a biomarker of successful delivery, were detected in mice receiving iNOS therapy. When combined with docetaxel, RALA/hOC-iNOS gene therapy also prolonged survival of metastases-bearing mice compared to mice receiving docetaxel only (data not shown). RALA/hOC-iNOS efficacy was also observed in a highly aggressive model of prostate cancer metastases (PC-3M-Luc-C6). Elevated nitrite levels can be easily monitored in serum, representing a potential non-invasive PD biomarker strategy.

RALA/hOC-iNOS therapy is highly applicable to cancers that exhibit a correlation between tumour stage and RUNX2 expression, including breast, prostate, ovarian and NSCLC cancers [7, 8]. It is anticipated that RUNX2 expression could act as a predictive companion biomarker to identify patients likely to respond to the RALA/hOC-iNOS therapy.

The inventors are currently investigating the effects of RALA/hOC-iNOS in clinically relevant PDX models and further validating RUNX2 expression as a companion biomarker.

INTELLECTUAL PROPERTY

A patent application has been filed covering the RALA peptide and its utility, including delivery of the hOC-iNOS construct (WO 2014087023 A1)

ORIGINATING INSTITUTE

This opportunity originates from Queens University Belfast, under the direction of Dr. Helen McCarthy.

REFERENCES

2. Bennett R, Yaakkundi A, McKeen HD et al., Nanomedicine, 2015 Sep 30 (ahead of print)

COMMERCIAL PARTNERSHIP OPPORTUNITY

Enabling Technology

Figure 2: iNOS gene therapy improves survival in mice bearing metastatic breast cancer. INOS gene therapy improves survival in mice bearing metastatic breast cancer. Female BALB/c SCID mice were inoculated with MDA-MB-231-Luc2 cells via the left ventricle. Mice received nanoparticles twice weekly for five treatments. (A) Bioluminescence (B) Survival curve: RALA/hOC-iNOS-treated mice survive significantly longer than mice treated with water or RALA alone. (C) Weight loss of mice treated with RALA/hOC-iNOS, RALA or water.