



THE JOINT PHARMACEUTICAL ANALYSIS GROUP

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JPAG Symposium at the APS UK PharmSci Conference 2014

University of Hertfordshire, Monday 8th September 2014

New medicines: future benefits and current analytical challenges

Scope:

This symposium reviewed some of the challenge for the analytical scientist arising from developments of new medicines and therapeutic regimes.

Six invited speakers gave their presentations at this event.

George Okafo; *Consultancy Director (SciNovo PTS), RD Platform Technology and Science, GSK* described the **Quality and regulatory challenges in characterising oligonucleotides**. The regulatory requirement had grown substantially over the past ten years, reflecting the requirements for characterisation of these compounds and the impurities. In the ten years there have been only three products approved – Vitravene, Macugen, and Kynamro. Currently many products are under development, generally falling into one of four classes; antisense, Si-RNA, immuno-stimulatory, aptomers.

Mr Okafo reviewed the phases in the development of a new medicine and the safety, efficacy, purity and consistency requirements. Oligonucleotides are classed as synthetic compounds; although they are not covered by ICH3 or ICH6, the ICH guidance still applies, as do the NCE quality standards.

Oligonucleotides present substantial analytical challenges because of their complex physico-chemical profiles and multi-stage chemical synthetic pathways. The starting materials are complex, impurities can combine to form "families" of impurities and "shortomers" can result from multiple causes. During synthesis, impurities arise from the starting material, the synthesis can produce short-mers and long-mers, sulphurisation can occur and trityl impurities form. Degradation produces other impurities.

Oligonucleotide separation methods include anion-exchange, ion-pair reverse phase chromatography and capillary gel electrophoresis; these techniques may cause denaturation. Mr Okafo described the use of "control points" as impurity fate mapping (to establish the limits of critical impurities), chemical in-process control (to understand the quality attributes of the drug substance and the reagents), and chain elongation (and impurities are not single components but are grouped by structural class and the analytical method chosen to resolve the parent from each structural class). He provided some examples of specifications applied to several examples of oligonucleotides.

In summary, the specifications for oligonucleotides evolve during the clinical development programme; the regulators will apply the ICH3 principles; plan control strategies early in development for detection and identification of impurities and continue throughout the development cycle.

Elena Bichenkova, *Reader in Medicinal Chemistry, University of Manchester*, gave a presentation on the **Characterisation of oligonucleotides and their analogues by spectroscopic methods**. These compounds, like small molecule compounds, must be well-characterised before regulatory approval is granted. The properties of these large molecular weight amphipaths with a high negative charge makes characterisation particularly challenging.

Spectroscopic methods such as UV-visible and fluorescence spectroscopy (including labelling with a fluorophor) allow hybridisation to be detected. Thermometric analysis with UV detection is used to detect hybridisation.



MS analysis is challenging because of the polyanionic structure; a soft ionisation method must be used for these labile compounds and the upper mass limits are 50,000 Da for MALDI and 120,000 Da for ESI, up to 120-mers. MALDI generates single charged ions which are relatively easy to interpret and can be used for analysing complicated mixtures.

NMR is also used and Dr Bichenkova showed typical ^1H NMR spectra of some oligonucleotide analogues, relating these to an understanding of the non-exchangeable protons occurring in some important amino-acids (guanine, adenine, etc). The main challenges to interpretation were the large number of protons generated, highly over-lapping resonance regions. J-coupling interactions arise from aromatic and CH_3 protons and sugar rings. 2-D NMR techniques are sometimes helpful, either using COSY [**C**ORrelated **S**pectroscop**Y**], or NOESY [**N**uclear **O**verhauser **E**nhancement **S**pectroscop**Y**]. ^{31}P NMR was also a useful tool. Overall NMR is the least sensitive technique but it does provide useful information for structural elucidation.

Hyphenated techniques for purity and identity include HPLC-MS, HPLC-NMR and HPLC-NMR-MS. Solvent suppression can be achieved through pulse sequencing. Techniques of structure elucidation were presented and the merits of HPLC-NMR and HPLC-MS were discussed. Each aspect was illustrated with reference to examples.

Jon Paul Sherlock, *Director, Product Development UK/US, AZ*, presented the **Regulatory challenges around novel manufacturing methods**. Mr Sherlock pointed out that it was generally recognised that there is a clear demand for pharma to move towards continuous processing and questioned why industry currently manufactures medicines the way it does. Can the industry afford not to change? The reasons for change were then outlined, including factors such as societal benefits (including environmental impact), before considering the regulatory perspectives. Challenges for implementation were discussed as different approaches are necessary in different situations and the necessity for regulatory acceptance was highlighted.

One of the main constraints to adopting a more continuous process is ensuring there is a clear understanding of the steps involved. Continuous processing can save time but potential confusion must be overcome to ensure it is successful. The need for pre-competitive collaboration was highlighted if the traditional methods of batch processing are to be replaced in the future. The presentation was well received by the audience and clearly of interest to those within the pharmaceutical industry.



Jon Paul Sherlock, AZ



*Zahraa Al-Ahmady,
University of Manchester*

Zahraa Al-Ahmady, *Nanomedicine Lab, Faculty of Medical & Human Sciences, University of Manchester* gave her presentation on **Theranostic design**, and introduced the topic by discussing the previously established proven safety profiles of liposomal cancer therapeutics. She identified that there are two particular limitations with this technique, namely heterogeneous extravasation and limited bioavailability. Ways to overcome the former were discussed using engineered image-guided liposomal vesicles, and the use of tumour-specific triggered drug delivery to overcome bioavailability problems was described. Several references were made to papers currently under review, highlighting the importance of this work and also its current relevance for cancer therapeutics.

Joanne Cooper, *NDA Analytics* gave her presentation on **Addressing the Antibody Drug Conjugate potency assay challenge**. Antibody Drug Conjugates or ADCs represent an emerging class of oncology drugs comprising a cytotoxic payload molecule linked chemically to a tumour-targeted monoclonal antibody. After internalisation of the ADC, the linker may be cleaved to release the cytotoxic drug. Dr Cooper emphasised the regulatory requirement for ADCs to be characterised with potency assays based on relevant biological properties related to both the safety and efficacy of the product, pointing out that the effector function or biological activity of the monoclonal antibody may also contribute to the overall mechanism of action.

Dr Cooper went on to illustrate the characterisation of Trastuzumab (Herceptin) ADCs in a short case study, including the use of Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) and Complement Dependent Cytotoxicity (CDC) assays, along with anti-proliferative assays using pertinent breast cancer cell lines such as SK-BR3. Concluding her talk, Dr Cooper highlighted the expectation that the final validated potency assay for release and stability testing should be capable of discriminating product quality changes such as glycosylation, aggregation or higher order structure.



To complete the presentations, Simon Hollingsworth, *Oncology Innovative Medicines & Early Development, AZ* gave a fascinating account of **Moving towards a personalised future of 21st Century healthcare** and the implications for pharmaceutical scientists. In comparison to sixty years ago, the ability to stratify cancers today according to their molecular and genomic signatures, and then design targeted therapeutics, has revolutionised the oncology landscape and created better medicines for patients.

For example, both Vemurafenib (late stage skin cancer) and Crizotinib (late stage lung cancer) received accelerated approvals due to clear efficacy and the ability to identify only those patients likely to respond, using companion diagnostic tests. Whereas this new paradigm reduces drug development costs and attrition rates dramatically, stratification (by definition) also creates challenges for clinical trial recruitment from ever-smaller patient populations. To overcome this, Dr Hollingsworth described novel collaborations between multiple Pharma companies whereby the trial is chosen for the patient instead of choosing the patient for the trial.

An example of such a so-called 'basket' trial is the MATRIX National Lung Trial in collaboration with Cancer Research UK, where patients can be allocated post-screening to one of 14 treatments from AZ or Pfizer. As well as providing better trial options for patients, 'basket' trials also allow cost-effective parallel testing of multiple therapeutic hypotheses and rapid detection of efficacy signals. In conclusion, Dr Hollingsworth noted that innovative trials will become the norm, enabling wider and earlier patient access to new therapies while simultaneously expediting development and regulatory pathways.

Presentation of the Geoffrey Phillips Analytical Science Award

****** *indicates that the presentation is eligible for this Award*

Noting the high quality of all the presentations, the JPAG Chairman announced that the 2014 winner of the Geoffrey Phillips Analytical Science Award was:

Amira Guirguis, School of Life and Medical Sciences, Department of Pharmacy, University of Hertfordshire



Amira Guirguis receiving the Award certificate from Andy Teasdale, the JPAG Chairman.

Submitted papers for podium presentation

Five contributed papers were given by younger authors, four ** of which were eligible for consideration for the Geoffrey Phillips Analytical Science Award. Dahlia Salman was the 2013 Award winner and was therefore not eligible for the award.

To determine who would be the winner of the JPAG Geoffrey Phillips Analytical Science Award for 2015, the speakers were assessed on the scientific content of their talk, the clarity and ease of understanding of the content and also in the way the speakers responded to questions.

1. Analysis of a Bristol amnesty bin as an indicator of current drugs trends **

Majdah Alotaibi, *Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, U.K*

Majdah Alotaibi described how she had used ^1H and ^{13}C NMR spectroscopy as the main analytical technique supported by electrospray ionisation-mass spectrometry (ESI-MS). 2D NMR (15N, H2BC, HMBC, DEPT) analysis in some cases to provide additional analytical data. She had determined that the samples contained drugs from different classes, class A (cocaine, crack cocaine, ecstasy), class B (mephedrone, flephedrone, cannabis), and class C (ketamine) as well as Legal highs, medicinal and herbal (cannabis) drugs. She concluded that analysis of amnesty bins like this would provide the police with useful intelligence information to aid better drug control.

2. A rapid in-vitro screen method for assessing the potential for precipitation of solubilised drugs in the small intestine **

J.S. Dickens, W. Lin, A. Patel & J. McDermott, *Quotient Clinical, Nottingham, UK*

Jordan Dickens described the use of solubilisation technologies for BCS II drugs can often lead to a window of super-saturation and precipitation within the small intestine, the degree of which is important for determining the exposure achieved for this class of drugs. He described how the "Spring and Parachute" effect by using precipitation inhibitors was becoming increasingly important. Jordan then described his work developing a rapid screening method to assess the impact of excipients on drug solubilisation and precipitation in a way that more accurately reflects the physiological process of gastric emptying than a traditional dissolution. He achieved this by utilizing a phase switch followed by monitoring the precipitation profiles of the drug from the different formulations.

3. Investigating the relationships between rapid thermal analyses and forced degradation using a complex, multi-domain protein as a model **

M.J. Robinson¹, P. Matejtschuk², C. Longstaff², P.A. Dalby¹, ¹*Department of Biochemical Engineering, UCL, Torrington Place, London, WC1E 7JE;* ²*National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, EN6 3QG*

Matthew Robinson described an increased need to understand and predict protein stability. In his work he utilised a multi-domain protein, tissue plasminogen activator (t-PA), which exhibited different phenomena in differing pH environments, to investigate whether the melting temperature (T_m) of a protein together with several orthogonal biophysical techniques, could be used to predict stability for complex proteins. He showed that correlations could be drawn between thermal scan experimental data and time-course experiments in terms of structure, monomer content and activity retention, thus predicting stability.

4 Investigation of 'legal high' substances, common cutting agents and adulterants using portable Raman spectroscopy **

A. Guirguis¹, S.B. Kirton¹, S. Fergus¹, M. Zloh¹ and J.L. Stair¹

¹*School of Life and Medical Sciences, Department of Pharmacy, University of Hertfordshire, Hatfield, UK*

Amira Guirguis described the use of handheld Raman spectroscopy to identify 'legal high' products, a technique that would be extremely useful in the field due to its portability. She obtained the spectra of 'legal highs', common cutting agents and adulterants tested in different packaging, batches and formulations. Comparing these to library signatures she was able to demonstrate that the technique showed promise in successful identification. However, she concluded that further work would be needed to optimise the classification methods for recently banned and internet 'legal' products.

5. The potential of NMR in oncology pharmacy: a forced degradation study of ifosfamide
D. Salman, J.-M. Peron, T. Goronga, J. Swinden, S. Barton and S. Nabhani-Gebara, *School of Pharmacy and Chemistry, Kingston University, London, UK*

Dahlia Salman, a previous winner Geoffrey Phillips Analytical Science Award, described how issues had arisen upon stability testing of IfosM over 19 days, where the methods provided results that conflicted with those from previous studies. Dahlia described her study aimed to investigate the robustness of analysis methods by force degrading Ifosfamide and analysing samples by HPLC and NMR in parallel. NMR (¹H, ³¹P) was used to investigate the degradation pathways and assist in structural elucidation of any detected products. She concluded that her results suggested that previously published HPLC methods for Ifosfamide did not detect the degradation products that were shown by NMR.

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