Poster

[P25-9] P25-9: Oncologic drugs (1)

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[P25-9-4] Quantification method for immune check point inhibitor, Nivolumab, using liquid chromatography tandem mass spectrometry

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Background

Nivolumab is an immune checkpoint inhibitor approved for the treatment of metastatic melanoma, non-small cell lung cancer, renal cell carcinoma and Hodgkin's lymphoma. Although dramatically response is observed in some patients, serious immune mediated side effects are major problems and risk of interstitial lung disease owing to EGFR-TKI using in post nivolumab treatment is reported. To examin the relationship of nivolumab sustain blood concentration and immune mediated side effects, we established LC-MS/MS method for the quantification of nivolumab in plasma.

Methods

Nivolumab selective trypsin digested peptide in plasma was simulated using Skyline (MacCoss Lab Software).plasma IgG including Nivolumab was purified by rProtein A sepharose Fast Flow (GE Healthcare). Proteins denaturation, reduction and alkylation of disulfide bind, trypsin digestion and solid phase extraction were performed using ProteinWorks eXpress Digest Start-up Kit (Waters). Chromatographic separation was performed on inertsil peptides C18 analytical column (GL science) with agilient 1280 LC system. The gradient mobile phase was composed of acetonitrile and water containing 0.1% formic acid. Nivolumab selective peptide was detected by quantum triple quadruple mass spectrometer (QTRAP4500, AbSCIEX). The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode with positive ion electrospray ionization.

Results

Three peptides (ASGITFSNSGMHWVR、 GLEWVAVIWYDGSK、 NTLFLQMNSLR) were identified as nivolumab selective peptide by simulation of skyline. ASGITFSNSGMHWVR peptide could be detected MRM mode and MRM transition was m/z 550.6→661.4. Although contaminating peak was observed in plasma IgG alone, nivolumab peak could be separated by gradient extraction. Linear range of nivolumab in plasma was 5~200μg/mL (R2=0.999). Accuracy was 90.8-109.0% and intra-assay precision was 2.6-14.4%.

Conclusions

Quantification method of nivolumab using LC-MS/MS was successfully developed. Quantification range covers clinical concentration of nivolumab. This LC-MS/MS method might be appliable for other therapeutic monoclonal antibodies including many immune checkpoint inhibitors in a development phase. Although it is unknown whether therapeutic drug monitoring of immune check point inhibitor is necessary, clinical research exploring relationship of nivolumab concentration and immune mediated side effects in non-small

cell lung cancer patient is ongoing in our hospital. We will present result of validation and the clinical research.