European Bioanalysis Forum

European Bioanalysis Forum, 1st Open Symposium: "Burning Issues in Bioanalysis"

1–2 December 2008, Hilton Hotel, Barcelona, Spain

The European Bioanalysis Forum (EBF) is a bioanalytical discussion group comprised of European pharmaceutical companies (26 members to date). The membership shares a common vision to advance the shared understanding of topical concerns through discussion of scientific, technological and regulatory issues of bioanalytical interest. The objective of the open symposium was to reach out to the broader European and global bioanalytical community, to report back on discussions EBF member companies have during their biannual closed meetings and, going forward, to provide guidance and recommendations to the European and global bioanalytical community. Pharmaceutical and biotechnology companies, contract research organisations (CROs), academia and instrument vendors were invited to attend to exchange scientific ideas and information on topical issues of mutual concern. The symposium included sessions on assay validation, metabolite quantification, instrumentation developments and protein quantification and immunogenicity. Experts and key opinion leaders were invited as guest speakers. A total of 251 delegates attended, representing a large percentage of the European bioanalytical community. The majority of attendees represented pharmaceutical companies (36%), CROs (42%), instrument vendors (15%) and academia (4%). In addition to 18 oral presentations, 51 posters were presented and there was a well-supported vendor exposition.

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Assay validation

The initial conference presentation was made by Silke Luedtke (Boehringer–Ingelheim, Germany), representing the European Bioanalysis Forum (EBF) and this covered the results of a survey among EBF member companies into best practices in method development and validation. There appears to be a high degree of common understanding due to extended discussion, except where topics are less well specified in the guidelines [1,2], such as acceptable carryover and the definition of outlier tests.

The next three speakers all focused on the hot regulatory bioanalytical topic of incurred sample reproducibility (ISR). Philip Timmerman (Johnson and Johnson, Belgium), representing EBF, presented the current EBF consensus following several surveys throughout 2007 and 2008. Interestingly, of 220 clinical studies conducted by EBF member companies to date that included ISR determination, only eight studies failed. EBF intends to publish the current consensus later in 2009. Binodh DeSilva (Amgen, USA) presented the Amgen position on ISR for macromolecules. Standard operating procedure (SOP) drafting for ISR is an ongoing process (currently working on revision 3) and an additional SOP on event investigation and resolution is also in production. The processes in place closely resemble the EBF position; however, in addition, they use a modified Bland–Altman statistical approach for the evaluation of acceptance criteria. Four example case studies were presented. The belief at Amgen is that the more thorough the method validation that is performed initially the less likely there are to be ISR problems [3], especially if focus is given to potential selectivity issues regarding the matrix from special populations. Finally, Michael Skelly (CDER, US FDA) discussed the latest FDA perspective on ISR. He stressed that FDA is now inquiring about ISR programmes and results during inspections and some deficiencies have been noted (e.g., programmes not yet implemented; acceptance criteria too liberal; sample size for reanalysis too small). The FDA recommends 5–10% reanalysis of study samples, documentation for ISR in all bioequivalance studies and if ISR criteria are not met a thorough investigation should be initiated.

To complete this session, Stephen White (GlaxoSmithKline, UK) described a new approach to the collection and handling of

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blood samples in bioanalysis. Dried blood spot technology, where blood is spotted on to a collection card coated with a lysing agent that denatures the blood proteins, has been around in clinical laboratories for more than 40 years. The cards are air dried and stored/shipped at room temperature, with discs being punched out of the dried blood spot for subsequent extraction and liquid chromatography (LC)–mass spectrometry (MS)/MS analysis. There are many potential advantages – small blood volumes (15 µl) being the main benefit for both preclinical and clinical studies along with simplified sample handling and shipping processes and cost reduction. The technique has been validated using more than 50 structurally diverse compounds and use includes investigational new drug (IND)-enabling studies and a first-time-in-human study (ongoing). The reported validation data looked encouraging, particularly the stability data with evidence of improved stability for metabolites and known unstable analytes.

Metabolite quantification

This session opened with a presentation from David Evans (Johnson and Johnson, USA, representing PhRMA) on the FDA Metabolites in Safety Testing Guidance (published February 2008) [4]. The guidance follows several years of debate. It recognises that a metabolite formed only in humans is rare, though there is greater potential to form metabolites at a disproportionately higher level in humans than in preclinical species. Two types of toxic mechanisms involving chemically stable metabolites and reactive metabolites have previously led to market withdrawals or black box warnings. The present guidance focuses on circulating metabolites that are at least 10% of parent drug systemic exposure at steady state but reactive metabolites could also elicit safety concerns as they can form drug–protein adducts, which potentially disrupt protein function or elicit an immune-based response, yet their formation is not always evident from the analysis of plasma only.

Dieter Zimmer (Novartis, Switzerland, representing EBF) presented an EBF survey on current member practice regarding metabolite quantification. The consensus appears to be that strategies are in place to quantify metabolites beginning in preclinical or clinical development. A tiered approach is adopted, with most companies (65%) first applying a fully validated method for metabolite quantification in the first regulatory preclinical study, with the metabolite methodology being completed after the human mass balance study.

Ronald de Vries (Johnson and Johnson, Belgium) and Richard Weaver (Servier, UK) completed this session, presenting their company perspectives on the topic with additional details of the UK Drug Metabolism Discussion Group position from Richard Weaver. Ronald described a tiered approach (screening, qualified and validated assays), which is endorsed by the Crystal City III conference report [2]. It is important to understand which quantification approach should be used at what stage and to understand the difference between qualitative, estimated and quantitative results. Richard's presentation included a metabolite classification similar to the one presented by David Evans (above). At Servier, the objective is to identify metabolites early and establish their metabolic fate in the human radiolabelled study (Phase I). Regarding bioanalysis, the aim is to perform an initial preliminary assessment with subsequent validation and to have a single assay for both parent and metabolite(s) of interest.

Technology developments

The evening speaker on day 1 was Josephine Bunch (Centre for Analytical Sciences, University of Sheffield, UK) who presented a fascinating talk on the use of imaging mass spectrometry and the scope and opportunity for elemental imaging via LA-ICP-MS. The laser ablation technique is well suited to both the acquisition of spatially resolved measurements of endogenous elements in both soft and hard tissue and to the measurement and mapping of metallodrugs, providing an alternative strategy to radiolabelling and autoradiography in preclinical investigations. In addition, measurement of element-tagged antibodies permits the measurement and mapping of peptides and proteins in biological tissue, previously the preserve of molecular MS. This application was illustrated by reference to the mapping of β-amyloid (and associated metal ions) in Alzheimer plaques and the imaging of cancer biomarkers (HER2 and MUC1) in breast cancer biopsies.

Each of the major MS instrument vendors – Waters, Agilent, ABI and Thermo – presented the latest innovations in MS.

The theme from each of the vendors was surprisingly uniform. All focused on data quality and greater sensitivity with three of the four vendors describing their particular

instrumental variation (the Xevo™ TQ MS, the Sciex QTrap® 5500 and the Orbitrap™) regarding the ability to acquire simultaneously both quantitative and qualitative data, clearly a helpful development in the era of ISR and the need to identify potential interfering metabolites. Agilent described their HPLC-Chip/QQQ system. The chip includes the analytical column (0.075 mm internal diameter), the enrichment column and the sprayer tip with nl/min flow. It has multiple potential applications from small to large molecules and in the drug metabolism pharmacokinetics area large sensitivity gains and cost savings are attractive advantages. The Agilent group have applied this technology to the dried bood spot system described by Stephen White, demonstrating that the possible sensitivity using the two approaches together can be as low as 50 pg/ml for a 10 µl blood sample.

Large molecules

The session was opened with a presentation from Isabel Buettel (Paul-Ehrlich-Institut, Germany) on the Commitee for Medicinal Products for Human Use Guideline on the 'Immunogenicity Assessment of Biotechnolgy-derived Therapeutic Proteins'. She first discussed the factors influencing immunogenicity after the application of a highly complex molecule to humans: considerations such as the 'foreign' character and size of the molecule, post-translational modifications, dose and length of treatment and, last but not least, process- and product-related impurities like host cell protein residues. Focusing on the European regulator's perspective on the influence of immunogenicity on efficacy and safety of the drug product, she discussed patient- aswell as product-related risk factors and their regulatory consequences. Finally she presented recommendations for an assay strategy to distinguish between neutralizing and non-neutralizing antibodies. Geoff Hale (BioAnaLab, UK) summarized the recommendations given by the CHMP immunogenicity guideline [5], several White Papers published by the AAPS [6–9] in the last few years and the view of his clients, respectively, on selected issues concerning screening and confirmatory assays as well as on validation procedures. He encouraged the audience to express its opinion on cut point definition, error rate and sensitivity used in a screening assay and discussed the pros and cons of the assay format used to confirm positive screening results. Hendrik Neubert (Pfizer, UK) completed the session by giving an insight in to protein quantitation by mass spectrometry using hyphenated techniques and compared the results with the data determined with immunological methods. In one of the presented case studies he focused on the determination and quantitation of biomarkers, an example that is challenged by the endogenous levels of the respective biomarker and requires a highly sensitive method. Hendrik demonstrated that highly sophisticated sample preparation techniques including isolation, digestion and purification steps allow the reproducible quantitation of large proteins by LC–multiple reaction monitoring to support clinical and preclinical studies.

Summary

Overall, a stimulating 2 days of presentations, discussions and networking on the dedicated topic of bioanalysis was conducted. The Steering Committee are planning the 2009 meeting already, so please keep the dates clear in your calendar: 2–4 December 2009, Barcelona, Spain. We look forward to welcoming you.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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