Antibody Drug Conjugates—A New Bioanalytical/Analytical Challenge

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Legend: Antibody drug conjugates consist of a specifically targeted monoclonal antibody, a linker moiety, and a highly potent small molecule drug. (Image from http://www.biooncology.com/bioonc/images/adc-main.jpg)

Antibody drug conjugates (ADCs) link precisely targeted antibodies to cytotoxic small molecule drugs with the goal of improving both the safety and efficacy of chemotherapeutics. Pivotal clinical trials of trastuzumab-DM1 for women with treatment-resistant HER2-positive breast cancer and of SGN-35 for relapsed/refractory Hodgkin's lymphoma represent the vanguard of several dozen ADCs currently in various stages of clinical development. According to Fred Regnier, Purdue University's John H. Law Distinguished Professor of Chemistry, Principal Investigator for the Purdue/IU Analytical Proteomics Team, and founder of Perfinity Biosciences, "The emergence of new isolation methods paired with stable isotope coding and mass spectrometry measurement of proteins, modified proteins, and their tryptic digests provides an unprecedented level of specificity and quantification in examining their structure, relative activity, and degradation in biological systems."

In ADCs the antibody acts primarily as a carrier to deliver the potent drug to the target tissue, thus limiting total body exposure to the free and potent small molecule, a strategy that can mitigate toxic side effects. However, ADCs pose several challenges, including the tendency for the covalently bound

small molecules to detach and circulate freely in the body, the difficulty of manufacturing conjugates reproducibly, and the metabolic fate of the conjugate and the antibody. Currently the analysis of ADCs requires performing four distinct assays on each plasma sample, two small molecule assays and two ELISA assays. The small molecule assays detect freely circulating drug and drug bound in ADCs. The ELISAs quantify total ADC and total free antibody.

MPI Research and Perfinity Biosciences are currently collaborating to streamline and validate this process. One option is to measure the free and bound small molecule levels by using immunosorbent technology to trap free drug for analysis, while the protein-containing eluate is collected and hydrolyzed to liberate bound drug for analysis. Thus, free and bound small molecules could be analyzed from the same serum sample rather than from duplicates. This process would remove any uncertainty about the liberation of the small molecule from the ADC during freeze-thaw procedures or during long-term storage. The proteins could be measured using two different ELISA assays: the small molecule free antibody could be measured by conventional ELISA with an antibody that recognizes the antibody/linker and the ADC, and the antibody-containing drug molecule could be measured by an ELISA that utilizes an antibody that recognizes the small molecule/linker portion of the ADC.

To further complicate analysis, ADCs are not homogenous products, but rather a mixture containing differing numbers of potent small molecules attached to each antibody. Therefore, the batch-to-batch distribution differences and the kinetics of the changing small molecule distribution in vivo may impact the clinical effectiveness of the compounds. Currently only gross averaged data are obtained, which may confound an accurate pharmacokinetic/pharmacodynamic prediction or batch potency determination. Simultaneous quantification of total ADC, in vivo fragments of the ADC, and drug conjugated at each site in the ADC can be achieved using MRM technology and carbon 13-labeled isopotomers of peptides and drug-bearing peptide conjugates. This analytical approach quantifies small molecules linked at specific locations on the antibody and tracks the binding and retention of the small molecule on the antibody to provide a more accurate indicator of the potency of each batch, as well as its potential in vivo performance.

Relative binding affinity with receptors also affects how well ADCs perform. For example, if antibody without any drug has a higher affinity for the receptor than antibody with drug, then the presence of drug-free antibody would block the desired binding of antibody-containing drug, potentially attenuating the ADC’s efficacy. Suppose, for instance, that two separate batches of 20 ADCs each have an average of

five small molecules bound per antibody. Would a batch in which all 20 ADCs contain five drug molecules possess the same toxicity and efficacy as a batch in which 10 antibodies contain 10 drug molecules and 10 antibodies are drug-free?

We believe an assay that traps the ADC using an immobilized receptor protein or antibody, followed by deglycosylation and enzymatic digestion of the protein, would yield a series of peptides that can be analyzed by liquid chromatography and mass spectrometry. The peptides that contain drug/linker would differ in retention and molecular weight from the analogous peptides that did not contain a linked small molecule. Tracking these peptides would permit the fate of specific sites of small molecule binding to the antibody to be tracked during processing and after in vivo administration. If sites that were more labile to in vivo release could be identified, then product specifications could limit the amount of ADCs containing linked drug at these labile sites. The result would be a safer and more potent ADC mixture.

Although ELISA assays are much more sensitive than mass spectrometry for analysis of manufacturing lots and administered therapeutic proteins, assay sensitivity may not be an issue. For example, a hypothetical ADC with a molecular weight of 150,000 daltons that required an overall limit of quantitation of 1 ng/ml in serum would need a 10-fold concentration (100 ng/ml) limit of quantitation for the MALDI target peptide, assuming that the ideal MALDI peptide had a molecular weight of 15,000 daltons and minimal heterogeneity. The hypothetical ADC with a molecular weight of 150,000 daltons that required an overall limit of quantitation of 1 ng/ml would need a 100-fold concentration (10 pg/ml) limit of quantitation for Ionspray target peptide, assuming an ideal Ionspray peptide with a molecular weight of 1500 daltons and minimal heterogeneity. This hypothetical case reasonably resembles actual experience with Herceptin, which has an average molecular weight of 145,531 daltons. Its published assay has a validated range from 5-100 ng/ml. The samples in the published ELISA are diluted 1000 to 2000-fold to correspond to an assay of 10-200 µg/ml. The published Cmax values for Herceptin are in the µg/ml range, suggesting that the sensitivity of many ELISA assays is too low for protein therapeutics.

We believe that new bioanalytical and analytical processes will be necessary to support emerging ADC therapeutics. To that end, MPI Research and Perfinity Biosciences, in consultation with Prof. Fred Regnier, are actively investigating innovative bioanalytical/analytical methods for ADCs. We are proud to be leading the scientific advances necessary to allow ADCs to achieve commercialization and widespread clinical application.

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