Background The US FDA has approved more than 100 antibody therapeutics (mAbs) and nearly 900 of them are currently being investigated in clinical trials. Accurate quantitation of antibody is critical for pharmacokinetics studies during the development of mAbs. In clinical settings, mAb monitoring has been applied to the dose adjustment for autoimmune disease. We recently showed that trough levels of mAbs can be a biomarker for cancer immunotherapy.1 Thus, the deployment of a rapid and universal platform for mAb monitoring may help streamlining processes ranging from drug development to clinical practice for a wide spectrum of diseases. However, mAb monitoring often depends on the development of an individual ligand binding assay, which is impractical to scale.2 Previously, we developed a method for mAbs quantitation that uses Fab-selective proteolysis of mAb followed by detection of signature peptides by LC-MS called nSMOL (nano-surface and molecular-orientation limited proteolysis) technology.3 So far, we have established the validated LC-MS conditions for over 35 mAbs.

Methods We confirmed that count per second (CPS) ratio between different signature peptides were constant thanks to negligible matrix effect in nSMOL assay. Therefore, we hypothesized that we could select optimal reference antibody and use that as a universal reference antibody for highly multiplexed mAb quantitation. To select a universal reference mAb (refmAb-Q), we picked 18 mAbs and compared quantitative reproducibility of each mAb from singleplex and 18-plex nSMOL assay with a concentration from LLOQ to HQC.

Results We found that the CPS ratio to trastuzumab was unique to each mAb and consistent when we arbitrarily picked trastuzumab. We identified optimal reference mAb candidates based on the several physicochemical properties of signature peptides. The refmAb-Q nSMOL assay showed perfect concordance with the conventional nSMOL with an authentic reference in clinical sample from ipilimumab-treated patients (NCT00495066), pembrolizumab-treated patients (NCT02575404), and patients with the combination immunotherapy of ipilimumab and nivolumab.

Conclusions This innovative refmAb-Q nSMOL platform may provide a practical solution for quantitating an ever-increasing number of mAbs from developmental to clinical use settings. We envision that wide adaptation of refmAb-Q nSMOL is possible for PK and TDM assays for mAbs in the treatment of cancer and autoimmune diseases. Furthermore, refmAb-Q nSMOL can be applied to the quantitation and structural analysis of individual autoantibodies and neutralizing antibodies.

REFERENCES