Proteins and Peptides as Pharmaceutical Agents

Matthew Blatnik, Ph.D. and Brian Furmanski, Ph.D.

Pharmaceutical Interest Group Workshop, ASMS 2013 Minneapolis

5:45 - 7:00 PM, Wednesday, June 12, 2013, Room 205 CD

Number of Attendees: ≥100 people

Workshop Pre-work

To identify panelists and to gauge the level of interest of the ASMS community we sent out a survey of open ended questions in April 2013 (see attached word document). The results of the survey identified ~ 40 people willing to participate as panelists or experts in large molecule mass spectrometry applications. The individuals were cross referenced utilizing Pubmed and LinkedIn then followed up with 6 phone interviews to identify the final panelists. Due to the success of the 2012 pharmaceutical interest group workshop on antibody drug conjugates (ADC's) and in the interest of the community, we decided to continue this year with more emphasis on ADC's. Jack Henion was identified as the led panelist and was asked to provide a 15 min overview to stimulate discussion on the workshop topic.

Workshop Format

The primary goal of this workshop was to learn from one another. This year's workshop briefly introduced some topics relating to large molecule therapeutics before shifting focus onto antibody drug conjugates (ADC's); the ADC space encompasses a wide variety of analytical skills and is an area that is gaining momentum. The success and outcome of this workshop was based primarily on audience and panelist discussion.

The workshop proceeded as follows:

1. (~ 5min) A brief synopsis of the Pharmaceutical Group by Matthew Blatnik Ph.D. (Pfizer Inc.) and Brian Furmanski Ph.D. (Siga Technologies).
2. (~15 min) An introduction to some potential topics to stimulate discussion led by Cornell Emeritus Professor Dr. Jack Henion (also co-founder, Chairman and CSO of Advion BioSciences)
3. (Remainder) Immediately following brief introductions, there will be a panel led conversation and the floor will open for audience participation. Open discussion between peers will proceed until the end of the workshop.

Panelists:

Jack Henion Ph.D. (Advion)
Sheng Gu Ph.D. (Biogen Idec)
Shawna Hengel Ph.D. (Seattle Genetics)
Da Ren Ph.D. (Amgen)

Workshop Discussion

Jack gave a 20 min overview of the topic which included: characteristics of proteins, peptides, and ADC biologics, strategies for sample prep/isolation, and a comparison of assay strategies including ELISA and LC-MS as complimentary tools (See power point deck). The short presentation was meant to capture the field in its current state, in addition it gave a specific example of a challenging issue in the industry of the characterization and quantification of antibody drug conjugates.

Very quickly, the audience engaged along with three key questions to start of the discussion with the general audience. Shawna Hengel (Seattle Genetics), Sheng Gu (Biogen Idec) and Da Ren (Amgen) provided critical insights into the characterization and quantification of proteins and peptides. Jack Henion was a superb moderator and went the extra mile by actively engaging the audience in discussion of the topic. The panel/audience led discussion included: preferred sample preparation and chromatography, choice of mass anaylzers qual/quan

workflows, drug antibody ratio (DAR) in vivo/ex vivo, and pegylation of novel protein theraputics. The discussion following Jack's overview was extremely constructive with a lot of audience participation and went over the allotted time.

Succession Plan

We propose shifting the name and focus of the Pharmaceutical workshop to the Antibody drug conjugate workshop since this is an up and coming area that needs a dedicated.

Workshop leadership is on a three year rotation which is as follows: 1st year co-chair, 2nd year chair and a 3rd year mentor. A new co-chair is identified every year from a call for workshop leaders to all the members of the group via email and at the workshop. Interested parties prepare a written statement of intent (no more than 100 words) to gauge their level of interest and willingness to take on the work required for a successful workshop. The current co-chair, Brian Furmanski, will move to the chair position as the experienced leader to plan and execute the 2014 workshop. Shawna Hengel is the new co-chair and will actively participate in the planning and execution of 2014 workshop. The outgoing chair, Matthew Blatnik, will remain as a mentor for 2014 discussions and communications.

ASMS Workshop on Large Molecule Therapeutics

Organized by Matt Blatnik and Brian Furmanski

Moderator, Jack Henion
Quintiles
12 June 2013

Issues for Large Molecule Biologics Compared with Small Molecule Drugs

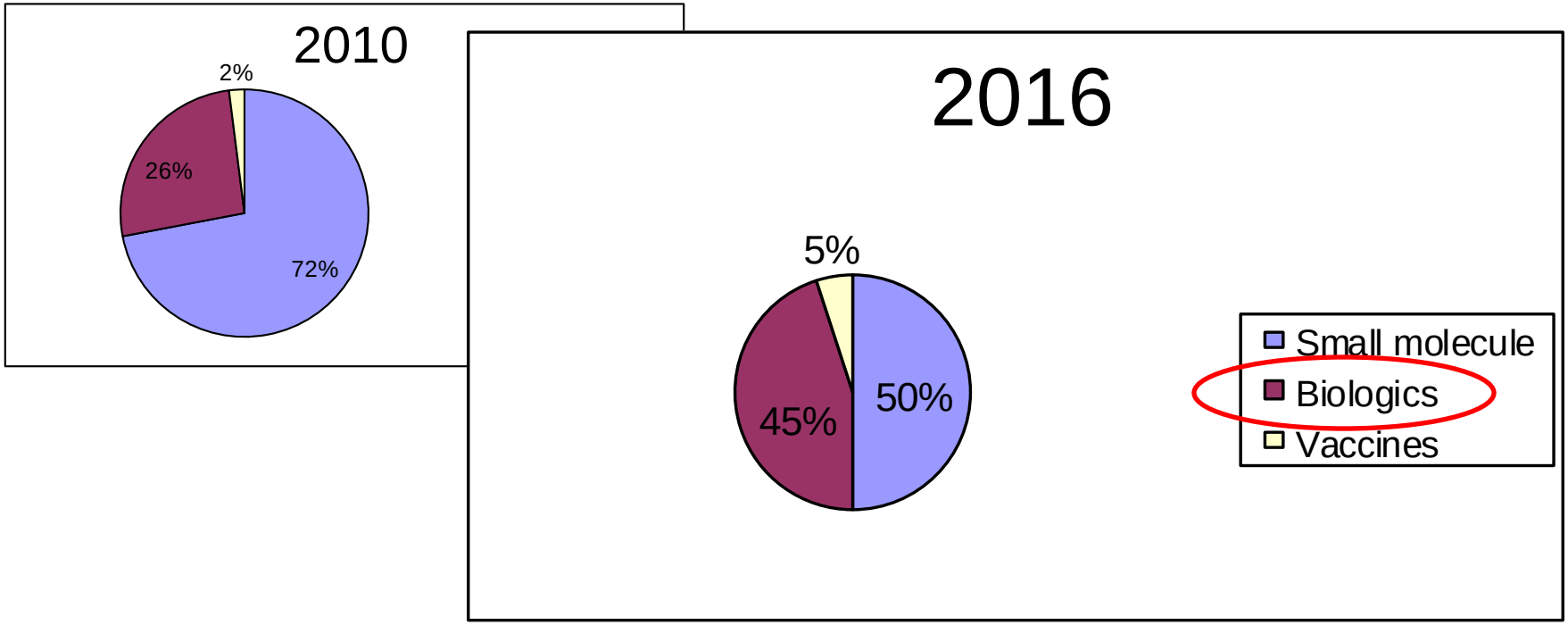
- Differences
  - Manufacturing, much larger molecular size, complex spatial structures, heterogeneous
  - Immunogenicity potential cannot be ignored
- Routes of elimination and observed PK
  - Small molecules metabolized by hepatic mechanisms in minutes or hours
    - Large molecules can exhibit half-lives of weeks
    - Typical focus is on AUC or total drug exposure
      - (less focus on metabolites)
- Routes of administration
  - Often intravenously, subcutaneously or intramuscular
    - Oral admin causes breakdown in gastro intestinal tract
  - In contrast to the less desirable IV, sub C, or IM administration
    - The frequency of administration is often less.
    - Also, newer automated admin techniques are developing
- Biotherapeutics benefits
  - Can be very selective and cause minimal disturbances in normal biological functions
- Peptide drug candidates: have very short half-lives
  - PEGylation improves PK behavior by extending the biologic half-life of peptides and reduces immunogenecity
  - Chemical modification to modify the PK profile
  - Binding with long-circulating serum proteins such as albumin.
- Antibody-Drug Conjugates (ADC's)
  - Currently a niche market with growing interest in subject
  - Very challenging bioanalysis strategies
- Bioanalysis methods will likely need to be revised
  - Sample preparation
  - Leverage LBA techniques
  - Chromatography
  - Mass Spectrometry

Issues for Large Molecule Bioanalyses

- Complexity of the macromolecule compared to small molecules
  - Heterogeneity of the drug
  - Stability in dose solutions and in *in vivo* conditions
  - Current challenges with
    - Sample preparation
    - Chromatography
    - Mass spectrometry
- Metabolism, limited prior experience
- Similarity to endogenous large molecules
- Detection limits

Growth of Biologics

Top 100 Drug Sales by Technology



Source: Parexel's Statistical Sourcebook, 2009/2010, pg. 24

"By 2015 biologic therapeutics are projected to be a $240B market"
– Binodh DeSilva, BMS.

Liquid Chromatography-Mass Spectrometry

Present

- SPE, Liq-Liq, Protein crash
- HPLC/UHPLC
- 1.7µm - 5µm particle
- 2 - 4.6 mm column diameter
- 1 - 10 minute gradients
- Primarily 1-Dimensional separation
- Triple-quadrupole and Selected Reaction Monitoring (SRM)
- High-flow rate heated ESI
- 0.1-1.0 mL samples

Future

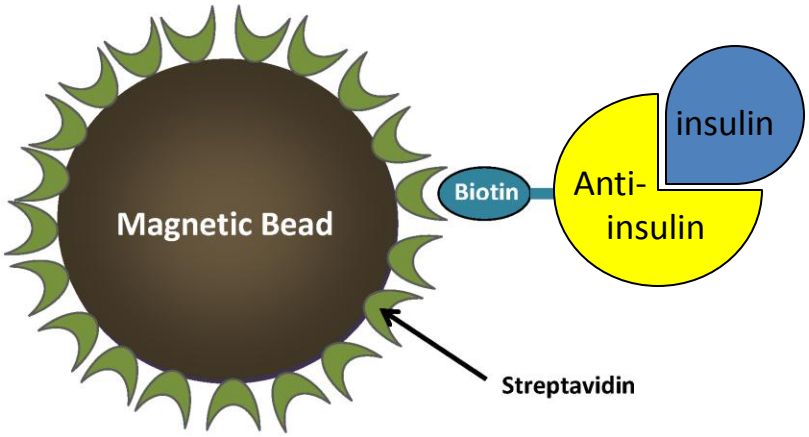
- Immunoprecipitation, Magnetic beads, Multi-Dimensional LC
- HPLC/UHPLC
- Sub-micron to 2µm particle
- 0.05 - 0.5 mm column diameter
- 0.25 - 5 minute gradients
- On-line extraction combined with multi-dimensional separation
- High Resolution Mass Spectrometry
- Nano electrospray ionization
- Microsampling; < 100 uL sample

Can we do Large Molecule Bioanalysis of *in vivo* Samples now?

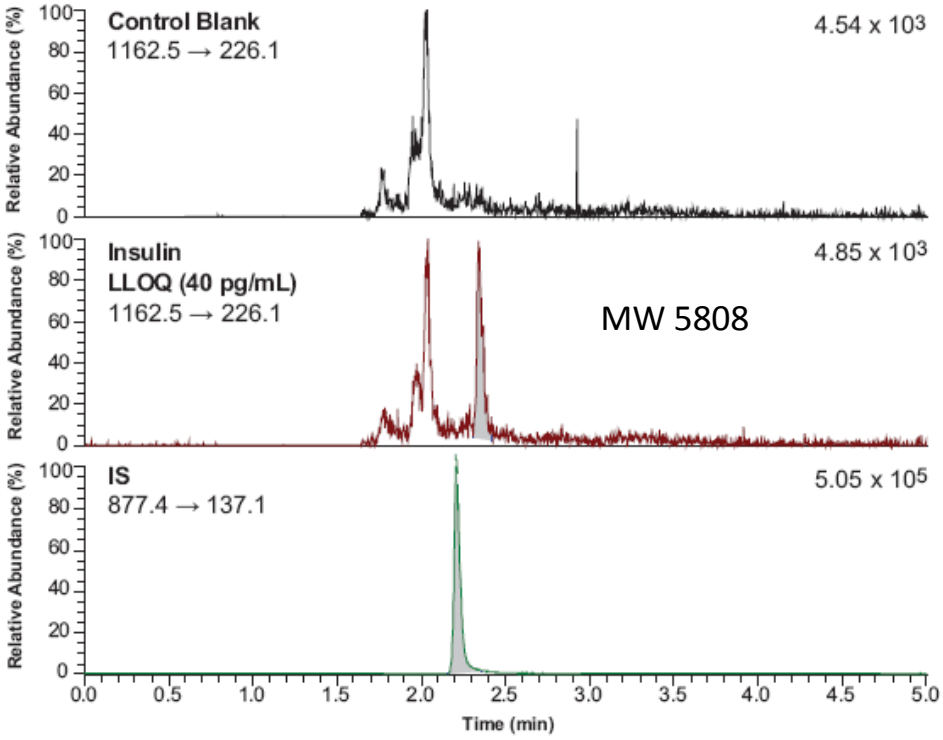
Nerve Growth Factor

MW = 5808.

Immunoprecipitation Methods: Extraction Procedure



- Combining immunoprecipitation and immunoaffinity capture chromatography with nanoLC-MS can match the sensitivity of ligand binding methods but with the selectivity of mass spectrometry



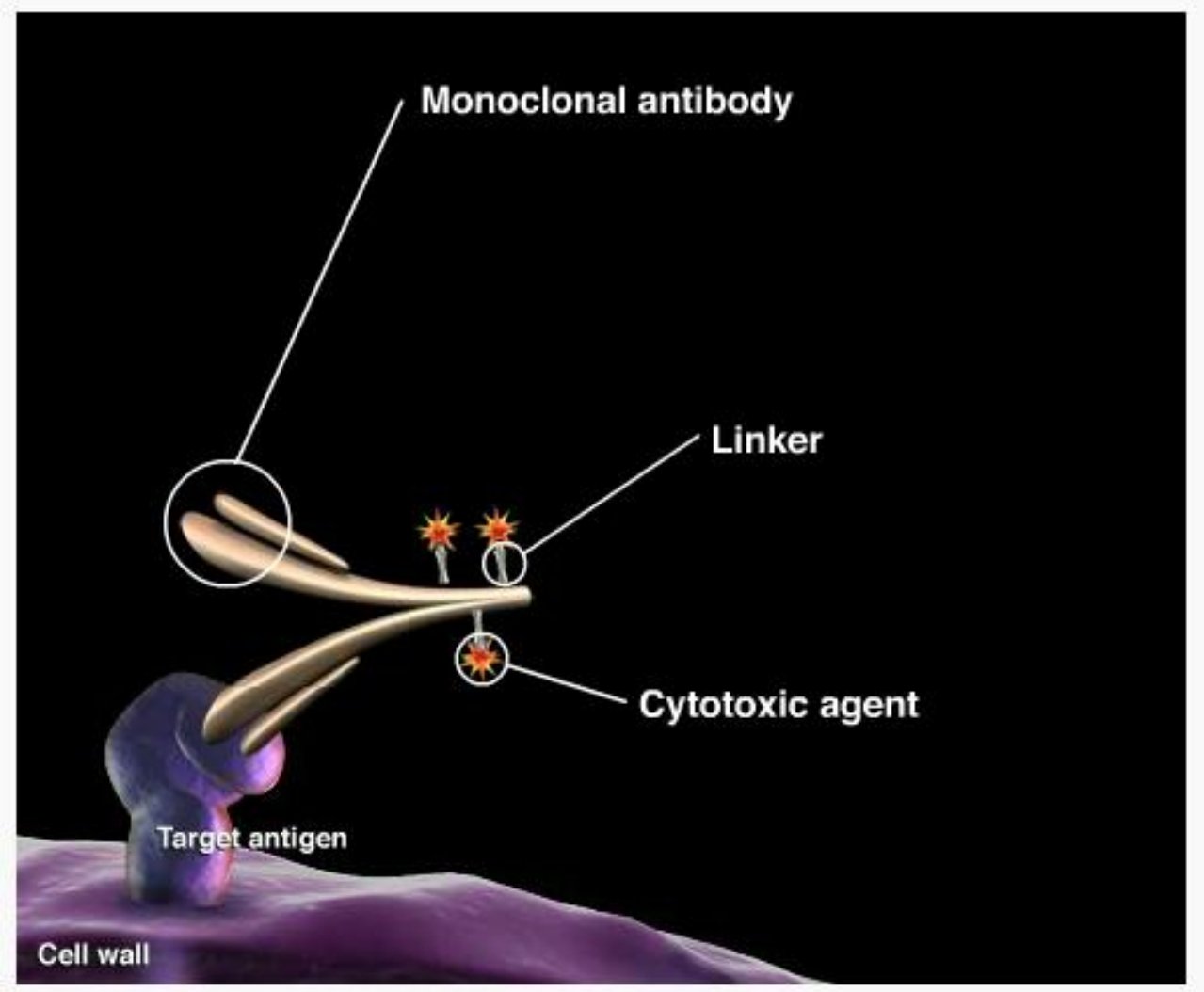
Validation of an immunoprecipitation and immunoaffinity LC-MS/MS assay for intact human insulin Kathlyn M. Porter; Lian Shan; Gary A. Schultz; Quintiles
Bioanalytical and ADME Labs,
ASMS 2012 Proceedings

Antibody Drug Conjugates (ADC's)
Growing Need for New Analytical Capabilities

- **Pharma pipeline is 30 in 2013**
  - 2009-2010: 8 ADC's entered clinic
  - 2011-2012: 17 ADC's entered clinic
  - 2013: currently 30 ADC's in the clinic
    - 15% of clinical-stage anticancer Ab-based pipeline
- **Pharma competitors pursue a diverse range of targets**
  - Small molecule competitors pursue same target
    - Minimal overlap in ADC pipeline
    - Pharmas pursue different targets

From A. Mullard, Nature Reviews, Drug Discovery, 12, May 2013 ,329-332

Antibody-Drug Conjugates



http://www.biooncology.com/research-education/adc/about-adcs

Definition of Terms

- **Antibody Drug Conjugate (ADC)**
  - **An antibody with a covalently linked payload drug**
- **Drug-to-Antibody ratio (DAR)**
  - **Stoichiometry ratio between cytotoxic drug and the Ab.**
- **Drug deconjugation**
  - **Unintended release of drug into circulation**
- **Anti-drug antibody**
  - **mAb reagent that can only bind to the ADC analyte via its drug portion**
- **Affinity capture**
  - **Use of specific reagent to selectively bind analyte of interest from the matrix to concentrate the target ADC and/or its payload**
- **HIC**
  - **Hydrophobic interaction chromatography**
- **SEC**
  - **Size exclusion chromatography**

Preferred Bioanalysis Techniques?

- Which of these ‘tools’ will be helpful?
  - ELISA/LBA’s
  - LC/MS
  - LC/UV
  - LC/F
  - HIC/UV
  - SEC/UV
  - SEC/MS

- NB: Some of these chromatographic techniques employ ‘unfriendly’ LC/MS mobile phases

Total-antibody ELISA and two (generic vs specific) conjugated-antibody ELISA's

Characterization of the drug-to-antibody ratio distribution for antibody–drug conjugates in plasma/serum

Keyang Xu*, Luna Liu, Randall Dere, Elaine Mai, Rebecca Erickson, Angela Hendricks, Kedan Lin, Jagath R Junutula & Surinder Kaur

Genentech, 1 DNA Way, South San Francisco, CA 94080, USA
*Author for correspondence:
Tel.: +1 650 225 4525
Fax: +1 650 225 1998
E-mail: xu.keyang@gene.com

Why do these differ?

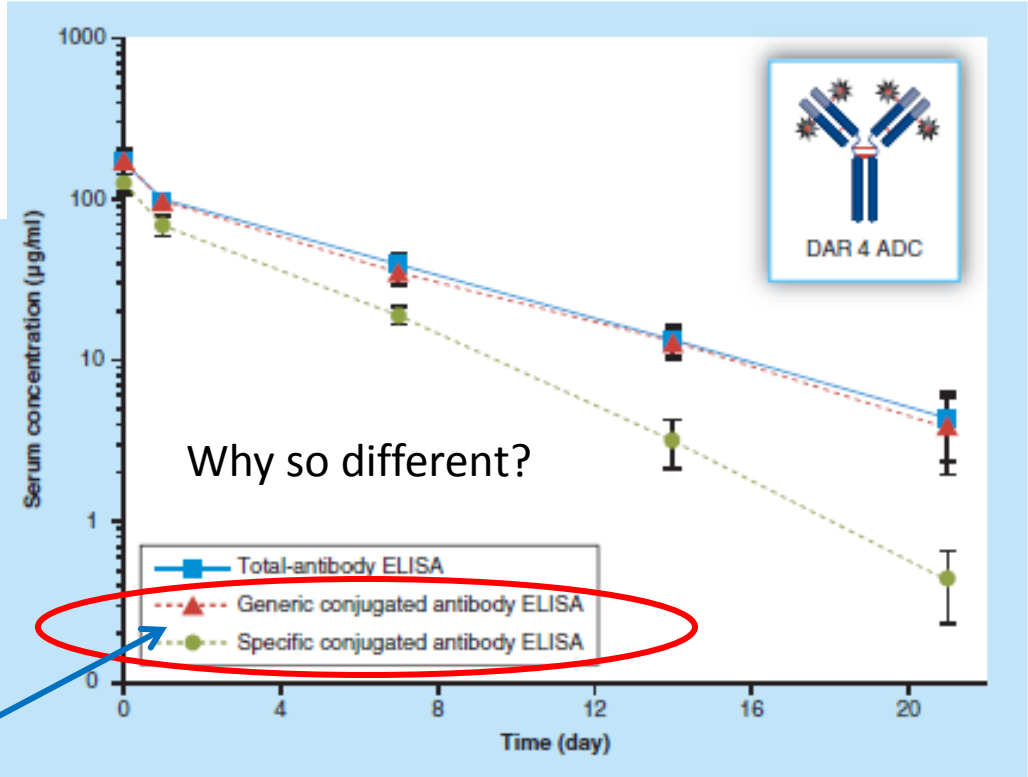


Figure 4. Serum concentration–time profiles following the first dose for a cynomolgus monkey toxicity study administered intravenously with an engineered cysteine-linked drug-to-antibody ratio 4 trastuzumab-valine-citrulline-monomethyl auristatin E at 6 mg/kg once every 3 weeks. Samples were analyzed by a total-antibody ELISA and two (generic vs specific) conjugated-antibody ELISAs.
ADC: Antibody–drug conjugate; DAR: Drug-to-antibody ratio.

Characterization of the drug-to-antibody ratio distribution for antibody–drug conjugates in plasma/serum

Keyang Xu*, Luna Liu, Randall Dere, Elaine Mai, Rebecca Erickson, Angela Hendricks, Kedan Lin, Jagath R Junutula & Surinder Kaur

Genentech, 1 DNA Way, South San Francisco, CA 94080, USA
*Author for correspondence:
Tel.: +1 650 225 4525
Fax: +1 650 225 1998
E-mail: xu.keyang@gene.com

"Clearly, the DAR distribution data obtained by affinity capture LC–MS played a critical role in ensuring accurate quantification of the ADC by appropriate ELISAs", K. Xu, et al.

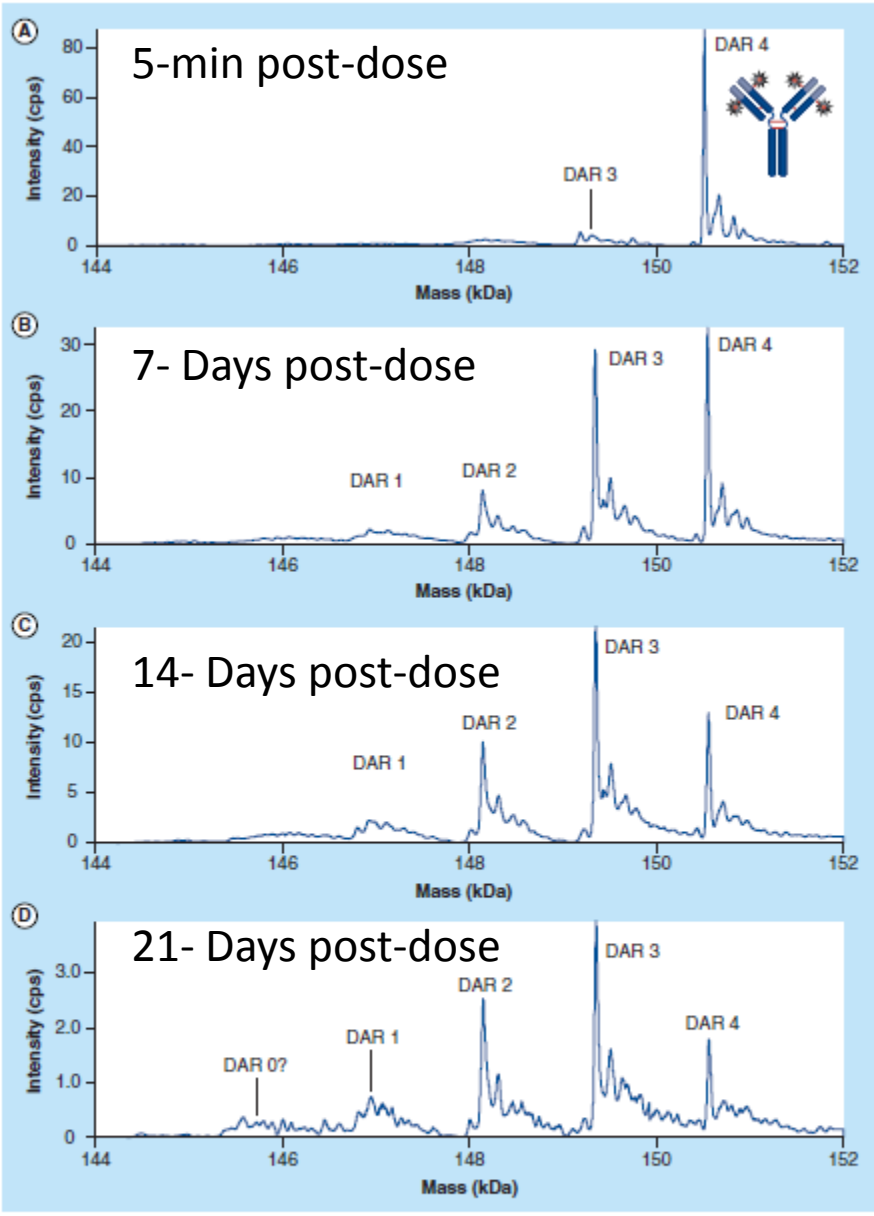


Figure 5. Changes in the drug-to-antibody ratio distribution in plasma for engineered cysteine-linked drug-to-antibody ratio 4 trastuzumab-valine-citrulline-monomethyl auristatin E following an intravenous dose of 6 mg/kg in cynomolgus monkeys. (A) 5 min post-dose; **(B)** 7 days post-dose; **(C)** 14 days post-dose; **(D)** 21 days post-dose. Samples were analyzed by affinity capture LC–MS using HER2 extracellular domain as the capture reagent. Minimal DAR 0 was generated from DAR 4 trastuzumab-valine-citrulline-monomethyl auristatin E due to fast succinimide ring hydrolysis to prevent complete drug deconjugation.
DAR: Drug-to-antibody ratio.

Can we measure trace level small-molecule payload drugs *in vivo* from microsamples?

Ultra-Trace Bioanalysis via 2D nano ESI SRM LC/MS

(prospects for the future?)

Miaoqing Shen[1], Li Sun[2], Kevin Bateman[2], and Jack Henion[1]

[1] Quintiles Bioanalytical and ADME Labs, Ithaca, New York, 14850
[2] Merck Research Laboratories, West Point, Pennsylvania, 19486

Wed Poster 422 ASMS 2013

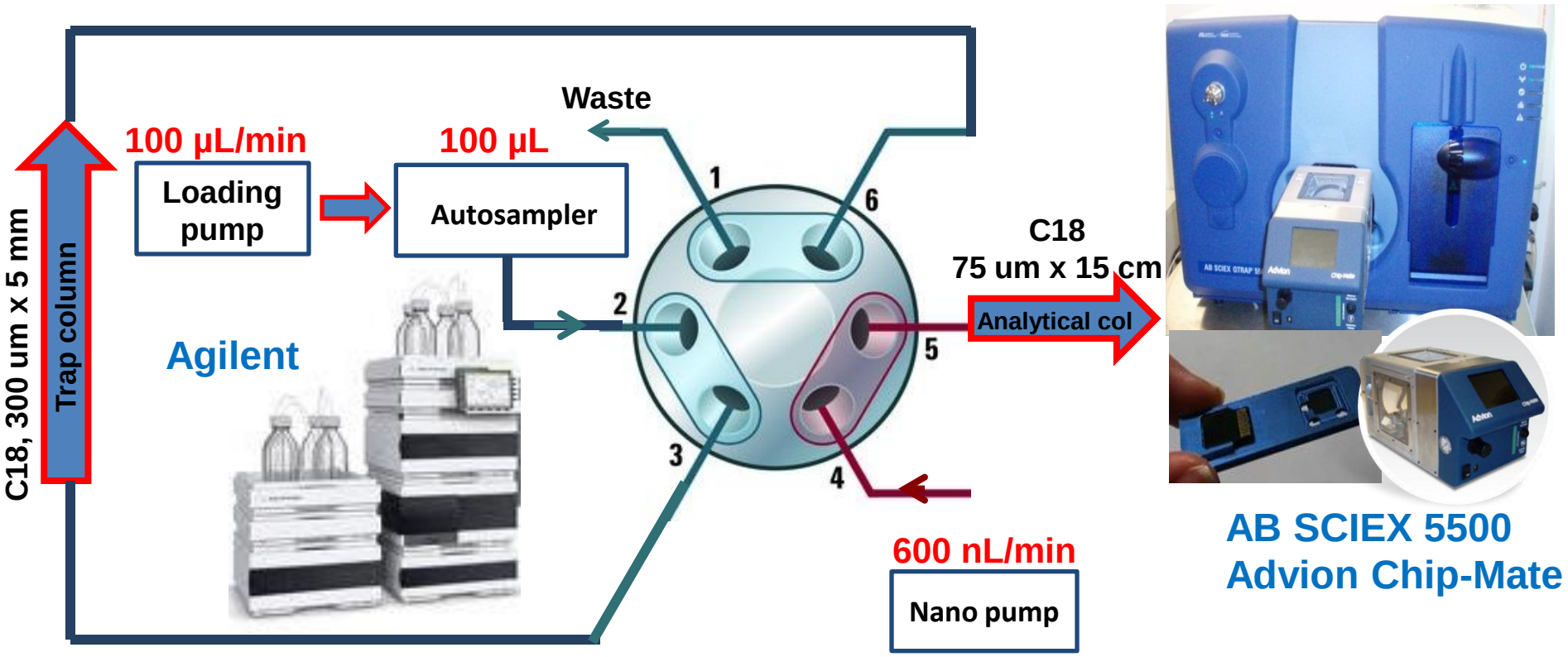
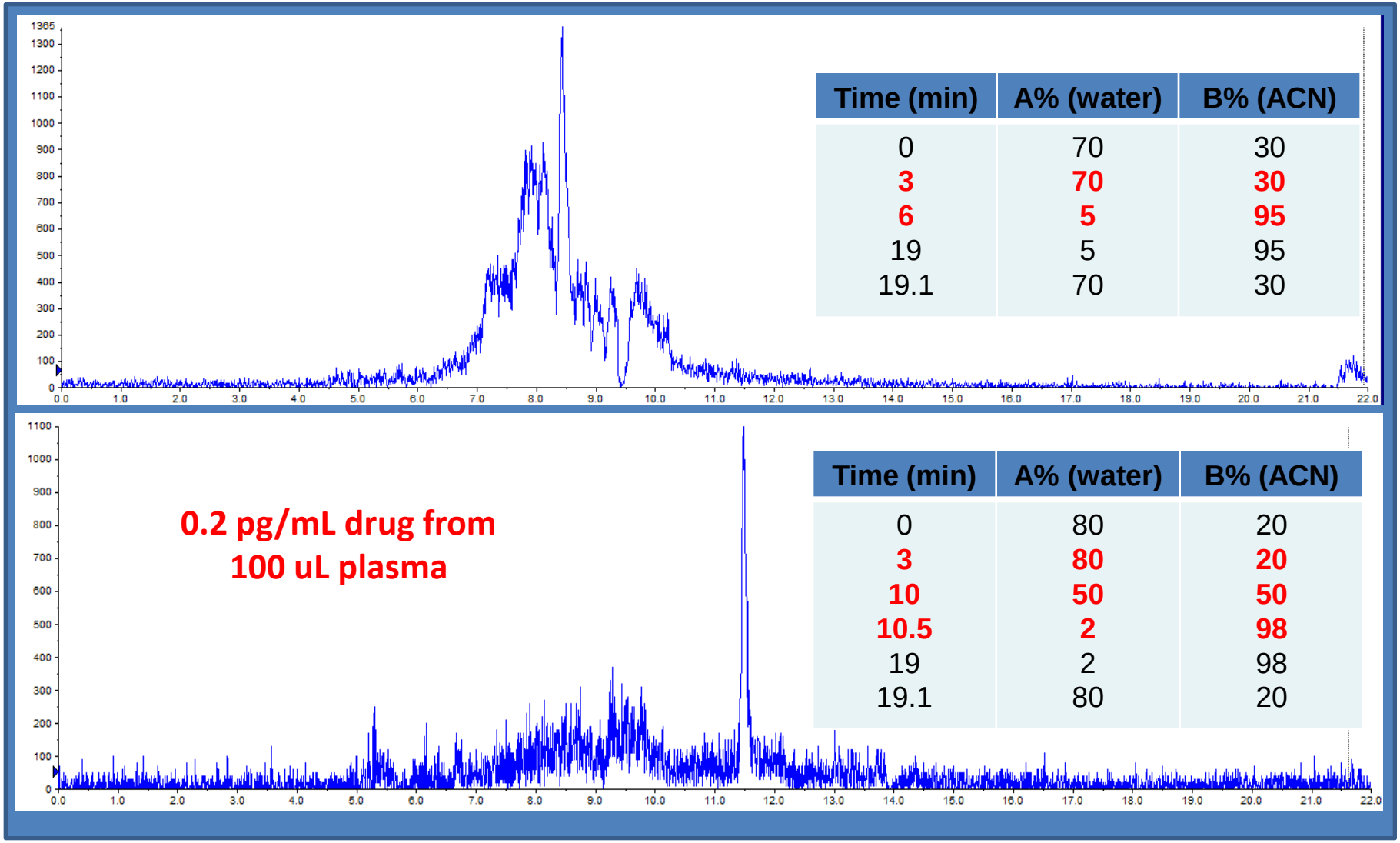


Figure 1: 2D Trap-Nano LC Configuration: AB SCIEX 5500 QTRAP and Nano LC coupled with nano ESI Chip-Mate. The samples were first loaded onto a trap column followed by switching to a nano LC analytical column operated at a flow rate of 600 nL/min. The trap column and nano LC column were both eluted via a gradient to high organic solvent composition to elute highly retained endogenous components.

Miaoqing Shen[1], Li Sun[2], Kevin Bateman[2], and Jack Henion[1] TP422.

❖ A nano LC gradient composition change resulted in a significant reduction of background chemical interferences.



Time (min)	A% (water)	B% (ACN)
0	70	30
3	70	30
6	5	95
19	5	95
19.1	70	30

Time (min)	A% (water)	B% (ACN)
0	80	20
3	80	20
10	50	50
10.5	2	98
19	2	98
19.1	80	20

Miaoqing Shen[1], Li Sun[2], Kevin Bateman[2], and Jack Henion[1] TP422.

Figure 7: **Extracted ion chromatograms for MK0518 at a concentration of 0.2 pg/mL from 100 µL of human plasma using different nano LC gradients.**

Where is the technology leading us?

- Integration of sample preparation and analysis
  - Sample preparation, analysis, automated data analysis, structure generation, LIMS, and data reporting.
  - LBA combined with LC/MS
- Sample Preparation
  - Immunoaffinity techniques
    - MIP's, 'nanobodies'?
  - Magnetic beads
  - Multi-dimensional 'step-down i.d.' LC
    - Load/inject entire sample
  - Automation
  - Non-denaturing conditions
- UHPLC separations
  - Higher peak capacity, less co-elution
- Multi-dimensional micro-columns (coupled to MS)
  - Separation modes which are 'orthogonal' to HPLC and UPLC
    - HIC (hydrophobic interaction chromatography)
    - SEC (size-exclusion chromatography
    - IC (ion chromatography)
    - CE (capillary electrophoresis)?
- For Improved detection limits
  - Nanoelectrospray coupled with nano LC
  - Better ionization efficiencies, less competition,

HRMS and Exact mass determination

  - Acquisition of qualitative and quantitative data, higher selectivity, more information

Panel Discussion Members

- Shawna Mae Hengel, Ph.D.
  - Scientist
  - Bioanalytical Development
  - Seattle Genetics, Inc.
- Sheng Gu, Ph.D.
  - Senior Scientist,
  - Analytical Biochemistry
  - Biogen Idec, Inc.
- Da Ren, Ph.D.
  - Principal Scientist
  - Process & Product Development
  - Amgen, Inc.

Suggested Key Topics for Discussion

- What is the preferred sample preparation approach?
- What is the preferred chromatography?
  - HPLC, UPLC, SEC, HILIC, HIC
- What is the preferred HRMS instrument in the future; e.g. QTOF, Orbitrap, FTMS, or ??