Biotherapeutics Interest Group Workshop
64th ASMS Conference and Allied Topics, June 5 - June 9, 2016, San Antonio, TX
Damian Houde, Ph.D. and Ashley Ruth, Ph.D.

The Biotherapeutics Interest Group (formerly the Protein Therapeutics Interest Group) workshop, entitled "Characterization of Protein Therapeutics by Mass Spectrometry", was held from 5:45 PM to 7:00 PM on Wednesday, Jun. 8, 2016. Approximately 150 people attended the workshop.

The primary goal of the workshop was to inspire and promote discussion on the use of mass spectrometry in the biopharamaceutical industry. We hoped an open a free-form conversation would allow the audience to speak freely and learn from one another. A pre-conference survey was created using SurveyMonkey to provide potential discussion topics for conversation within the workshop (see survey below). The workshop started with a brief introduction from the co-chairs, and then a panel of five selected industrial professionals joined the stage (Charles Cheng, Amgen; Guodong Chen, Bristol-Myers Squibb; Sarah Rogstad, FDA/CDER/OTR/DPA; Jacquelynn Smith, Pfizer; Martin Eysberg, Antec). The panelists introduced themselves and the discussion began on the most relevant survey topics of interest to the workshop. A few slides from the survey results, included below, on various topics related to protein therapeutics characterization served as a starting point for our discussion.

The discussion began with a focus on "emerging technologies". There was a healthy debate on how to define when/if a technology is emerging vs. has emerged. Technologies such as covalent labeling and hydrogen/deuterium exchange mass spectrometry (HDX-MS) were used as examples. Several people from various companies suggested that techniques such as HDX-MS have already emerged, while techniques like top-down analysis are still emerging. Some of the audience engaged the FDA representatives into the discussion, specifically asking their opinion on and inquiring into how many filings (to their knowledge) contain "emerging/ed" techniques such as HDX-MS. The representatives from the FDA are only able to offer their opinion and not the opinion of the FDA; nevertheless they did share information on the number of filings with these techniques. One filing contained top-down analysis and three contained HDX-MS analysis. Some of the audience used this as evidence that these techniques are still emerging and not yet emerged. After some discussion (25-30 minutes), it appeared as though there was no clear definition on how to define an emerging vs. emerged technology and at this point, it is still very subjective.

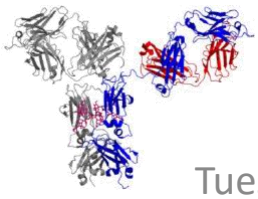
Our conversation then moved on to discuss the use of quantitative mass spectrometry as a multi-attribute method (MAM) for characterization, quality control testing and disposition of biologics. Several people reiterated that mass spectrometry plays a critical role in biopharmaceutical characterization in discovery through development and onto commercialization and MAM has the potential to replace several conventional electrophoretic and/or chromatographic methods currently used in QC to release therapeutic molecules. They suggested that MAM represents an optimized analytical solution, which can help focus on the attributes of the therapeutic molecule that are essential for function and implement QbD principles across process development and manufacturing. Many others in the audience disagreed with this and felt that methods such as MAM cannot replace the profiling of attributes such as charge distribution by imaging isoelectric focusing and/or aggregation by SEC.

Overall, the audience was very engaged and there was a lot of informative discussion. The workshop was adjourned around 7pm. Next year, Ashley (Gucinski) Ruth will be joined by Charles Cheng from Amgen as the Biotherapeutics Interest Group workshop organizers.

Slides presented to the audience at the Biotherapeutics Workshop.

Biotherapeutics Interest Group Workshop

Characterization of Protein Therapeutics by Mass Spectrometry



ASMS 2016

Tuesday, June 7, 5:45 – 7:00 pm

Damian Houde Biogen
Ashley Ruth FDA/CDER/DPA*

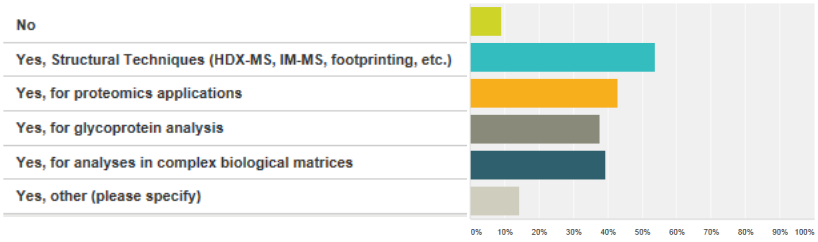
**Disclaimer: Any comments reflect the views of the author and should not be construed to represent the FDA's views or policies.*

Panelist Introductions

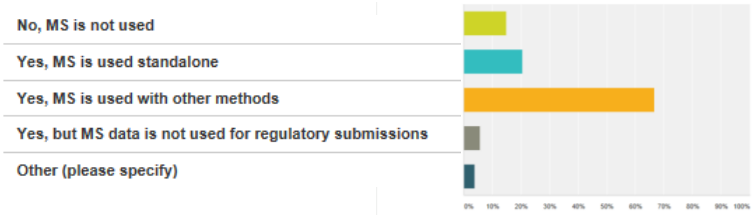
- Guilong (Charles) Cheng, *Amgen*
- Guodong Chen, *Bristol-Myers Squibb*
- Sarah Rogstad, *FDA/CDER/OTR/DPA*
- Jacquelynn Smith, *Pfizer*
- Martin Eysberg, *Antec*

Workshop Focus Areas

- **Emerging Technologies -** ***where are they used?***



- **Comparability -** ***is mass spectrometry used?***



Survey Questions for Discussion: Emerging Technologies

- Are emerging technologies more suitable for early or late stage biotherapeutics characterization?
- What are the largest challenges (technical and/or regulatory) that may hinder the use of MS for biotherapeutics?
- Are currently available software packages sufficient to properly use this data?
- How should this data be displayed to non-experts?

Survey Questions for Discussion: Emerging Technologies

- What role can emerging technologies play in HCP, SVA or trisulfide analysis?
- What impact could the integration of CE-MS play for biotherapeutics analysis?
- Do you see a value in IMS or differential mobility to improve workflows?
- What are the limitations of using HDX for comparability assessments?

Survey Questions for Discussion: Comparability

- How can we define the scope of comparability? What tests are necessary for biotherapeutic characterization?
- What role can MS play in determining comparability? How does this differ for determining biosimilarity or interchangeability?
- Does this include emerging technologies (ion mobility, H/DX, etc.)?
- What considerations should be made when displaying differences in quality attributes to non-specialists?

Biotherapeutics workshop survey and results

ASMS - 2016 Biotherapeutics Interest Group Workshop

Question Summaries | Data Trends | Individual Responses

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57 responses

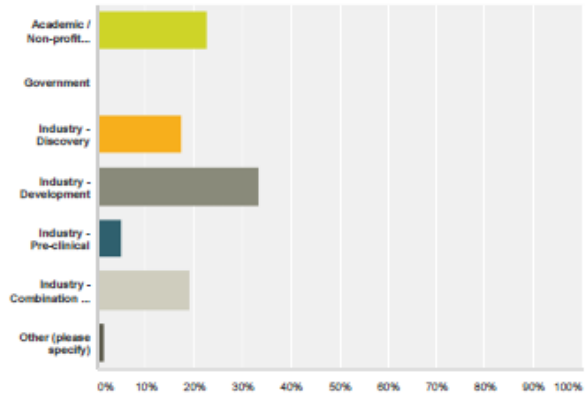
73 days (4/1/2016 - now)

5 views

Q1

What is your primary work focus?

Answered: 57 Skipped: 0



Answer Choices	Responses	
Academic / Non-profit institution	22.81%	13
Government	0.00%	0
Industry - Discovery	17.54%	10
Industry - Development	33.33%	19
Industry - Pre-clinical	5.26%	3
Industry - Combination of Above	19.30%	11
Other (please specify) Responses	1.75%	1
Total		57

Q2

What is your specific biotherapeutic focus area?

Answered: 57 Skipped: 0

Q2

What is your specific biotherapeutic focus area?

Answered: 57 Skipped: 0

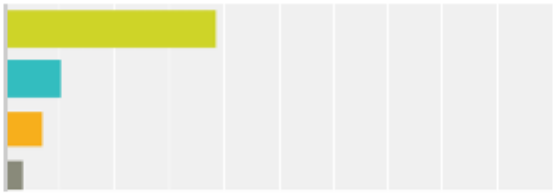
Antibodies

ADCs

Biosimilars

Vaccines

Recombinant Proteins

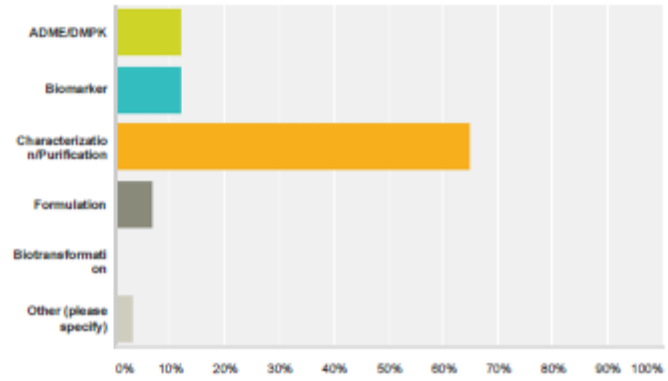


Answer Choices	Responses	
Antibodies	38.60%	22
ADCs	10.53%	6
Biosimilars	7.02%	4
Vaccines	3.51%	2
Recombinant Proteins	24.56%	14
Therapeutic Peptides	8.77%	5
Oligonucleotides	1.75%	1
Other (please specify) Responses	5.26%	3
Total		57

Q3

What is your group's primary support function?

Answered: 57 Skipped: 0

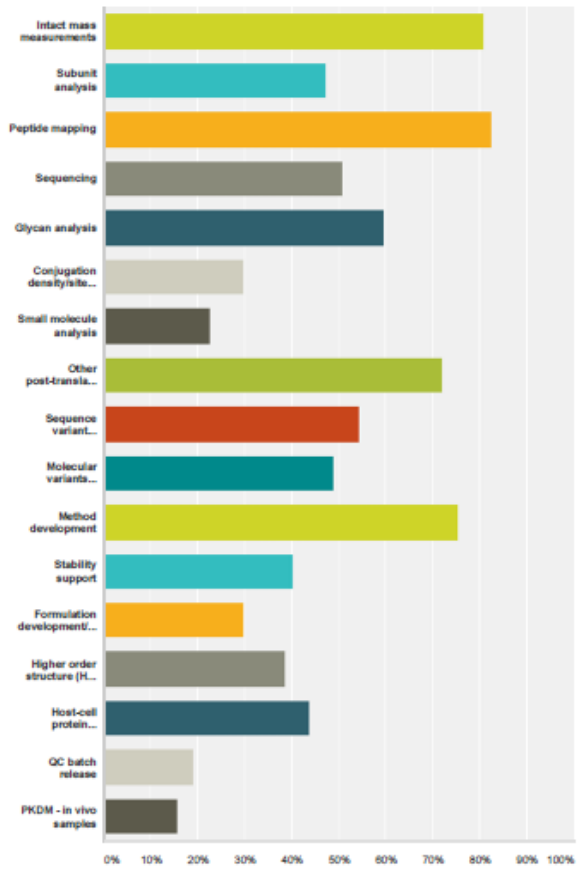


Answer Choices	Responses	
ADME/DMPK	12.28%	7
Biomarker	12.28%	7
Characterization/Purification	64.91%	37
Formulation	7.02%	4
Biotransformation	0.00%	0
Other (please specify) Responses	3.51%	2
Total		57

Q4

What do you use mass spectrometry for? (Choose all that apply)

Answered: 57 Skipped: 0



Answer Choices	Responses	
Intact mass measurements	80.70%	46
Subunit analysis	47.37%	27
Peptide mapping	82.46%	47
Sequencing	50.88%	29
Glycan analysis	59.65%	34
Conjugation density/site-occupancy	29.82%	17
Small molecule analysis	22.81%	13
Other post-translational modifications	71.93%	41
Sequence variant analysis	54.39%	31
Molecular variants (charge, N- and C-terminal heterogeneity, glycation, etc.)	49.12%	28
Method development	75.44%	43
Stability support	40.35%	23
Formulation development/support	29.82%	17
Higher order structure (H/D exchange, radical footprinting, etc.)	38.60%	22

Total Respondents: 57

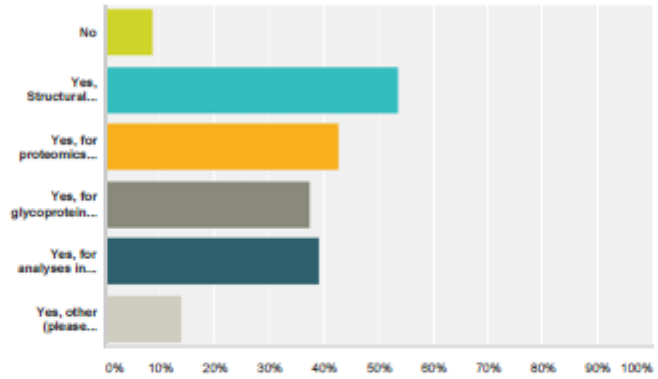
Answer Choices	Responses	
Host-cell protein ID/quantitation	43.86%	25
QC batch release	19.30%	11
PKDM - in vivo samples	15.79%	9

Total Respondents: 57

Q5

Are emerging MS technologies used in your laboratory? (Choose all that apply)

Answered: 56 Skipped: 1

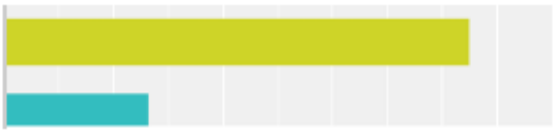


Answer Choices	Responses	
No	8.93%	5
Yes, Structural Techniques (HDX-MS, IM-MS, footprinting, etc.)	53.57%	30
Yes, for proteomics applications	42.86%	24
Yes, for glycoprotein analysis	37.50%	21
Yes, for analyses in complex biological matrices	39.29%	22
Yes, other (please specify) Responses	14.29%	8
Total Respondents: 56		

Q6

Where are emerging MS technologies being used in your laboratory? (Choose all that apply)

Answered: 53 Skipped: 4



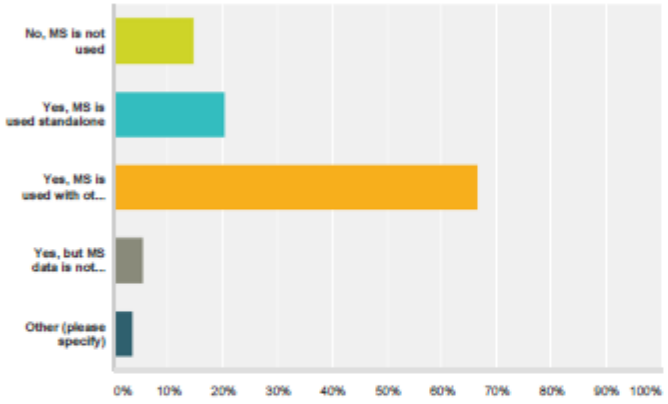
Answer Choices	Responses	
Characterization	84.91%	45
QC	26.42%	14
Discovery	49.06%	26
Development	60.38%	32
Other (please specify) Responses	3.77%	2

Total Respondents: 53

Q7

Is mass spectrometry used for comparability assessments in your laboratory? (Choose all that apply)

Answered: 54 Skipped: 3



Answer Choices	Responses	
No, MS is not used	14.81%	8
Yes, MS is used standalone	20.37%	11
Yes, MS is used with other methods	66.67%	36
Yes, but MS data is not used for regulatory submissions	5.56%	3
Other (please specify) Responses	3.70%	2

Total Respondents: 54

Q8

This year's workshop will focus on two areas: emerging MS technologies for biotherapeutics characterization and comparability. Do you have any questions you would like the panel to address?

Answered: 17 Skipped: 40

Use of IM for comparability
4/12/2016 6:22 PM

emerging technologies in HCP, SVA or trisulfide analysis
4/11/2016 10:37 AM

how to define the scope of comparability. What are must to do to characterize biotherapeutics and any emerging MS technlogy to make it faster and better.
4/10/2016 8:01 AM

Nanoparticle analysis
4/7/2016 6:23 AM

Why hasn't Biopharma, Proteomics, and Metabolomics adopted the SCIEX CESI 8000 PLUS platform as it provides fully intact analysis and can be interfaced to Waters, Thermo, Bruker and SCIEX Mass Spec Systems.
4/6/2016 11:26 AM

What are the biggest challenges, technological, and regulatory, which may hinder use of MS for

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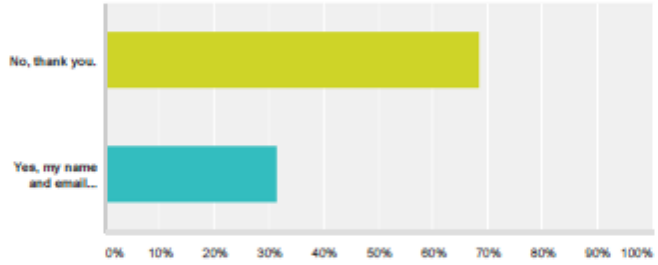
Q9

Are you interested in being on the panel for this year's workshop?

Answered: 54 Skipped: 3



Answer Choices		Responses	
No, thank you.		68.52%	37
Yes, my name and email address are:	Responses	31.48%	17
Total			54

Q10

What other topics would you like this workshop to address about biotherapeutics characterization this year or in the future?

Answered: 17 Skipped: 40

None
4/11/2016 10:37 AM

Intact Analysis for mAbs, ADC's, HCPs, Proteins, and Peptide Mapping using the CESI 8000 PLUS System for front end separation.
4/6/2016 11:26 AM

1. Disruptive technologies for de novo sequencing 2. Top-down HDX methods
4/6/2016 10:10 AM

1] The REALITIES of moving high res MS to QC; 2] Living with imperfect science but perfect reproducibility
4/6/2016 8:58 AM

applications
4/6/2016 8:52 AM

PK of ADCs in plasma using MS
4/6/2016 2:57 AM

Challenges in characterization of Monoclonal antibodies