Oligonucleotides: The Next Big Challenge For Analytical Chemistry

Chromatographic Society Symposium at GSK Ware 27 and 28th October 2010

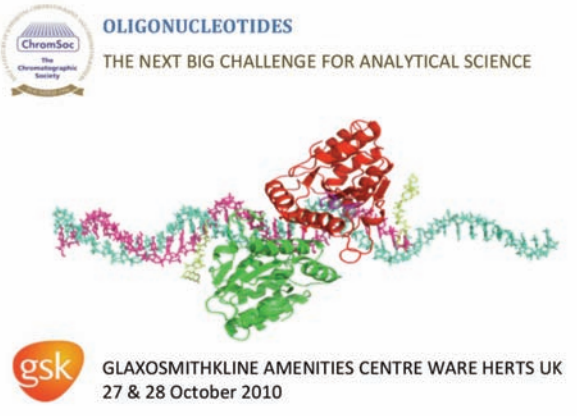
*by George Okafo[1] & Chris Bevan[2],**

[1]Scinovo, GSK, Ware, SG12 0DP - [2]The Chromatographic Society, c/o Meeting Makers, Jordanhill Campus, 76 Southbrae Drive, Glasgow, G13 1PP

**Corresponding author: chromsoc@meetingmakers.co.uk*

Research and development of oligonucleotides as therapeutic medicines is experiencing exponential growth and interest within academia, the biotechnology and pharmaceutical industries [1]. This has largely been driven by several key historical events including:

1. The regulatory approval of the first antisense oligonucleotide (ASO) for cytomegalovirus infection Vitravene® (1998)[2],
2. Approval of Macugen®, an aptamer for treatment of wet macular degeneration (2005) [3]
3. The discovery by Drs Andrew Fire and Craig Mello of gene silencing by RNA interference (RNAi) (published 1998, Nobel Prize, 2006).[4]



RNAi technology has been used to validate gene targets, and both RNAi and ASO technologies have been used to interfere with targets that are considered non-druggable by traditional small molecule interventions [5]. The early Nobel Prize-winning work by Fire and Mello demonstrated that some genes in C.elegans can be switched off by the introduction of double stranded small interfering RNA (siRNA) to block the normal translation of a specific messenger RNA to the protein product [4]. This early identification of the process has been extended to mammalian cells and the molecular process characterized.

Oligonucleotide therapeutics

Oligonucleotide therapeutics can be divided into a number of different classes based on mechanism of action;

1. siRNA's and microRNA (miRNA) use RNA interference to degrade messages via a phylogenetically conserved regulatory mechanism [6][7]

Anti-sense oligonucleotides (ASO) working either through steric hindrance or RNA degradation via RNAase H degradation of double stranded RNA.[8] Receptor or soluble mediator antagonism using single stranded oligonucleotide ligands such as aptamers and speiglemers.[9] Each oligonucleotide class has a different mechanism of action; some work by blocking a specific gene (siRNA, ASO) [6] [8] or family of genes (miRNA) to down-regulate the production of a harmful protein [7], others stimulate the body's immune system (sequence dependant ASO and siRNA) [10] or work by interacting with a specific gene sequence to restore function to a defective protein (exon skipping ASO) [11]. As scientific knowledge of oligonucleotides grows coupled with increased evidence of clinical efficacy of these compounds as drugs, the pharmaceutical industry has considered it worth investing billions of dollars to develop oligonucleotide-like drugs to treat diseases ranging from viral infections [12], immuno-inflammation [13] and certain cancers (acute myeloid luekeamia) [14] to rare diseases like Duchenne muscular dystrophy [11], thrombic thrombocytopenic purpura [15], thought to be caused by genetic mutations.

The analytical challenge

Unlike small molecule NCE's, oligonucleotides present some uniquely different analytical challenges due to their larger size (>3kDa and between 20 and 40 units in length) and multiple charges. Also, oligonucleotides can adopt complex secondary structures involving highly specific folding and other intra-molecular conformations which play a vital role in their biological activity. Traditional approaches have used in-vitro bioassays to measure biological activity as an indirect measure of secondary structure. Nowadays, more modern analytical approaches are being developed to complement these indirect methods and to provide a more direct assessment of oligonucleotide conformation, not only in the formulated drug product, but also in biological systems. To meet these challenges, analytical chemists have had to develop novel state-of-the-art analytical solutions to address the types of scientific problems encountered in oligonucleotide R&D. For these reasons it is timely to hold a symposium to share current best practices in the analysis of these challenging molecules.

The Symposium

This highly topical symposium has attracted international specialists and pharma experts in this area who will be describing their work and revealing the analytical challenges they face.

- Dr. Vesela Encheva (LGC, Middlesex, UK) describing the characterisation of oligonucleotide mixtures using electrospray ionisation mass spectrometry (ESI MS). Her work has demonstrated the utility of ESI MS for the detection and base composition determination of oligonucleotides ranging in size from 80 to 200 base pairs. Her methodology has been directly applied to real samples and successfully used for genotyping human mitochondrial DNA.
- Ken Cook (Dionex, UK) will describe high resolution ion exchange separations of a broad spectrum of oligonucleotides with automated off-line desalting for MS. Particular reference will be made to aberrant linkage isomers of RNA, thioation and an automated off-line desalting protocol for MS which can be introduced as a second dimension separation. Separation of a broad spectrum of oligonucleotides is possible and examples of control of the separation by pH, temperature and counter ion used will be given.
- Dr. Mark J Dickman (ChELSI Institute, University of Sheffield, UK) will explain the principles and applications of RNA chromatography. Mark has elucidated the mechanism of the separation of RNA using ion pair reverse phase chromatography. The high-resolution separation of double stranded (ds) RNA was observed, in a similar manner to dsDNA under non denaturing conditions. The versatility of the application of RNA chromatography has been extended to a wide range of important applications including; purification of synthetic oligoribonucleotides.
- Resident scientist Dr George Okafo and colleagues from Scinovo, GSK, Ware are developing radio-labelling strategies for synthetic therapeutic oligonucleotides and will describe their procedures in some detail. Also from GSK, Nigel Richardson and Paul Newstead will discuss the practical implications for developing related impurity HPLC/UHPLC methods for thiolated oligonuleotides and will present the findings from their investigations-.
- Dr. Nadim Akhtar (Astra Zeneca) will discuss pulmonary delivery of Oligonucleotides; focussing on analytical, formulation and regulatory considerations in early development.
- From Roche Kulmbach GmbH, Dr. Bernhard Noll will present various analytical methods used in the siRNA drug development process, with a special focus on HPLC-MS. Examples of in-depth data analysis will be presented and the challenges associated with process development, method development and transfer will be demonstrated using case studies. Setting of release specifications with regard to regulatory requirements will also be discussed.
- Dr. Kathy Ackley from Girindus will describe the use of orthogonal analytical methods for analysis of impurities in oligonucleotides.
- Dr. William van Dongen (Proxylab, Netherlands) will describe the development of UPLC methods for characterisation of synthetic oligonucleotide drugs. Synthetic oligonucleotides are short nucleic acid chains, typically 15-35 nucleotides long, which can direct gene expression in a sequence specific manner. This class of therapeutic agents target the diseases literally at the nucleus resulting in a broad therapeutic range. In his presentation, the process of analytical method development and qualification for oligonucleotide products will be outlined.

Any analyst who wishes to stay at the forefront of the subject will benefit from the knowledge shared at this symposium and should make every effort to attend. For details on how to register for this exciting symposium, please contact The Chromatographic Society through our secretariat Meeting Makers using the contact details listed above.

References

1. *K. Appasani, Pharmacogenomics, 2007, 8(8), p889-893*
2. *US FDA: Drug approval package:vitravene (fomivirisen sodium intraveal injectable) injection; www.fda.gov/cder/foi/nda/98/20961_vitravene.html*
3. *US FDA:FDA approves new drug treatment for age-rlated macular degeneration; www.fda.gov/bbs/topics/news/2004/new01146.html.*
4. *DP Hamilton, R. Zimmerman, Nobel Discovery Already Sparked Hunt for Drugs, Wall Street Journal, Oct 3rd 2006.*
5. *E. Bellott, European biopharmaceutical Review, 2008, 44 - 52.*
6. *Castanotto D and Rossi JJ. Nature 2009; 457(7228);426-433*
7. *Petri A, Lindow M and Kauppinen S.. Cancer Res. 2009;69(2);393-395.*
8. *Sahu NK, Shilakari G, Nayak A and Kohli DV,. Curr. Pharm. Biotechnol. 2007;8(5);291-304.*
9. *Purschke G, Radtke F, Kleinjung F and Klussmann S. Nucleic Acids Res. 2003; 31;3027-3032.*
10. *Kreig AM.. Nat Rev. Drug Discov. 2006; 5(6);471-484.*
11. *A. Aartmus-rus, W.E.Kaman, J.T. den Dunnen, G.J. van Ommen, J.C.van Deutekim,.Mol.Ther.14(3) (2006);401-407.*
12. *S. Agrawal: Trends Biotechnol. Sci. 10 (1992) 3499-3507.*
13. *K. Sobczak, N. Bangel-Ruland, J. Semmler, H. Lindemann, R. Heermann und W.-M. Weber , HNO (2009), 1106 – 1112*
14. *G. Degols, J.P. Leonitti, N. Mechti, B. Lebleu. Nucl. Acids. Res. 19 (1991) 945-948.*
15. *M.D. Joel Moake, Best Practice & Research Clinical Haematology 22(4) (2009) 567-576.*