BioAnalytix

Accelerating the Development of Biologic Therapeutics through Advanced Analytics

Advances in Biologic Development: Science Letter 1.0

In Vivo CQA Mapping—Opportunities in De-risking and Advancing Biologic Drug Development

We are experiencing unprecedented innovation, growth and opportunity within the biopharmaceutical industry, with increasingly promising yet complex biologic therapeutic platforms in development, including advanced monoclonal antibodies (mAbs), recombinant human proteins, enzyme-replacement therapies, fusion proteins, antibody-drug conjugates, bi-specific drugs and gene and cell therapy approaches. However, despite significant advances in therapeutic platform development, almost 90% of biologic drug candidates fail from Phase I to Approval, and over 50% of biologics still fail in Phase III [1-3]. As a key element of biologic development today, precise characterizations of a drug candidate's structures, post translational modifications (PTM's) and determinations of its Critical Quality Attributes (CQA's) are essential in defining, optimizing and controlling development programs from early candidate design through bioproduction scale-up, CMC package development, clinical trials, BLA submission and post-launch commercialization.

However, such precise characterizations are typically conducted on a drug candidate in its formulation buffer (*'in vitro'*), while the drug's key structural attributes may be directly altered, metabolized, cleared or enhanced as soon as it enters its actual target biologic system (*'in vivo'*), directly impacting the drug's actual stability, safety and efficacy in the patient. Therefore, a more effective understanding of a biologic candidate's structural attributes *in vivo*, along with a determination of patient exposures to specific drug structures and PTM's through a full PK time course, may enable a significantly more effective, de-risked biologic development program from candidate selection through dosing determinations, CMC program development and potentially clinical trial design and analysis.

Science of *In Vivo* CQA Mapping:

Historically, general biologic drug PK levels have been assessed by ELISA in a pre-clinical model or clinical subject, while related drug sub-structures and PTM's have rarely been assessed *'in vivo'*, partly because it has been technically difficult to effectively recover, quantify and precisely characterize individual protein structures and PTM's from biologic fluids. Recently, however, multi-attribute affinity purification approaches have been combined with high-precision LC-MS profiling to effectively quantify and assess the impact of specific biologic drug structures and related PTM levels *in vivo* [4-5]. These advanced '*In Vivo* CQA mapping' methods utilize specifically targeted drug extraction and preparation strategies, in conjunction with high-sensitivity LC-MS based structural characterizations of the biologic, to generate both a detailed quantitative PK profiling of the drug (covering multiple peptides simultaneously), as well as a highly precise profiling of the drug's structural attributes, metabolites and PTM's, all as assessed through a full PK time course directly from pre-clinical and/or clinical samples (serum, plasma or other tissue types). Specific *in vivo* CQA's may include glycosylation, sequence variants, terminal truncation, chain cleavage, disulfide variants and typical modifications such as deamidation, oxidation, pyroE, glycation and hydroxylation. Further, these specific structure assessments may also be evaluated relative to drug stability *in vitro*, clearance, patient exposure to individual attributes and clinical response by patient or patient group. This correlation of a biologic drug's structural attributes to patient exposure levels and performance *in vivo* may be used to define, optimize and guide early-stage candidate selection, dosing levels into Phase I, later-stage bioproduction strategies and decisions, CMC and control packages and potentially clinical trial designs and analysis going forward.

[1]Thomas DW, Burns J, Audette J, Carroll A, Dow-Hydelund C, Hay M. Clinical Development Success Rates 2006-2015. BIO Industry Analysis, 2016.
[2]Grignolo A, Pretorius S. Applied Clinical Trials 2016, 25(8). http://www.appliedclinicaltrialsonline.com/phase-iii-trial-failures-costly-preventable
[3]Sachs et al. JAMA 2014, 311(4):378-84.
[4]Li et al. mAbs 2016, 8(5):961–968.
[5]Li et al. mAbs 2016, 8(6):1079-87.

Key Words: Biotherapeutic; mass spectrometry; critical quality attributes; in vivo; PK quantitation, pre-clinical and clinical study design and analysis, PTM.

About BioAnalytix: BioAnalytix (www.bioanalytixinc.com) is a pharmaceutically oriented spin-out from the Barnett Institute of Chemical and Biological Analysis at Northeastern University. Founded in 2012 by thought leaders in biologic drug development, regulatory strategy and advanced analytics, BioAnalytix works with leading pharmaceutical companies to develop and apply advanced analytic technologies, methods and data analysis in enabling, improving and accelerating biologic therapeutics from development through market.

Learning More and Next Steps: We will be pleased to connect and discuss these approaches and potential *In Vivo* CQA mapping projects in your biologic development programs any time. Please feel free to contact us directly or visit us at our scientific posters at the WCBP Meeting in January.

Very best,

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