

**Drug Metabolism and Pharmacokinetics Interest Group Report of the Interest Group Workshop
63rd ASMS Conference, May 31 to June 4, 2015 St. Louis MO**

More DMPK Knowledge from Less Sample – Leveraging Modern LC-MS Instruments for Small Sample Amounts

The Drug Metabolism and Pharmacokinetics (DMPK) Interest Group Workshop was held on Monday June 1 from 5:45 to 7:00 pm. Coordinator Mustafa Varoglu and Co-Coordinator Kevin Bateman led the meeting by introducing the session. Approximately 110 scientists attended the workshop demonstrating interest in drug metabolism and pharmacokinetics. An expert panel shared their perspectives to spur discussion on the workshop topic. The strong attendance and active attendee participation in the discussion provide a good endorsement for continuing the DMPK-IG in future years.

A brief business meeting was held at the beginning of the workshop to review the status of the current Oral Sessions, solicit ideas for future DMPK Oral Sessions and Workshops, as well as a call for nominees and volunteers for future DMPK-IG sessions.

1. Review of the DMPK IG Goals

The DMPK Interest Group goals of providing a discussion forum to MS practitioners in drug metabolism, pharmacokinetics, qualitative and quantitative, non-regulated bioanalysis include sharing:

- Recent advances in techniques and methodologies for metabolite identification and pharmacokinetic bioanalysis
- Interpretation of, and application of related guidance documents (i.e. MIST, ICH M3, DDI, expl. IND)
- Sharing of best practices across industry and academia
- Provide input on ASMS conference program of interest to scientists working in DMPK
- Reach out and coordinate with related groups to complement scope and broaden outreach to scientific community

2. Solicitation for nominations for 2016 and future DMPK IG Coordinators

2015: Mustafa Varoglu, Alnylam Pharmaceuticals (Coordinator) – mvaroglu@alnylam.com
Kevin Bateman, Merck & Co (Co-Coordinator) – kevin_bateman@merck.com

List of nominated DMPK-IG coordinators:

Outgoing 2015: Mustafa Varoglu

2016: Kevin Bateman

2016 and future nominees: Phil Tiller (RMI Laboratories), Ahmed Aman (Ontario Inst. Of Cancer Research), Chet Bower (GSK), Hongying Gao (Pfizer), Sattanathan Paramasivan (ARMGO Pharma)

3. Update on the DMPK Interest Group's Impact on the 2014 ASMS Program

We thank the ASMS Program Vice President of Programs Vicki Wysocki for being receptive to our requests and proposals for a comprehensive set of DMPK oriented oral sessions for the 2015 meeting. This responsiveness was reflected in the increased number of DMPK oriented Oral sessions compared to 2014. In addition the DMPK-IG workshop was returned to the Monday evening time slot. The DMPK oriented oral sessions in 2015 were:

Mon AM: Advances in Software and Hardware to Improve DMPK Workflows

Mon PM: Quantitative Analysis in Drug Discovery for Small Molecules

Tues AM: LC-MS Approaches to Combine Translational PK/ PD Biomarkers with Small Molecule ADME Workflows

Tues PM: Imaging: Pharmaceuticals and Metabolites

Weds AM: Application of Stable Isotope Labeling in MS Analysis of Small Molecules and Proteins (Metabolomics)

Weds PM: Antibodies and Anti-body Drug Conjugates (Large and Small Molecule)

Thurs AM: Ion Mobility: Small Molecules, Pharmaceuticals, and DMPK

Thurs PM: Applying New LC MS Techniques to Solve Challenging Drug Metabolism Problems

4. Suggestions on ASMS 2015 Oral Session Topics from DMPK-IG Attendees

The attendees agreed that the current topics were still of high interest and supported expanding on them on the 2016 program. Attendees provided many suggestions with some topics such as Quantitative and Qualitative Analysis requested as regular sessions each year as well several other sessions in new areas.

As an interest group we wish to continue to work with the ASMS Vice President of Programs to identify potential Oral Session topics and Oral Session Chairs. In order to support a strong DMPK focus in future ASMS meetings the DMPK IG encourages people to submit DMPK focused abstracts for oral sessions to the 2016 ASMS.

Based on Attendee and DMPK IG Member feedback; the DMPK-IG requests retaining the DMPK-IG Workshop on Monday night from 5:45 to 7 pm.

DMPK-IG attendees offered the following potential Oral Session DMPK topics and areas of interest:

Session Title/ Topic	Day/ Time
Quantitative Analysis in Drug Discovery for Small Molecules	Mon AM
MS of Nanoparticles/Nanomaterials? in Drug Discovery and Development	Mon PM
Utility of Microflow and Nanoflow LC-MS for Small Molecule Quantitation OR: Assay miniaturization in DMPK	Tues AM
MS Solutions for Drug Metabolism Challenges	Tues PM
Microsampling and Microdosing: Opportunities and Challenges for DMPK	Wed AM
HRMS for Quantitation in Drug Discovery and Development	Wed PM
DMPK of Biologics: Challenges and Solutions	Thu AM
Peptide and Protein Quantitation for Drug Discovery and Development	Thu PM

Alternate or Additional Topics for Oral Sessions:

DMPK Workflows Enabled by New LC-MS Techniques

“Quantitation without Standards” OR “New Approaches to Quantitative Analysis”

Combining DMPK and Small Molecule Biomarker Workflows

Translational PK/ PD combined with ADME workflows

Isotopes and Drug Metabolism

In additions several potential future workshop ideas were proposed:

- How low should we go?
- How do in silico models compare to experimental DMPK results.
- TOF vs. Orbitrap in DMPK Workflows.
- DMPK Challenges of Covalent Inhibitors

5. Discussion Topic: “More DMPK Knowledge from Less Sample – Leveraging Modern LC-MS Instruments for Small Sample Amounts”

To understand the state of microsampling knowledge in the DMPK-IG membership, and guide the discussion group topics, a survey was conducted in winter/ spring 2015. The results were summarized as an introduction before the three panel speakers shared their perspectives on Discovery, Development and Diagnostic aspects of Microsampling for approximately 25 minutes. The survey received 85 responses from the membership and included 23 detailed written responses.

Some broad trends from the survey were: microsampling is used across all phases of drug discovery and development with an emphasis on discovery, the most commonly sampled matrix was blood (including plasma and serum) with many other fluids (CSF, saliva), and tissues (including biopsy and single cells and their lysates) examined. The survey indicated the major advantages were felt to be the small sample sizes and the ability to reduce the number of animals used, while the major challenges were perceived as lack of automation and potential loss of the ability repeat analyses if detection limits were insufficient.

Based on this background the three panel speakers provided their expert perspectives:

1. Discovery Micro-sampling:
 1. Rationale, Impact, and Methods: (Dan Kassel, Ph. D., SciAnalytical Strategies)
 2. Specific Examples and Applications: (Walter Korfmacher, Ph. D., Sanofi)
2. Development (Regulated) Micro-sampling:
 1. Reasons, Impact, Methods, & Examples (including Tissue Distribution (Chester Bowen, Ph. D., GSK))
3. Future Perspectives - Clinical Diagnostics, Other Matrices (Dan Kassel)

Dan Kassel presented an overview of the current instrumentation advances and sampling development that has enabled discovery microsampling to provide similar results as traditional plasma sampling. Walter Korfmacher provided specific examples of whole blood microsampling and serial sampling in mice using capillary microsampling and MITRA (Neoteryx) devices for reproducible sampling of a fixed 8 or 10 μ L volume and the overlap of time-concentration PK curves that could be achieved with these techniques compared to traditional larger volume sampling. Dr. Korfmacher emphasized the ability to sample whole blood by these techniques and the steps necessary to obtain good results

Chet Bowen provided the perspective of using microsampling in the regulated development environment with examples using the Drummond capillary device for plasma, MITRA tips, paper and non-cellulose based cards, and solid phase microextraction for the direct sampling of blood. The advantages were identified as the reduction in number of animals and the smaller amount of drug product needed for large studies. The validation steps needed for microsampling were of a similar number to conventional plasma and blood sampling, however for dried blood spot analysis an evaluation of drying time and hematocrit was identified as important. Dr. Bowen provided a brief overview of tissue analysis with microsampling and the ability to generate higher concentration samples with less dilution than with conventional sampling.

Dr. Kassel provided a final perspective on using microsampling in the diagnostic arena with existing and potential applications identified as inborn errors of metabolism for neo-natal screening, bacterial strain identification, and wellness testing for vitamins and stress hormones, and patient driven blood collection to reduce the impact of fasting. Many of the current assays are immunoassay based however LC-MS combined with microsampling is being explored. Opiate testing for therapeutic drug monitoring was identified as an area that could advance to the doctor's office setting.

Following the presentations, the audience and Panel Members engaged in an extended discussion with additional viewpoints from the audience adding many points of discussion to the panel members' introduction:

- IACUC considerations and resistance to allowing multiple blood draws via tail snips from mice for serial bleeds
 - This was a concern in an academic setting, however one of the panel members shared that this was not a problem in their company or at the CRO's they worked with as there were other methods to obtain blood than tail snips.
- Automation of microsampling was raised by the audience (and in the survey) however several audience members had identified automation solutions for microsample collection and

- processing.
- The ongoing development of the FDA dried blood sampling Guidance and the uncertainty of the final protocols
 - However this was countered by an audience members from Merck and Janssen commenting that this technique was being used in TK studies and Phase 3 studies.
 - There was a large amount of audience discussion on sampling plasma vs. whole blood and the steps needed to bridge the different matrices.
 - Challenges in dried blood spot analysis from a simple spotting of the samples:
 - A large pharma audience member described a clinical trial experience in which DBS concentrations derived from venous blood vs. blood from a finger prick did not match each other and that this may have been caused by differences in paper chromatography that occurred at different sites.
 - Another member described challenges of saliva samples with high water content and the impact of the capillary tip touching or not touching the paper and the spread of water and analyte across the paper.
 - There was panel and audience agreement that these apparently simple techniques still require training and validation in the workflow.
 - The need to validate drying times as well as hematocrit levels for MITRA tips
 - A Neoteryx employee and audience member provided the advice to dry the tips for at least 20 hours and this may reduce inter-compound variability that is occasionally observed with some analytes.
 - Large molecules and microsampling:
 - Published reports exist:
 - One mouse, one pharmacokinetic profile: quantitative whole blood serial sampling for biotherapeutics, Pharm Res, 2013, 31, 731-738.
 - Capillary microsampling and analysis of 4 μ L blood, plasma and serum samples to determine human to α -synuclein elimination rate, Bioanalysis, 2013, 5, 449-462.
 - Metabolite ID and microsampling:
 - Published reports exist :
 - A strategy for identification of drug metabolites from dried blood spots using triple-quadrupole/linear ion trap hybrid mass spectrometry, RCMS, 2005, 19, 1984-1992
 - Extra aliquots can be pulled from DBS samples.
 - Hands on experience was shared:
 - For whole blood analysis a 10 fold 75% aqueous acetonitrile dilution enables direct analysis.
 - For MITRA tips further handling should occur within 1 hour of collection
 - Displacement pipets should be used for capillary handling.

Current Officers for the ASMS DMPK Interest Group:

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