Overview

Monoclonal antibodies (mAbs) represent currently the therapeutic agent category experiencing the most important progression due to their therapeutic potency and specificity. Some patents regarding mAbs are going to end in the near future, giving the opportunity to alternative actors to produce and market the same protein which is defined in biopharmaceuticals as biosimilar. Here, capillary zone electrophoresis coupled to tandem mass spectrometry by the intermediate of an ultra-low flow interface (CESI-MS/MS) was used to characterize marketed mAbs and their respective candidate biosimilar simultaneously over different facets of their primary structure using a sole sample. Injection of 200 fmol of digested peptides, CESI-MS/MS data enabled to obtain simultaneously 100% sequence coverage, structures of 15 glycoforms and the characterization of all PTMs hot-spots present on the studied mAbs samples. CESI-MS/MS allowed to conclude regarding the biosimilarity study between approved mAbs and biosimilar candidate. Characterization results allowed to specifically point out the facets of the candidate which were not complying to be considered as a biosimilar.

Methods

Studied samples. Trastuzumab, cetuximab in their final formulation and respective candidate biosimilar (Trastuzumab-B and Cetuximab-B) were characterized by CESI-MS/MS and the obtained data confirmed for biosimilarity assessment.

Samples treatment. Tryptic digestion in solution of mAbs. Digestion buffer: 50 mM bicarbonate ammonium, reducing reagent: diithothreitol 100 mM, alklyation reagent: iodoacetamide 100 mM. Sample peptide final concentration: 0.3 µg/µL.

Results

Amino acids sequence characterization / comparison

100 % sequence coverage (no misalignments / PTMs) 1 AA difference characterized for trastuzumab biosimilar

Trastuzumab glycoprofiling / profiles comparison

Cetuximab glycoprofiling / profiles comparison

Different glycoforms characterized between cetuximab and biosimilar (glycans containing murine sialic acids)

Conclusions

Data obtained from solely one injection of each sample tryptic digest allowed to characterize each mAbs simultaneously over several levels defining their primary structure. Biosimilarity study results indicated that in both cases, candidate biosimilars exhibited minor differences compared to the original mAb. In the case of trastuzumab-B, one AA difference compared to the original mAb led to this conclusion while in the case of cetuximab-B uncontrolled expression of murine glycans motifs to human metabolism ended in the rejection candidate biosimilar.

Acknowledgements

Authors would like to thank AB Sience for the loan of a prototype CESI system and a 5600 Triplet TOF® mass spec. The authors would like to express their gratitude to Dr. M. Amelene (Sience, Les Ulis, France) for his support and Dr Joseph Visaka (Merck, West Point, PA) for critical discussions around antibody structural characterization.

References


