Protein Therapeutics Interest Group Workshop Report
60th ASMS Conference and Allied Topics, May 21, 2012, Vancouver, BC

Protein Therapeutics Workshop

Protein Therapeutics Interest Group (PTIG) workshop was held from 5:45 PM to 7 PM on Monday, May 21, 2012. Approximately, 175 to 200 scientists attended the workshop. The group was responsible for two oral sessions as well: "Biotherapeutics and Their Impurities and Degradants: Structural Characterization and QC" and "Biotherapeutics and Biomarkers: New Developments in Quantitative Analysis". For the first time a short course entitled "Practical Mass Spectrometric Characterization of Protein Therapeutics" was provided for conference attendees on the weekend. The oral sessions, short course and workshop were all well attended and contained considerable discussion on the topic of biotherapeutics. Taken together these show a continued strong interest in protein therapeutics within the ASMS community and, specifically, the topics related to the characterization and quantitation of the post-translational modifications on them.

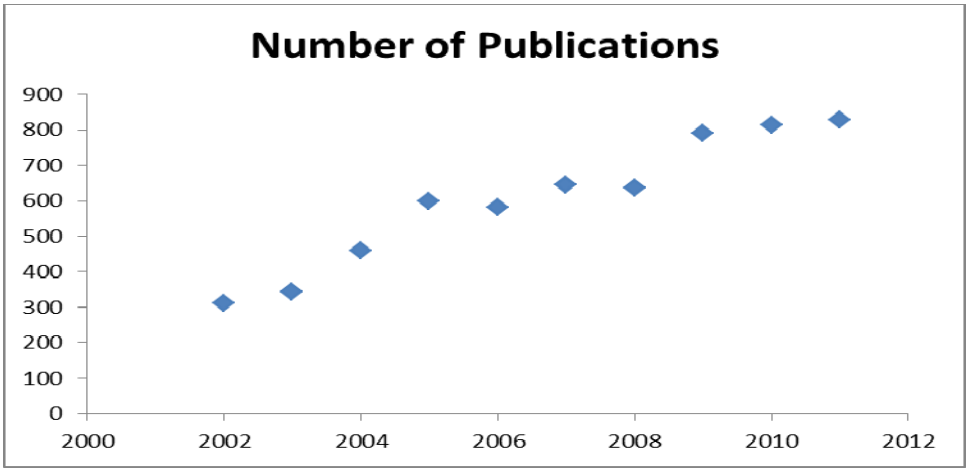

Figure1. The number of publication on protein characterization by mass spectrometry in the past 10 years.

The workshop was organized around two areas of focus in the biotherapeutic industry, the characterization and quantification of the host cell proteins (HCPs) and antibody drug conjugates (ADCs).

(1) **General Protein Characterization/quantification**: (Sheng Gu)
Sheng Gu (Biogen Idec) gave a very brief introduction and history of the workshop, the general methodologies used for the characterization of protein therapeutics, and highlighted the expanding fields in the industry. The increased rate of publications clearly demonstrates the demand for new and improved methods for mass spectrometry-based analysis of biotherapeutics.

(2) **Characterization and quantification of HCPs**: (Weibin Chen and Justin Sperry)

Weibin Chen (Waters) provided an excellent introduction to the mass spectrometry-based method for HCP identification and quantification. The identification was achieved by an almost complete and automated workflow which utilizes two dimensional separation (RP-RP) and data-independent MS/MS acquisition. The results from the mass spectrometry-based approach were compared to the results obtained from ELISA (the traditional method for quantitation), and a similar trend was observed. Questions from the audience were mainly focused on the comparison between the data obtained from ELISA and LC-MS/MS. The LC-MS/MS experiment is intended to aid process development and to demonstrate process consistency and enable HCP monitoring throughout the development lifetime of a biotherapeutic. It was clearly demonstrated that the LC-MS/MS approach may afford a broader HCP coverage versus kit-based ELISA methods.

Justin Sperry (Pfizer) presented an experiment using TMT stable isotope tags to afford relative quantitation of HCPs in different lots of Qbeta VLP. This method only targeted cysteine containing peptides, but it covered over 80% of the proteins in the proteome. Six-plex TMT tagging experiments showed that the batch-to-batch consistency was within a two-fold change for most proteins and a four-fold change for all proteins detected. The identified proteins consisted of ribosomal protein, nucleotide binding protein and metabolic pathway related proteins. A critical question regarding the method was raised—whether the quantified HCP proteins are all high abundance proteins? This particular method cannot distinguish between high and low abundance proteins because there is an enrichment step. It was mentioned, however, that by a traditional 2D approach with no enrichment that only a portion of the HCPs were identified, indicating that these were the most abundant HCPs in the VLP.

(3) **Characterization of Antibody Drug Conjugates (ADCs)** (Jingiie Mo and David Hambly)

Jingjie Mo (BMS) presented a general mass spectrometry-based experimental design for ADC characterization. The discussion centered around a small molecule attached to the antibody using a one-step chemical reaction. The modification is labile and CID MS/MS could not determine the exact modification site on the antibody scaffold. The general strategy for ADC characterization is to evaluate the reaction efficiency by determining the intact ADC first, which also affords the conjugation ratio and the heterogeneity of the product. The next step for ADC characterization was peptide mapping using ETD MS/MS to determine the precise modification sites. It was mentioned that the conjugated drug significantly affected the electrospray ionization, thus MALDI was used to determine the molecular weight before and after conjugation. Questions regarding approaches to accurately quantify the amounts of drug loading by mass spectrometry and whether or not they were consistent with other orthogonal methods were raised.

David Hambly (Amgen) presented a case study regarding the effect of buffer conditions on the conjugation efficiency and ratio for ADCs. Antibody drug conjugation using DOTA-NHS to couple a metal chelator to lysine residues was studied using UPLC-MS-based methodology. The apparent ratio of moles of DOTA per mole of antibody (DAR) changed significantly when there was minimal variation in phosphate buffers. A systematic study utilizing 96-well reaction plates and an automated LC-MS workflow was carried out in about a week. The comparison of the deconvoluted mass spectra determined the concentration of phosphate and NaCl has major impacts on the conjugation rate. The discussion focused on the throughput of the method, the change of ionization and the impact of high concentration salt on desalting efficiency.

The meeting was adjourned around 7pm with very positive responses!

Respectively submitted,

Sheng Gu, Ph.D.
Justin Sperry, Ph.D.
Coordinators of Protein Therapeutics Interest Group