RESEARCH ARTICLE

Verification between Original and Biosimilar Therapeutic Antibody Infliximab Using nSMOL Coupled LC-MS Bioanalysis in Human Serum

Noriko Iwamoto1, Kotoko Yokoyama1, Megumi Takanashi1, Atsushi Yonezawa2,3, Kazuo Matubara2 and Takashi Shimada1*

1Leading Technology of Bioanalysis and Protein Chemistry, Shimadzu Corporation, Kyoto, Japan; 2Department of Clinical Pharmacology and Therapeutics, Kyoto University Hospital, Kyoto, Japan; 3Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

Abstract: Background: Infliximab (IFX) is a chimeric therapeutic monoclonal antibody targeting tumor necrosis factor alpha (TNFα)-mediated inflammatory immune diseases. However, despite of an initial good clinical response, decrease in response to long-term treatment is a common observation.

Objective: Recent studies suggest that IFX level in circulation has a correlation with clinical bioavailability. Therefore, the management of IFX dosage for individual manifestation by IFX monitoring may be valuable for the improvement of therapeutic response and outcomes.

Method: In order to develop a broad IFX therapeutic monitoring in human serum, we have developed the validated IFX bioanalysis for Remicade™ and its biosimilar product using our nano-surface and molecular-orientation limited proteolysis (nSMOL) technology coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The nSMOL chemistry has a unique property of Fab-selective proteolysis, and makes it possible a global bioanalysis for many monoclonal antibodies.

Results: The quantitation range of IFX in serum was from 0.293 to 300 µg/ml with good linearity. Quantitation verification at the concentrations of 0.293, 0.879, 14.1 and 240 µg/ml was within 1.56-7.53% of precision and 98.9-111% of accuracy using H-chain signature peptide SINSATHYAESVK. Moreover, cross-verified bioanalysis of Remicade quantitation using biosimilar standard, and its opposite combination, obtained an identical and inter-comparative results.

Conclusion: The nSMOL strategy has the potential as a practical therapeutic monitoring technology in IFX therapeutic applications.

Keywords: Infliximab, biosimilar, nSMOL, LC-MS, bioanalysis, clinical pharmacokinetics, therapeutic drug monitoring.

1. INTRODUCTION

1.1. Background

Infliximab (IFX) is a chimeric monoclonal IgG1 kappa antibody targeting tumor necrosis factor-alpha (TNFα) signaling inhibitor approved by Food and Drug Administration in US at 1998 [1], and is widely treated to immunological basis of inflammatory diseases such as rheumatoid arthritis (RA), [2] psoriatic arthritis (PsA) [3], Behcet syndrome (BD) [4], ankylosing spondylitis (AS) [5], plaque psoriasis (PPs) [6], inflammatory bowel disease (IBD) [7], Kawasaki disease (KD), [8] and Crohn disease (CD) [9]. The original IFX Remicade™ has already expired its patent, and several biosimilar antibodies are available in the market [10]. Biological products such as therapeutic antibodies have a diverse category, and generally high-molecular-weight compounds [11-13]. Biosimilar is defined as a biological product that has highly similarity of character and no clinical and bioactive properties from an approved reference product [14]. However, the potential implications of the glycosylation profile changes in antibody production, especially in biosimilars, are one of the key factor for clinical efficacy and/or outcomes. And new technologies for multiple monitoring the glycosylation patterns have been recently reported using the selectivity and quantitation potentials of mass spectrometry [15]. There are currently two biosimilar products approved by FDA and EMA (CT-P13 from Celltrion/Hospira/Nihon-Kayaku and SB2 from Biogen/Merck). In Japan, CT-P13 is only a...
1.2. Significance of Infliximab Monitoring

Pharmacokinetic properties of IFX vary dependent on each disease. The half-life of IFX in circulation can be affected by combined immunosuppressive agents, and the concentration of TNFα and/or C-reactive protein (CRP). Moreover, some study showed that more than 23% of patients with CD met a secondary failure to IFX treatment for a year after IFX maintenance [17, 18]. Therefore, it should become significant that the therapeutic concentration management by IFX trough monitoring is improved with clinical response and prognosis [19-22]. And IFX treatment can be result in the formation of anti-infliximab antibodies (anti-drug antibodies, ADA). Filip et al. reported that ADA to IFX were detected in over 60% of patients in the treatment of CD, and ADA was associated with a decreased response to long-term efficacy [23]. The formation of ADA is certainly affected to immunogenicity, therefore a guide for clinical decision on next treatment strategy for secondary failure or partial response using anti-TNF biopharmaceuticals has been designed based on IFX and ADA monitoring [24-26]. These study strongly suggest that therapeutic drug monitoring (TDM) of IFX should support the therapeutic response to immunological basis of inflammatory diseases [27-29].

1.3. Current Technologies in Infliximab Monitoring

Currently, ligand-binding assay (LBA) is widely used to monitor IFX level, for example enzyme-linked immunosorbent assay (ELISA), digital ELISA, radioimmunoassay (RIA), and immunochromatography [30]. However, there are some remarkable issues in selectivity, linear dynamic range, cross reactivity, and the differences in quantitative data between various ELISA methods [31, 32]. Moreover, our data showed that coexistence of ADA was direct source of quantitative inhibition in ELISA assay, and the level of quantitative inhibition changed in dependence on the type of idiotypic antibody clones or its specific ligand [33]. Therefore, cross validation study should be necessary for IFX monitoring by LBA. To overcome these issues, clinical monitoring test such as TDM and pharmacokinetics should be carried out with the identical and practical assay method to exclude unexpected errors.

1.4. Development of LC-MS/MS based Global Therapeutic Antibody Monitoring

The feasibility of monitoring IFX and other therapeutic antibodies in human serum by liquid chromatography and tandem mass spectrometry (LC-MS/MS) has recently been advanced in several clinical laboratories [17, 34, 35]. Quantitation of tryptic signature peptide in antibodies can provide the high-sensitivity and selectivity, expectation for the prevention from IFX-specific binding substrates. Several approaches using LC-MS/MS have been reported such as fractionated pellet digestion, whole serum digestion, and hybrid LBA-LC-MS/MS, but these are limited applications [36]. In antibody bioanalysis by LC-MS/MS, there are several essential issues, such as separation ability in the LC, the ion suppression effect of the target molecule due to coexisting excess ions in the MS, maintenance of the ESI interface, and more than anything, universal use purpose for any antibodies. Recently, we have reported a sole LC-MS/MS bioanalysis method independent of a variety of antibodies [37]. The nSMOL (nano-surface and molecular-orientation limited proteolysis) chemistry is designed as a Fab-selective proteolysis of IgGs which maintaining antibody sequence specificity while minimizing additional analyte peptides and trypsin contamination. In this principle, trypsin immobilized on the surface of nanoparticle reacts by the physicochemically limiting trypsin access to the antibody substrate utilizing the diameter difference of immobilized trypsin particle and IgG collection resin pore. IgG molecules gather into the pore via Fc. Therefore, as a consequence, Fab is oriented to the reaction solution. We have already developed fully validated assays for multiplexed quantitation using nSMOL for many monoclonal antibodies [38-42]. These results show the significant value of regulated LC-MS analysis. In this report, we have discussed the validated analysis of IFX in human serum for TDM application and its biosimilar reciprocal verification using the same condition of IFX assay.

2. MATERIALS AND METHOD

2.1. Chemicals

nSMOL™ Antibody BA kit for monoclonal antibody quantitation and reaction socket tubes was commercially available from SHIMADZU Corporation (Kyoto, Japan). Infliximab original Remicade™ was obtained from Mitsubishi Tanabe Pharma (Osaka, Japan), and biosimilar Infliximab NK™ (CT-P13) was from Nippon-Kayaku (Tokyo, Japan). Individual three male and female human serums was from Kohjin Bio (Saitama, Japan). P14R, a synthetic peptide for IFX reference species KEGG Drug en-try D02598), Rituximab (RTX, D02994), Brentuximab vedotin (BRX, D09587), and Cetuximab (CTX, D03455) in Fig. (1). The tryptic peptide identification was achieved by high-resolution LCMS-IT-TOF MS (SHIMADZU, Kyoto, Japan) and Mascot (Matrix Sciences, London, UK) in-house proteome database server. And these identified data were confirmed and organized by the information-based software Skyline (MacCoss, University of Washington) [43]. The LCMS conditions were as follows: solvent A, 0.1% aqueous formic acid; solvent B, acetonitrile with 0.1% formic acid; column, L-column2 ODS, 2.1x150 mm, 2 µm, 10 mm pore (Chemicals Evaluation and Research Institute, Tokyo, Japan); column temperature, 40°C; flow rate, 0.2 ml/min; gradient program, 0-5 min: %B=3, 5-35 min: %B=3-30 gradient, 35-46 min: %B=95, 46-55 min: %B=3. MS and MS/MS spectra were obtained with the desolvation line and heat block temperature at...
200°C, respectively. Nebulizer nitrogen gas flows were set to 1.5 liter/min. Drying gas pressure was 100 kPa. Ion accumulation time was 30 msec for MS, and 70 msec for MS/MS analysis. MS/MS analysis was performed by automated data dependent mode. Ar pulse time into ion trap cell was 125 µsec. The electrode of collision-induced dissociation (CID) cell was set at -1.5 V. Using the identified peptide structure, predicted CDR (complementarity-determining region)-containing peptides were selected as candidate signature peptides after comparison with ClustalW results.

2.3. Signature Peptide Selection of IFX in Human Serum

The selected candidate signature peptides were verified by the addition of each antibody in serum and the preparation of a set of diluted samples. nSMOL reaction proceeded as follows: All control and test samples were filtered by 5 µm and then by 0.65 µm filter devices to remove debris. All protocols were followed by nSMOL kit protocols with minor modification. Briefly, a 5 µL aliquot of antibody-spiked human serum was diluted 10-fold in washing solution 1. The IgG fraction in serum was collected in 12.5 µL of immunoglobulin collection resin with gentle vortexing at 25°C for 5 min. Nonspecific binding to resin was washed twice with 300 µL of washing solution 1 and then with 300 µL of washing solution 2. And washed resin was suspended in 75 µL of enhanced reaction solution containing 10 fmol/µL P14R peptide. nSMOL proteolysis was accomplished by 10 µL FG-beads on trypsin with gentle vortexing.

Fig. (1). ClustalW sequence alignment of Infliximab (IFX), Rituximab (RTX), Brentuximab vedotin (BRX), and Cetuximab (CTX) of a) H-chain alignment and b) L-chain alignment. Black area is shown as a framework structure with common amino acid sequences. And, Gray is similar amino acid position. White and blank are shown the different and deletion amino acid position. Red line squares show the candidate signature peptide for IFX quantitation by nSMOL bioanalysis.
at 50°C for 5 h under saturated vapor atmosphere. nSMOL reaction was quenched by addition of quenching solution. The peptide solution was collected by centrifugation (10,000 g for 1 min) and magnetic separation to remove collection resin and FG-beads. These analytes were transferred into low protein binding polypropylene vials, and then analyzed by triple quadrupole LCMS-8050 (SHIMADZU). The LC-MS conditions were as follows: solvent A, 0.1% aqueous formic acid; solvent B, acetonitrile with 0.1% formic acid; column, Shim-pack GISS C18, 2.1x50 mm, 1.9 μm, 20 nm pore (SHIMADZU); column temperature, 50°C; flow rate, 0.4 ml/min; gradient program, 0-1.5 min: %B=5, 1.5-4 min: %B=5-45 gradient, 4-4.8 min: %B=95 with flow rate 1 ml/min, 4.8-5.2 min: %B=1 with flow rate 1 ml/min, and 5.2-6 min: %B=5. MS spectra were obtained with the ESI probe temperature, desolvation line and heat block at 300°C, 250°C and 400°C, respectively. Nebulizer, heating, and drying nitrogen gas flows were set to 3, 10, and 10 liters/min, respectively. The dwell time was set to 10 msec for each transition. MRM (multiple reaction monitoring) monitor ions of peptide fragments were imported from the measured values of structure-assigned fragments by high-resolution LC-MS analysis. CID gas of argon partial pressure in Q2 cell was set to 270 kPa. The electrode voltage of Q1 pre bias, collision cell Q2, Q3 pre bias, parent and fragment ion m/z were performed by optimization support software (LabSol). Peptide structure-indicated MRM signals with good correlation to drug concentration were selected as the signature peptide transitions of each antibody drugs. Finally, for the setting of MRM transition, we selected the most marked b- or y-ion series fragment as a quantitation, and additional fragment as a structure confirmation. In Infliximab assay, peptides SINSATHYAESVK (aa.55-67 on H-chain), YASESMGIPSR (aa.50-61 on L-chain), and ASQFVGSSIHWWYQQR (aa.25-39 on L-chain) were selected as signature peptides for Infliximab quantitation. Each MRM chromatogram of three signature peptides is shown in supplementary materials (Fig. S1-S3).

2.4. Method Verification between Original and Biosimilar IFX

The analytical method was first validated in accordance with the Guideline on Bioanalytical Method Validation in Pharmaceutical Development from Notification 0711-1 of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, the Ministry of Health, Labour and Welfare, dated July 11, 2013. The summary of validation data was shown in Tables 1 to 7. All validation sample set were prepared and stored at -80°C for 24 h or more before each validation assay. The concentrations of IFX in human serum were set from 0.293 to 300 µg/ml with 2-fold serial dilution as 11 calibration samples. The concentrations of lower limit of quantification (LLOQ), low quality control (LQC), middle quality control (MQC) and high quality control (HQC) were set 0.293, 0.879, 14.1 and 240 µg/ml, respectively.

3. RESULTS

3.1. Selection of IFX Signature Peptides for Practical Clinical Bioanalysis

Selected signature peptides of IFX bioanalysis are shown by ClustalW alignment in Fig. (1). We have several criteria for the selection of good signature peptide: peptides with about 8 to 15 amino acid sequences, with no missed cleavage of trypsin, exclusion of close to the position of conserved disulfide bonding on the framework. Five candidate peptides were detected and identified from CDR-containing domain on Fv region. Fig. (2) shows the 3D configuration on antibody structure, so that nSMOL reaction was very successfully reacted on high Fv-selective manner similar to our previous reports like Trastuzumab, Bevacizumab, and

![Fig. (2). Signature peptide configuration of IFX into 3D antibody structure. Green color shows the signature peptides from IFX H-chain, and red is from L-chain. nSMOL successfully reacted as Fv-selective manner.](image-url)
Table 1. MRM transition summary for Infliximab signature peptide candidate.

| Selected Peptide | Region             | Optimal MRM Condition | Role       |
|------------------|--------------------|-----------------------|------------|
|                  | Transition Mass Filter [m/z] | Q1 [V] | Collision [V] | Q3 [V] |                |
| SINSATHYAESVK    | H-chain of CDR2    | 469.8>603.9 (y11 ^+) | -46        | -16     | -34          | Quantitation   |
|                  |                    | 469.7>547.0 (y10 ^+) | -46        | -17     | -28          | Structure      |
| GLEWVAEIR        | H-chain of CDR2    | 536.8>773.4 (y6 ^-)  | -45        | -19     | -34          | Quantitation   |
|                  |                    | 536.8>488.4 (y4 ^-)  | -45        | -18     | -24          | Structure      |
| SAVYLQMTDLR      | H-chain of CDR3    | 649.0>876.4 (y8 ^-)  | -40        | -24     | -32          | Quantitation   |
|                  |                    | 649.0>763.3 (y6 ^-)  | -40        | -23     | -40          | Structure      |
| ASQFVGSSHWYQQQR  | L-chain of CDR1    | 598.8>631.5 (y10 ^+) | -44        | -17     | -32          | Quantitation   |
|                  |                    | 598.8>754.5 (y12 ^-) | -44        | -21     | -30          | Structure      |
| YASESMGIPSAR     | L-chain of CDR2    | 642.8>834.4 (y8 ^-)  | -40        | -25     | -30          | Quantitation   |
|                  |                    | 642.9>359.4 (y3 ^-)  | -40        | -22     | -10          | Structure      |
| P14R             | Internal standard  | 512.1>292.3 (b3 ^-)  | -40        | -20     | -20          | Quantitation   |
|                  |                    | 512.1>389.3 (b4 ^-)  | -40        | -16     | -28          | Structure      |

Table 2. Calibration curve reproducibility of SINSATHYAESVK H-chain peptide from Remicade.

| Set Conc. [µg/ml] | Back-calculated Conc. [µg/ml] | Accuracy (%) |
|------------------|--------------------------------|--------------|
|                  | 1          | 2            | 3            | 1          | 2            | 3            |
| 0.293            | 0.283     | 0.286        | 0.302        | 96.7       | 97.7         | 103          |
| 0.586            | 0.609     | 0.606        | 0.528        | 104        | 103          | 90.1         |
| 1.17             | 1.21      | 1.19         | 1.32         | 104        | 102          | 113          |
| 2.34             | 2.31      | 2.26         | 2.41         | 98.5       | 96.6         | 103          |
| 4.69             | 4.78      | 4.87         | 4.88         | 102        | 104          | 104          |
| 9.38             | 9.82      | 8.93         | 9.83         | 105        | 95.2         | 105          |
| 18.8             | 18.4      | 19.0         | 19.2         | 98.1       | 101          | 102          |
| 37.5             | 37.4      | 38.6         | 37.7         | 99.7       | 103          | 100          |
| 75.0             | 74.9      | 79.0         | 72.3         | 99.8       | 105          | 96.4         |
| 150              | 156       | 145          | 145          | 104        | 96.9         | 96.8         |
| 300              | 276       | 293          | 280          | 92.0       | 97.6         | 93.5         |

Nivolumab, etc. To best our knowledge, nSMOL technology is a novel and broad approach for the Fv-selective bioanalysis of therapeutic monoclonal antibodies. And in Table 1, MRM conditions for candidate signature peptides of IFX and P14R synthetic internal standard are summarized by using LCMS-8050/8060 (SHIMADZU). We recommend that MRM transitions of quantitation and structure confirmation should be acquired at the same time because of the selectivity verification by its structure indexes from several biological matrices.

3.2. nSMOL Assay Validation Using Signature Peptide SINSATHYAESVK

The assay was validated in accordance with the Guideline on Bioanalytical Method Validation in Pharmaceutical Development from Notification 0711-1 of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, the Ministry of Health, Labour and Welfare, dated July 11, 2013 for low-molecular-weight drug compounds. And for the other two peptides YASESMGIPSAR and ASQFVGSSHWYQQQR validation data, we have summarized in supplementary materials (Table S1-S6).

3.2.1. Linearity and Calibration Standard

The linearity of nSMOL bioanalysis (Table 2) was evaluated by the analysis of 11 calibration standards (zero sample, 0.293, 0.586, 1.17, 2.34, 4.69, 9.38, 18.8, 37.5, 75.0, 150, and 300 µg/ml) using linear regression model including the pharmacokinetic study of IFX. The calibration plot was used the 1/Area2 weighting. The accuracy at LLOQ was 96.7-103%, and other concentration set were 90.1-113%. And using the same condition of Remicade, NK calibration set provided almost the same results (Table 3). These data suggest that original and biosimilar product have a very similar conformation and equal nSMOL reaction efficiency.
Table 3. Calibration curve reproducibility of SINSATHYAESVK H-chain peptide from NK.

| Set Conc. [µg/ml] | Back-calculated Conc. [µg/ml] | Accuracy (%) |
|------------------|-------------------------------|--------------|
|                  | 1                | 2          | 3          | 1          | 2          | 3          |
| 0.293            | 0.287            | 0.278      | 0.284      | 98.1       | 94.9       | 96.9       |
| 0.586            | 0.657            | 0.673      | 0.581      | 112        | 115        | 99.1       |
| 1.17             | 1.02             | 1.16       | 1.27       | 87.6       | 98.8       | 105        |
| 2.34             | 2.42             | 2.19       | 2.51       | 103        | 93.7       | 107        |
| 4.69             | 4.96             | 5.03       | 4.74       | 106        | 107        | 101        |
| 9.38             | 9.89             | 8.80       | 10.2       | 105        | 93.8       | 109        |
| 18.8             | 19.6             | 20.1       | 18.3       | 104        | 107        | 97.2       |
| 37.5             | 36.7             | 38.5       | 38.0       | 97.7       | 103        | 106        |
| 75.0             | 71.9             | 70.6       | 69.7       | 95.9       | 94.2       | 92.9       |
| 150              | 146              | 152        | 143        | 97.1       | 101        | 95.4       |
| 300              | 302              | 300        | 298        | 101        | 100        | 99.4       |

Table 4. Carryover test of SINSATHYAESVK.

| Compounds | Run | LLOQ Area | Max Area | CO Sample Area | Area% |
|-----------|-----|-----------|----------|----------------|-------|
|           |     | [0.293 µg/ml] | [300 µg/ml] |                |       |
| Remicade  | 1   | 9,626      | 5,601,867 | 709            | 7.37  |
|           | 2   | 9,366      | 5,201,490 | 887            | 9.47  |
|           | 3   | 8,487      | 5,363,308 | 849            | 10.0  |
| NK        | 1   | 8,321      | 5,987,371 | 1,151          | 13.8  |
|           | 2   | 8,128      | 5,734,701 | 661            | 8.13  |
|           | 3   | 8,113      | 5,533,250 | 606            | 7.47  |

3.2.2. Carryover Test

The carryover test was performed by analyzing 3 replicates for IFX peptide from Remicade and NK just after the analysis of maximum concentration set (300 µg/ml). The carryover was calculated as the response cps value in the blank serum compared with the LLOQ sample. In nSMOL analysis, low level of carryover was observed for the IFX peptide being about 10%, having no influence on this analysis (Table 4).

3.2.3. Precision and Accuracy in Inter- and intra-assay

Precision and accuracy data were confirmed by the analysis of human serum validation sample at LLOQ, LQC, MQC, and HQC concentration of Remicade (Table 5) and NK (Table 6). The intra-day and inter-day precision and accuracy were determined by analyzing 5 QC sample sets at 4 concentrations on 3 different days. These data were summarized in Table 5 for Remicade: precision and accuracy of run 1, 98.9% and 7.53% at LLOQ, 99.5-111% and 1.56-6.60% at other set; run 2, 94.8% and 11.7% at LLOQ, 97.5-104% and 2.02-6.78% for other; run 3, 111% and 5.65% for LLOQ, 97.7-106% and 0.78-10.2% for other; 102% and 10.4% for LLOQ, 98.2-105% and 2.19-8.83% for other set of intra-assay (N=15) results. And same as in NK assay results were shown in Table 6.

3.2.4. Processed Sample Stability

The processed sample stability after nSMOL reaction at 5°C for 24 and 48 hours were demonstrated at the concentration of LQC and HQC. Accuracy of LLOQ and HQC were 101-103% and 93.3-95.3, respectively (Table 7).

3.2.5. Cross Verification between Original and Biosimilar IFX

In order to apply a broad TDM application for many inflammatory immune diseases by nSMOL bioanalysis, cross verification assay of Remicade quantitation by NK standard set, and its opposite combination, was carried out and summarized in Tables 8 and 9. In these two combination, precision and accuracy at LLOQ were 104% and 92.9%, 7.54% and 8.27%; 91.8-102%, 1.48-9.11% at other concentration set, respectively.
Table 5. Precision and accuracy data of SINSATHYAESVK from Remicade.

| Run | Set Conc. | Concentration [µg/ml] | 0.293 | 0.879 | 14.1 | 240 |
|-----|-----------|-----------------------|-------|-------|-------|-----|
| Observed | | | 0.275 | 0.915 | 14.9 | 245 |
| | | | 0.303 | 1.04 | 14.7 | 238 |
| | | | 0.290 | 0.898 | 15.2 | 238 |
| | | | 0.318 | 0.974 | 14.9 | 234 |
| | | | 0.263 | 1.03 | 14.0 | 239 |
| Mean | | | 0.290 | 0.971 | 14.7 | 239 |
| SD | | | 0.02 | 0.06 | 0.45 | 3.72 |
| CV (%) | | | 7.53 | 6.60 | 3.06 | 1.56 |
| ACC (%) | | | 98.9 | 111 | 105 | 99.5 |

| Observed | | | 0.254 | 0.954 | 14.6 | 247 |
| | | | 0.295 | 0.846 | 15.0 | 230 |
| | | | 0.253 | 0.812 | 14.5 | 238 |
| | | | 0.327 | 0.926 | 14.9 | 225 |
| | | | 0.260 | 0.851 | 14.3 | 231 |
| Mean | | | 0.278 | 0.878 | 14.6 | 234 |
| SD | | | 0.03 | 0.06 | 0.30 | 8.49 |
| CV (%) | | | 11.7 | 6.78 | 2.02 | 3.63 |
| ACC (%) | | | 94.8 | 99.9 | 104 | 97.5 |

| Observed | | | 0.310 | 0.792 | 14.8 | 234 |
| | | | 0.337 | 0.886 | 15.0 | 237 |
| | | | 0.301 | 0.986 | 15.1 | 232 |
| | | | 0.334 | 0.793 | 15.0 | 234 |
| | | | 0.343 | 0.955 | 14.7 | 236 |
| Mean | | | 0.325 | 0.882 | 14.9 | 235 |
| SD | | | 0.02 | 0.09 | 0.18 | 1.84 |
| CV (%) | | | 5.65 | 10.2 | 1.18 | 0.78 |
| ACC (%) | | | 111 | 100 | 106 | 97.7 |

N=15

Table 6. Precision and accuracy data of SINSATHYAESVK from NK.

| Run | Set Conc. | Concentration [µg/ml] | 0.293 | 0.879 | 14.1 | 240 |
|-----|-----------|-----------------------|-------|-------|-------|-----|
| Observed | | | 0.278 | 0.822 | 14.6 | 213 |
| | | | 0.217 | 0.824 | 14.8 | 220 |
| | | | 0.279 | 0.779 | 14.4 | 211 |
| | | | 0.273 | 0.877 | 14.5 | 232 |

(Table 6) Contd....
| Run | Set Conc. | Concentration [µg/ml] | 0.293 | 0.879 | 14.1 | 240 |
|-----|-----------|-----------------------|-------|-------|------|-----|
|     |           | Mean                  | 0.271 | 0.800 | 14.2 | 228 |
|     |           | SD                    | 0.03  | 0.04  | 0.22 | 8.99|
|     |           | CV (%)                | 9.96  | 4.46  | 1.52 | 4.07|
|     |           | ACC (%)               | 90.0  | 93.3  | 103  | 92.0|
| 2   | Observed  | Mean                  | 0.240 | 0.885 | 14.1 | 245 |
|     |           | SD                    | 0.02  | 0.01  | 0.17 | 8.62|
|     |           | CV (%)                | 8.44  | 1.15  | 1.17 | 3.70|
|     |           | ACC (%)               | 88.1  | 100   | 100  | 97.0|
| 3   | Observed  | Mean                  | 0.317 | 0.899 | 13.8 | 230 |
|     |           | SD                    | 0.01  | 0.04  | 0.49 | 3.35|
|     |           | CV (%)                | 4.28  | 4.83  | 3.38 | 1.47|
|     |           | ACC (%)               | 105   | 103.7 | 104  | 95.3|
|     | N=15      | Mean                  | 0.276 | 0.871 | 14.4 | 227 |
|     |           | SD                    | 0.03  | 0.05  | 0.37 | 8.61|
|     |           | CV (%)                | 10.8  | 5.75  | 2.54 | 3.79|
|     |           | ACC (%)               | 94.2  | 99.1  | 102  | 94.8|

Table 7. Processed sample stability of quantitation data using signature peptide SINSATHYAESVK.

| Time   | Store at 5°C | Remicade [µg/ml] | NK [µg/ml] |
|--------|--------------|------------------|-----------|
|        |              | 0.879            | 240       |
|        |              | 0.879            | 240       |
| 24 hours| Mean          | 0.893            | 224       |
|        | ACC%          | 102              | 93.3      |
| 48 hours| Mean          | 0.888            | 229       |
|        | ACC%          | 101              | 95.3      |

4. DISCUSSION

nSMOL chemistry is Fab-selective proteolysis with maintaining antibody sequence specificity while minimizing a large excess of tryptic peptide analyte. Therefore, the analytical validation condition can be easily set in a short time. In LC-MS/MS based bioanalysis, excess analyte injection causes the severe ionization suppression effect. This issue has been essential for the development of regulated LC-MS bioanalysis for the practical clinical application. In this study, we have developed the validated IFX and its biosimilar analysis in human serum within the guideline criteria for pharmaceutical development, and this method is expected to
be applied to many therapeutic antibody monitoring for inflammatory immune diseases. In RISING study of RA, trough concentration of IFX level at week 54 showed the good references for clinical responses, and higher serum level of IFX above 1 µg/ml provided more effective responses and prognosis [44]. The nSMOL validation data indicate that our new assay can be enough analyzed at this reference levels.

The recent NOR-SWITCH trial switching from original IFX to biosimilar showed that CT-P13 was similar in safety and immunogenicity to the continued treatment for the CD, RA, ulcerative colitis, and several inflammatory immune diseases, and that switching to biosimilar is allowable [45]. However, the author also recommended that these results cannot be generalized to other biosimilar antibodies because of the possibility for too wide clinically differences of 15% margin. Same as this significant switching study, we propose that the data of comparative clinical assays or bioanalysis can be performed on the identical platform by demonstrating the cross validation between original and biosimilar antibodies. nSMOL assay coupled with LC-MS/MS can be provided the bioanalysis data for monoclonal antibodies with less errors within 15% because this technology is based on biochemical and physicochemical proof [46].

Recently, several reports showed that IFX treatment is advocated for the management of immune-related adverse events (irAEs) like enterocolitis or pneumonitis induced by the immune checkpoint inhibitors [47, 48]. In the case of irAE management using IFX, serum or plasma concentration of IFX may be related to the inflammatory observation. Moreover, IFX monitoring should be carried out in coexistence with other anti-immune checkpoint antibodies, and be strictly separated from the interference of other antibodies. The nSMOL have already been succeeded the multiplex antibody bioanalysis, and we suggest that this approach have a

Table 8. nSMOL assay verification of biosimilar NK using original Remicade standard.

| Compounds | Set Conc. | Concentration [µg/ml] |
|-----------|-----------|----------------------|
|           | 0.293     | 0.879               |
|           | 14.1      | 240                 |
| Observed  | 0.318     | 0.806               |
|           | 14.3      | 229                 |
|           | 0.291     | 0.970               |
|           | 14.7      | 229                 |
|           | 0.333     | 0.765               |
|           | 13.6      | 248                 |
|           | 0.306     | 0.855               |
|           | 14.5      | 234                 |
|           | 0.274     | 0.878               |
|           | 14.6      | 240                 |
| Mean      | 0.304     | 0.855               |
|           | 14.4      | 236                 |
| SD        | 0.02      | 0.08                |
| CV (%)    | 7.54      | 9.11                |
| ACC (%)   | 104       | 97.2                |

Table 9. nSMOL assay verification of original Remicade using biosimilar NK standard.

| Compounds | Set Conc. | Concentration [µg/ml] |
|-----------|-----------|----------------------|
|           | 0.293     | 0.879               |
|           | 14.1      | 240                 |
| Observed  | 0.295     | 0.957               |
|           | 14.2      | 217                 |
|           | 0.259     | 0.839               |
|           | 13.7      | 231                 |
|           | 0.244     | 0.883               |
|           | 13.8      | 212                 |
|           | 0.268     | 0.891               |
|           | 14.0      | 212                 |
|           | 0.295     | 0.827               |
|           | 13.8      | 230                 |
| Mean      | 0.272     | 0.879               |
|           | 13.9      | 220                 |
| SD        | 0.02      | 0.05                |
| CV (%)    | 8.27      | 5.84                |
| ACC (%)   | 92.9      | 100                 |

nSMOL have already been succeeded the multiplex antibody bioanalysis, and we suggest that this approach have a
potential to apply to this clinical trials or drug-drug interaction assay.

CONCLUSION
In conclusion, we have developed the validated LC-MS/MS based IFX monitoring in circulation using our nSMOL technologies. This approach will be expected to develop an optimal administration strategy in RA or other inflammatory immune diseases. And this approach will be adjusted for many monoclonal antibody or biosimilar analysis and rapid application for new clinical trials.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

HUMAN AND ANIMAL RIGHTS
No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS
This work was partly supported by the Research on Regulatory Harmonization and Evaluation of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics from the Japan Agency for Medical Research and Development, AMED.

SUPPLEMENTARY MATERIAL
Supplementary material is available on the publisher’s website along with the published article.

REFERENCES

[1] Elliott, M.J.; Maini, R.N.; Feldmann, M; Kalden, J.R.; Antoni, C.; Smolen, J.S.; Leb, B.; Breedveld, F.C.; Macfarlane, I.D.; Bijl, H.; et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet. 1994, 344, 1105-1110.

[2] Smolen, J.S.; Landewe, R.; Bijlsma, J.; Burmester, G.; Chatzidi-onysiou, K.; Dougados, M; Nam, J.; Ramaro, S.; Voshaar, M.; van Vollenhoven, R.; Aletaha, D.; Aringer, M.; Boers, M.; Buckley, C.D.; Buttgerest, F.; Bykerk, V.; Cardiel, M.; Combe, B.; Cutolo, M.; van Eijk-Hustings, Y.; Emery, P.; Finckh, A.; Gabay, C.; Gomez-Reino, J.; Gosse, L.; Gottenberg, J.E.; Hazes, J.M.W.; Huizinga, T.; Jani, M.; Karateev, D.; Kouloumas, M.; Kvien, T.; Li, Z.; Mariette, X.; Melnich, I.; Myser, E.; Nash, P.; Pavelka, K.; Poor, G.; Richez, C.; van Riel, P.; Rubbert-Roth, A.; Saag, K.; da Silva, J.; Stamm, T.; Takeuchi, T.; Westhovens, R.; de Wit, M.; van der Heijde, D. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. Ann. Rheum. Dis. 2017, 76, 960-977.

[3] Merola, J.F.; Lockshin, B.; Mody, E.A. Switching biologics in the treatment of psoriatic arthritis. Semin. Arthritis. Rheum. 2017, 47, 29-37.

[4] Hisamatsu, T.; Ueno, F.; Matsumoto, T.; Kobayashi, K.; Koganei, K.; Kunisaki, R.; Hira, F.; Nagahori, M.; Matushita, M.; Kobayashi, K.; Kishimoto, M.; Takeno, M.; Tanaka, M.; Inoue, N.; Hibi, T.

The 2nd edition of consensus statements for the diagnosis and management of intestinal Behcet's disease: Indication of anti-TNFalpha monoclonal antibodies. J. Gastroenterol. 2014, 49, 156-162.

Elalouf, O.; Elkayam, O. Long-term safety and efficacy of infliximab for the treatment of ankylosing spondylitis. Ther. Clin. Risk Manag. 2015, 11, 1719-1726.

Kawakami, H.; Matsumoto, Y.; Abe, N.; Katori, Y.; Takahashi, K.; Tsuboi, R.; Okubo, Y. Perioperative management of tumor necrosis factor-alpha blocker-treated psoriatic patients: Case reports and review. J. Dermatol., 2016, 43, 190-193.

Mitrev, N.; Vande Casteele, N.; Seow, C.H.; Andrews, J.M.; Connor, S.J.; Moore, G.T.; Barclay, M.; Begun, J.; Bryant, R.; Chan, W.; Corte, C.; Ghaly, S.; Lemberg, D.A.; Kariyawasam, V.; Lewindon, P.; Martin, J.; Mountfield, R.; Radford-Smith, G.; Slo-bodian, P.; Sparrow, M.; Toong, C.; van Langenberg, D.; Ward, M.G.; Leong, R.W. Organisation IBDS the Australian Inflammator-y Bowel Diseases Consensus Working Group. Review article: Consensus statements on therapeutic drug monitoring of anti-tumour necrosis factor therapy in inflammatory bowel diseases. Aliment. Pharmacol. Ther., 2017, 46, 1037-1053.

Kawasaki, T.; Naoe, S. History of Kawasaki disease. Clin. Exp. Nephrol., 2014, 18, 301-304.

Geczy, K.B.; Bemelman, W.; Kamm, M.A.; Stoker, J.; Khanna, R.; Ng, S.C.; Panes, J.; van Assche, G.; Liu, Z.; Hart, A.; Levesque, B.C.; D’Haens, G. World Gastroenterology Organization IBD-ites ESoC, Roberts Clinical T, World Gastroenterology Organization International Organisation for Inflammatory Bowel Diseases IBD-ites ESoC, Roberts Clinical T. A global consensus on the classification, diagnosis and multidisciplinary treatment of perianal fistulating Crohn's disease. Gut, 2014, 63, 1381-1392.

Beck, A.; Reichert, J.M. Approval of the first biosimilar antibodies in Europe: A major landmark for the biopharmaceutical industry. Mabs, 2013, 5, 621-623.

Lee, C.; Jeong, M.; Lee, J.J.; Seo, S.; Cho, S.C.; Zhang, W.; Jaquez, O. Glycosylation profile and biological activity of Remicade(R) compared with Flixa(R) and Remsima(R). Mabs, 2017, 9, 968-977.

Hong, J.; Lee, Y.; Lee, C.; Eo, S.; Kim, S.; Lee, N.; Park, J.; Park, S.; Seo, D.; Jeong, M.; Lee, Y.; Yeon, S.; Bou-Assaf, G.; Sosic, Z.; Zhang, W.; Jaquez, O. Physicochemical and biological characterization of SB2, a biosimilar of Remicade(R) (infliximab). Mabs, 2017, 9, 364-382.

Fang, J.; Doneeau, C.; Alley, W.R. Jr.; Yu, Y.Q.; Beck, A.; Chen, W. Advanced assessment of the physicochemical characteristics of Remicade(R) and Inflectra(R) by sensitive LC/MS techniques. Mabs, 2016, 8, 1021-1034.

Hudson, M.; Tascilar, K.; Suisa, S. Comparative effectiveness research with administrative health data in rheumatoid arthritis. Nat. Rev. Rheumatol., 2016, 12, 358-366.

Toyama, A.; Nakagawa, H.; Matsuda, K.; Sato, T.A.; Nakamura, Y.; Ueda, K. Quantitative structural characterization of local N-glycan microheterogeneity in therapeutic antibodies by energy-resolved oxonium ion monitoring. Anal. Chem., 2012, 84(22), 9655-9662.

Fiorino, G.; Ruiz-Agüello, M.B.; Maguregui, A.; Nagore, D.; Correale, C.; Radice, S.; Gilardi, D.; Allocca, M.; Furfaro, F.; Martinez, A.; Danese, S. Full interexchangeability in regard to immunogenicity between the infliximab reference biological and biosimilars CT-P13 and SB2 in inflammatory bowel disease. Inflamm. Bowel Dis., 2018, 24(3), 601-606.

E.I. Amrani, M.; van den Broek, M.P.; Gobeli, C.; van Maarseveen, E.M. Quantification of active infliximab in human serum with liquid chromatography-tandem mass spectrometry using a tumor necrosis factor alpha based-pre-analytical sample purification and a stable isotopic labeled infliximab bio-similar as internal standard: A target-based, sensitive and cost-effective method. J. Chromatogr. A., 2016, 1454, 42-48.

Tighe, D.; Hall, B.; Jeyarajah, S.K.; Smith, S.; Breslin, N.; Ryan, B.; McMnara, D. One-year clinical outcomes in an IBD cohort who have previously had anti-TNFa trough and antibody levels assessed. Inflamm. Bowel Dis., 2017, 23(7), 1154-1159.

Vaughn, B.P.; Sandborn, W.J.; Cheifetz, A.S. Biologic concentration testing in inflammatory bowel disease. Inflamm. Bowel Dis., 2015, 21, 1435-1442.

Steenholm, C.; Bendtzen, K.; Bryskov, J.; Thomsen, O.O.; Ainsworth, M.A. Clinical implications of measuring drug and anti-drug antibodies by different assays when optimizing infliximab treat-
Verification between Original and Biosimilar Therapeutic Antibody Infliximab

in the treatment of. Post hoc analysis of a randomized controlled trial. Am. J. Gastroenterol., 2014, 109, 1055-1064.

[21] Laine, J.; Jokiranta, T.S.; Eklund, K.K.; Vakevainen, M.; Paolakka, K. Cost-effectiveness of routine measuring of serum drug concentrations and anti-drug antibodies in treatment of rheumatoid arthritis patients with TNF-alpha blockers. Biologics, 2016, 10, 67-73.

[22] Favalli, E.G.; Raimondo, M.G.; Becciolini, A.; Crotti, C.; Biggio, G.; M.; Caporali, R. The management of first-line biologic therapy failures in rheumatoid arthritis: Current practice and future perspectives. Autoimmun. Rev., 2017, 16(12), 1185-1195.

[23] Baert, F.; Noman, M.; Vermeire, S.; Van Assche, G.; D’Haens, G.; Carbonet, A.; Rutgeerts, P. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn’s disease. N. Engl. J. Med., 2003, 348, 601-608.

[24] Bartelds, G.M.; Kriekelaart, C.L.; Nurmohamed, M.T.; van Schouwenburg, P.A.; Lems, W.F.; Twisk, J.W.; Dijkmans, B.A.; Aarden, L.; Wolbink, G.J. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. JAMA, 2011, 305, 1460-1468.

[25] Marin, J.C.; Sendek, J.; Cornillie, F.; Popp, J.W. Jr.; Black, S.; Blank, M.; Gil, A.; Van Stappen, T.; Hamann, D.; Rispen, T.; Therien, L.; Chun, K.; Shankar, G. Comparisons of serum infliximab and antibodies to-infliximab tests used in inflammatory bowel disease clinical trials of Remicade(R). AAPS J., 2017, 19, 161-171.

[26] Ungar, B.; Anafy, A.; Yanai, H.; Ron, Y.; Yavzori, M.; Picard, O.; Fudin, E.; Loebenstein, R.; Kopylov, U.; Chowers, Y.; Dotan, I.; Eli-akim, R.; Ben-Horin, S. Significance of low level infliximab in the absence of anti-infliximab antibodies. World J. Gastroenterol., 2015, 21, 1907-1914.

[27] Muller, D.; Meric, J.C.; Paingaud, G.; Ducourau, E.; Magdelaine-Beuzelin, C.; Valat, J.P.; Goupille, P.; Centre National de la Recherche Scientifique UG. Infliximab concentration monitoring improves the control of disease activity in rheumatoid arthritis. Arthritis Res. Ther., 2009, 11, R178.

[28] Feuerstein, J.D.; Nguyen, G.C.; Kupfer, S.S.; Falck-Ytter, Z.; Singh, S.; American Gastroenterological Association Institute Clinical Guidelines Committee. American Gastroenterological Association Institute Guideline on Therapeutic Drug Monitoring in Inflammatory Bowel Disease. Gastroenterology, 2017, 153(3), 827-834.

[29] Nagore, D.; Rapin, N.; Antón, I.; Harper, J.; Martinez, A. Response to: Comparison of infliximab drug measurement across three commercially available ELISA kits. Pathology, 2017, 49(3), 332-334.

[30] Price, S. Rheumatoid arthritis: Monitoring serum concentration of infliximab might improve RA disease control. Nat. Rev. Rheumatol., 2010, 6, 66.

[31] Hansen, C.H.; Yang, D.; Koussa, M.A.; Wong, W.P. Nanowire-linked immunosorbent assay (NLISA) for fast, sensitive, and specific protein detection. Proc. Natl. Acad. Sci. USA, 2017, 114, 10367-10372.

[32] Lee, M.W.; Connor, S.; Ng, W.; Toong, C.M. Comparison of infliximab drug measurement across three commercially available ELISA kits. Pathology, 2016, 48, 608-612.

[33] Imaeda, H.; Andoh, A.; Fujiyama, Y. Development of a new immunoassay for the accurate determination of anti-infliximab antibodies in inflammatory bowel disease. J. Gastroenterol., 2012, 47, 136-143.

[34] Iwamoto, N.; Hamada, A.; Shimada, T. Antibody drug quantitation in coexistence with anti-drug antibodies on nSMOL bioanalysis. Anal. Biochem., 2017, 540-541, 30-37.

[35] Jourdil, J.F.; Lebert, D.; Gautier-Veyret, E.; Lemaître, F.; Bonaz, B.; Picard, G.; Tonini, J.; Stanke-Labesque, F. Infliximab quantitation in human plasma by liquid chromatography-tandem mass spectrometry: Towards a standardization of the methods? Anal. Bioanal. Chem., 2017, 409, 1195-1205.

[36] Willrich, M.A.; Murray, D.L.; Barnidge, D.R.; Ladwig, P.M.; Snyder, M.R. Quantitation of infliximab using clonoty peptides and selective reaction monitoring by LC-MS/MS. Int. Immunopharmacol., 2015, 28, 513-520.

[37] Kleinnijenhuis, A.J.; Ingola, M.; Toersche, J.H.; van Holthoorn, F.L.; van Dongen, W.D. Quantitative bottom up analysis of infliximab in serum using protein A purification and integrated mulC-electrospray chip IonKey MS/MS technology. Bioanalysis, 2016, 8, 891-904.

[38] Iwamoto, N.; Shimada, T.; Umino, Y.; Aoki, C.; Aoki, Y.; Sato, T.A.; Hamada, A.; Nakagama, H. Selective detection of complemtarity-determining regions of monoclonal antibody by limiting protease access to the substrate: Nano-surface and molecular orientation limited proteolysis. Analyst, 2014, 139, 576-580.

[39] Iwamoto, N.; Umino, Y.; Aoki, C.; Yamane, N.; Hamada, A.; Shimada, T. Fully validated LCMS bioanalysis of Bevacizumab in human plasma using nano-surface and molecular-orientation limited (nSMOL) proteolysis. Drug Metab. Pharmacokinet., 2016, 31, 46-50.

[40] Iwamoto, N.; Shimada, T.; Terakado, H.; Hamada, A. Validated LC-MS/MS analysis of immune checkpoint inhibitor Nivolumab in human plasma using a Fab peptide-selective quantitation method: nano-surface and molecular-orientation limited (nSMOL) proteolysis. J. Chromatogr. B. Analit. Technol. Biomed. Life Sci., 2016, 1023-1024, 9-16.

[41] Iwamoto, N.; Shimomura, A.; Tamura, K.; Hamada, A.; Shimada, T. LC-MS bioanalysis of Trastuzumab and released emtansine using nano-surface and molecular-orientation limited (nSMOL) proteolysis and liquid-liquid partition in plasma of Trastuzumab emtansine-treated breast cancer patients. J. Pharm. Biomed. Anal., 2017, 145, 33-39.

[42] Iwamoto, N.; Takashiki, M.; Hamada, A.; Shimada, T. Multiple LCMS bioanalysis of brentuximab vedotin, rituximab and cetuximab towards therapeutic drug monitoring application by combined calibration curve using fab-selective limited proteolysis nSMOL. Clio. Pharmaco. Biopharm., 2016, 5(4).

[43] Prakash, A.; Tomazela, D.M.; Frenwen, B.; Maclean, B.; Merrihew, G.; Peterman, S.; Maccoss, M.J. Expediting the development of targeted SRM assays: Using data from shotgun proteomics to automate method development. J. Proteome Res., 2009, 8, 2733-2739.

[44] Takeuchi, T.; Miyasaka, N.; Inoue, K.; Abe, T.; Koike, T.; RISING study. Impact of trough serum level on radiographic and clinical response to infliximab plus methotrexate in patients with rheumatoid arthritis: Results from the RISING study. Mod. Rheumatol., 2009, 19, 478-487.

[45] Jorgensen, K.K.; Olsen, I.C.; Goll, G.L.; Lorenzen, M.; Bolstad, N.M.; Haavardsholm, E.A.; Lundin, K.E.A.; Mork, C.; Jahnson, J.; Kvien, T.K.; NOR-SWITCH study group. Switching from originator infliximab to biosimilar CT-P13 compared with maintained treatment with originator infliximab (NOR-SWITCH): A 52-week, randomised, double-blind, non-inferiority trial. Lancet, 2017, 389, 2304-2316.

[46] Jenkins, R.; Duggan, J.X.; Aubry, A.F.; Zeng, J.; Lee, J.W.; Cojocaru, L.; Dufeld, D.; Garofolo, F.; Kaur, S.; Schultz, G.A.; Xu, K.; Yang, Z.; Yu, J.; Zhang, Y.J.; Vazvaei, F. Recommendations for validation of LC-MS/MS bioanalytical methods for protein biotherapeutics. AAPS J., 2015, 17, 1-16.

[47] Minor, D.R.; Chiu, K.; Kashani-Sabet, M. Infliximab in the treatment of anti-CTLA4 antibody (ipilimumab) induced immune-related colitis. Cancer Biother. Radiopharm., 2009, 24, 321-325.

[48] Spain, I.; Diem, S.; Larkin, J. Management of toxicities of immune checkpoint inhibitors. Cancer Treat. Rev., 2016, 44, 51-60.