Development of *in vivo* imaging techniques to determine the biodistribution of antisense oligonucleotides in dystrophin deficient muscular dystrophy

Umar Burki, Blain A1, Wilson I1, Launay G1, Coursindel T2, Maxwell R1, Carroll M1, Laval S1, Gait, M2, Straub V1.

1Institute of Genetic Medicine, Newcastle University, International Centre for Life, Central Parkway, Newcastle upon Tyne, NE1 3BZ, UK
2MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 0QH, UK

**BACKGROUND**

Antisense-oligonucleotide (AON) induced gene skipping is one of the most promising strategies for treating Duchenne muscular dystrophy, with the first generation of AONs advancing to phase 3 clinical trials. These AONs have been shown to induce specific gene skipping leading to increased dystrophin expression and muscle function in vivo. However, the efficacy of these AONs is limited by their poor pharmacokinetic properties, including poor targeting and uptake by key affected tissues such as the heart and diaphragm. Therefore, the next generation of AONs have been conjugated to *cell penetrating peptides* (CPP), which have shown early promise in pre-clinical studies. Several studies have already demonstrated enhanced pharmacokinetic properties of conjugated AONs together with improved tissue targeting and an overall increase in efficacy. The *in vivo* activity of conjugated AONs is sensitive to even small changes in the sequence of CPPs; therefore several modified peptide sequences have been generated for pre-clinical evaluation.

However, optimisation of these peptides is currently limited by the lack of non-invasive methods for evaluating the changes to biodistribution profiles *in vivo*. One promising method is the addition of small radioactive 18F ligands to the AONs and using PET imaging to monitor and evaluate the biodistribution of AONs *in vivo*.

**AIM**

The aim of this project is to develop a robust PET imaging platform for determining the biodistribution and pharmacokinetics of conjugated AONs *in vivo*.

**METHODOLOGY**

**PET biodistribution data:**

The PMO and PPMOs will be labelled with 18F ligand (Fig 2) and injected via tail vein in anesthetized mdx mice. For increased throughput three mice can be scanned simultaneously using a small animal PET imaging scanner (Fig 3). The animals will be scanned over a 2-3hr duration to collect the PET data, which will be processed to determine the biodistribution of these oligos (Fig 4).

**VALIDATION**

Validation of PET biodistribution data requires a method of directly quantitating PPMO levels in tissue lysate. Currently this is mainly done using basic HPLC methods, which have a detection limit of about 75nM. However, a novel ultrasensitive fluorescent ELISA method, has been described for quantitating phosphorothioate oligos in blood samples, with a far superior detection limit of about 50pM. Therefore, for the first time, this exciting new approach is currently being adapted for detecting PMO levels in tissue lysate (Fig 5).

**Importance of Pharmacokinetics:**

To accurately determine the biodistribution of PPMOs, it is essential that this is done when the distribution levels are at their peak. The most common method for determining this is by creating a pharmacokinetic profile of the compound from blood samples. Again this is currently done using inadequately sensitive HPLC methods. Therefore the ultrasensitive ELISA method is also being developed for quantitating PPMO levels in blood samples (Fig 5).

**REFERENCES / ACKNOWLEDGEMENTS**


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**SUMMARY**

PET imaging technique has great potential for being developed into a robust platform for determining the biodistribution of AONs. If successful this study will provide essential information regarding the potential efficacy and toxicity of new generation AONs, which will subsequently lead to more effective clinical therapies.

In addition to PET imaging other novel methods will also be developed including ELISA and HPLC/MS methods for determining pharmacokinetic and stability profiles of AONs *in vivo*.