Establishing strategies to meet the bioanalytical needs of Oligonucleotide Therapeutics in Pre-clinical Models and beyond

Neil Henderson, Nucleotide Bioanalysis Group, AstraZeneca Gothenburg
EBF 2015 Open Meeting, Barcelona
Bioanalytical needs of oligonucleotide therapeutics

Outline of presentation

• Introduction to Nucleotide Bioanalysis Group, AstraZeneca
  • Who are we & why are we here

• Oligonucleotide therapeutics
  • What are they and how do they work

• Overview of bioanalytical approaches
  • Oligonucleotide (PK bioanalysis)
  • modRNA derived-protein ((PK) PD bioanalysis)
  • modRNA (PK bioanalysis)

• Summary
**Bioanalytical needs of oligonucleotide therapeutics**

**Nucleotide Bioanalysis Group overview**

<table>
<thead>
<tr>
<th>Gothenburg NucBio Team</th>
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<td><img src="image.png" alt="Group Image" /></td>
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Group remit – expand the role of the (BA/TK) group beyond small molecules in **New Modality area** (e.g. RNA, Oligonucleotides and peptides) for toxicology and PK, PK/PD studies

<table>
<thead>
<tr>
<th>Bioanalytical work arena</th>
<th>Type</th>
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<tr>
<td>Investigational</td>
<td>GLP</td>
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<td>Formulation studies</td>
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<td>PK, PK/PD, TK</td>
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Gothenburg NucBio Team

**Rationale for group**

- Increased demand for pre-clinical bioanalysis from **New Modality projects**

**Mode of action of New Modality drugs**

- **Regulation of protein synthesis**
  - Oligonucleotides and active metabolites
  - ASO
  - anti-miR

**Increased induction of protein synthesis**

- mRNA
  - modified mRNA

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Setting the scene: DNA to mRNA to protein
Bioanalytical needs of oligonucleotide therapeutics

Setting the scene: DNA to mRNA to protein
Nucleotide Bioanalysis group (BA/TK)
Setting the scene: biological action of new modalities

Anti-miR (microRNA) inhibit the endogenous inhibitor; resulting in increased levels of target mRNA accessible for translation into protein.
Antisense oligonucleotide (ASO) blocks translation /increases degradation of target mRNA
Nucleotide Bioanalysis group (BA/TK)

Setting the scene: biological action of new modalities

Chemically modified mRNA (modRNA) functions as direct template for protein translation
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Setting the scene: Potential of New Modality drugs?

Potential to target any type of protein

Potential to target any region of body

Potential to target any disease area
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Setting the scene: what are we measuring?

Drug

- Oligonucleotides and active metabolites
- ARGO (Antisense Oligos)
- anti-miR (microRNA)
- human
- modified mRNA
- mRNA (messenger)

Pharmacodynamic (PD) endpoint

- Proteins and peptides
- Bioactivity (efficacy)

- rodent
- swine
- non-human primate

human

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Setting the scene: how are we measuring

- **Target**
  - Oligonucleotides and active metabolites
  - Proteins and peptides
    - Protein (human)
    - Protein (non-human)
    - Induced
    - Endogenous

- **Method used**
  - LC-MS
  - LC-MS
  - LBA
  - Branched DNA (bDNA)

- **Future methods**
  - HPLC-FL
  - Hybrid (PCR)
  - ELISA
  - Quantitative real time PCR (qRT-PCR)

**modified mRNA**
- Human
- mRNA

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Setting the scene: demands on data generated

Choosing method depends upon

- Preclinical species
- Matrix type
- Matrix volume
- Target
- Method approaches available
- Analytical throughput
- What the data will be used for
- Quality standard required
- Hypothesis being tested

Quality standards depend upon

- Investigational
- GLP
- Wider acceptance criteria
- Line of sight
- Regulatory guidelines
- Meet internal quality demands
- Tiered approach
- Meet external quality demands

Speed, quality, direct the science, enhance the project understanding

Q1 2016

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Case studies:

- PK Measurements (oligonucleotides and active metabolites)
- (PK) PD Measurements (modRNA)
- PK Measurements (modRNA)
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Case studies:

• PK Measurements (oligonucleotides and active metabolites)

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Oligonucleotides and active metabolites

Proteins and peptides

modified mRNA

ASO

anti-miR
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Case study 1: Oligonucleotide PK analysis

Use of LC-MS

Q3 2015

Develop a method to analyse investigational PK dose of Oligo Y

Targeting moiety helps direct oligo

Average oligo size approx. 20 nucleotides

"clean" profile of entire molecule + metabolites

Signal intensity

Retention time

Expect high concentrations of drug to build up in tissues

Current LC-MS methods are Fit for Purpose

ASOs have chemical modifications for increased stability

Focusing on the modifications allows identification of "cleaner" profiles

Challenges with LC-MS methods

Sample preparation steps take time

Rate of Column turnover

Sharing equipment with small molecule bioanalysts

Risk of instrument contamination

Potential for Carryover

Dedicated LC-MS solely for oligo work is desirable

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Case studies:

• PK Measurements (oligonucleotides and active metabolites)

• (PK) PD Measurements (modRNA)

• PK Measurements (modRNA)

Oligonucleotides and active metabolites

Proteins and peptides

modified mRNA

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Case study 2: Establishing suitable assays

Balancing the right method with the question that requires answering is important

- Formulation experiments need quick turnaround of data
- Timecourse experiments
- Small in vivo sample sizes

Identifying a suitable assay

- Assay to measure total level of Protein W
- Purified protein spiked into (large volume of pooled) plasma or tissue homogenates (in vitro)
- Can we identify assays with suitable qualities including low sample volume and large dynamic ranges?

Screening assays

Low sample volume

- Volume
  - 15 uL (LBA Y)
  - 50 uL (LBA X)

Large dynamic range

- Dynamic range
  - 10 - 10000 pg/mL (LBA Y)
  - 30 - 2000 pg/mL (LBA X)

LBA-Y has desirable assay properties for single ("one shot") analysis batches

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Case study 3: Establishing suitable assays

Detecting full length proteins

Degradation of an encoded protein can lead to inactivation of a protein

- Full length protein
- Protein (n – 5 amino acids)

Functional

Non-functional

May need methods that are specific for full length proteins

Digestion of the protein into discrete fragments followed by LC-MS

LBA approach using an antibody pair that are raised against N-terminus and C-terminus

- Commercial reagents/kit available
- Generate in-house reagents

Direct correlations between circulating protein level and biological activity over time can then be demonstrated

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Case study 4: Establishing suitable assays

Adapting to situation & innovating where possible

Q1 2015
Measure secreted protein-V

Only had one anti-protein V polyclonal antibody

Unique target
No commercial reagents

In-house polyclonal antibody

Q3 2015
New antibody produced in-house

Now have potential to make a “sandwich” LBA

In-house polyclonal antibody

New monoclonal antibody

Gyrolab method used
3 hr turnaround
No sample clean up
Small sample volumes

LC-MS method used
2 day sample prep

An assay with quicker turnaround time was desirable for screening

Immunoaffinity column purification with polyclonal antibody had limited success

Multiple assays are used within the same project depending upon the question being asked

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Case studies:

• PK Measurements (oligonucleotides and active metabolites)

• (PK) PD Measurements (modRNA)

• PK Measurements (modRNA)
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Case study 5: PK analysis of (modified) mRNA

Branched DNA (bDNA) method

ELISA-like workflow

Incubation steps

Wash steps

Add a chemiluminescent substrate

Specifically capture mRNA

Amplify signal

Read catalysed substrate
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Case study 5: PK analysis of (modified) mRNA

How are we using bDNA method?

Purified modRNA for use as Reference Standard & QCs

Instrumentation

Luminometer

Not a dedicated RT-PCR platform

Dynamic range

Rt-PCR

bDNA

2.5 – 250 fg/ul

Sensitivity

vs.

Throughput

Evaluation criteria

Plasma / Tissue

Non encapsulated

Encapsulated

Non protected

Protected

Nuclease-free buffer only

Matrix matched vehicle

Drug

modRNA

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Take home message

An exciting year in the life of NucBio Group

Huge Bioanalytical Demand for New Modalities

One size approach fits all project requests

Tiered approach to evaluate assays to meet questions asked

Learnings from previous Method Approach X

Method Approach 1

Method Approach 2

The next step in project

Appropriate method for task

Quality data to Project team to aid decisions on progression of project
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Summary

• New Modalities are an exciting area of drug discovery/development with potential to treat any disease
• Bioanalytical approaches can be simple or complex depending upon the needs of the project
• A combination of bespoke methods and commercially available kits are used
• Applying a “fit for purpose” tiered approach strategy generates confidence in assay performance and data quality for Investigational studies and GLP studies
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  - Glen Hawthorne

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  - Petra Thulin
  - Daniel Linden

- Collaborators
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Setting the scene: biological action of new modalities

**Antisense oligonucleotide (ASO)**
blocks translation / increases degradation of target mRNA

**DNA**

**nucleus**

**mRNA**

**protein**

Anti-miR (microRNA)
inhibit the endogenous inhibitor; resulting in increased levels of target mRNA accessible for translation into protein

**miR**

**mRNA**

Chemically modified mRNA (modRNA) functions as direct template for protein translation

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