Zolbetuximab – Methodology for Physicochemical Compatibility and Microbial Challenge Studies

- In-use stability studies were performed from the perspective of not only the physicochemical compatibility with the diluent and the materials of medical apparatus, but also the microbial growth to accommodate the proposed preparation and administration conditions.[1]
- Available information on the methodologies used for the physicochemical compatibility and microbial challenge studies is provided below.[1]

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Physicochemical compatibility study[1]

- The physicochemical compatibility studies were performed for reconstituted zolbetuximab DP and infusion solution.
- DP vials were reconstituted with sterile water for injection to achieve a 20 mg/mL DP solution.
- Reconstituted DP was further diluted with sterile 0.9% w/v sodium chloride injection (referred to as infusion solution or diluted DP) to achieve targeted zolbetuximab concentration (2 mg/mL).
- To cover a wider range of concentrations, infusion solutions for evaluation were prepared at 1.5 mg/mL and 5 mg/mL.
- Reconstituted DP was held in glass vials and infusion solutions were held in bag/container with infusion tubing, and in-line filter at 2 - 8°C or 30°C for compatibility assessments.
- Infusion solutions prepared using CSTDs were also evaluated.
- The materials evaluated in compatibility studies and the study conditions in each study are summarized in Table 1 and Table 2, respectively.

Table 1. Materials evaluated in physicochemical compatibility studies[1]

Evaluated item	Materials of main parts contacting with infusion solution
IV bag/container	• PE • PP • PVC [plasticizer: DEHP, TOTM] • Ethylene propylene copolymer • EVA copolymer • PP and SEBS copolymer • Glass bottle for administration use
Infusion tubing	• PE • PVC [plasticizer: DEHP, DEHT, TOTM] • PB • Elastomer modified PP
In-line filter membrane	• PES • PS
Closed system transfer device	• PP • PE • Stainless steel • Silicone (rubber/oil/resin) • PI • PVC • PVC [plasticizer: TOTM] • ABS copolymer • MABS copolymer • Thermoplastic elastomer • PTFE • Polycarbonate • PES • Acrylic copolymer

	• PBT • PB • EVA copolymer
Central port	• Silicone rubber • Titanium alloy • PVC [plasticizer: TOTM]

Table 2. Study conditions in physicochemical compatibility studies[1]

Evaluation summary	Zolbetuximab concentration	Temperature	Evaluated storage period
Stability of reconstituted drug product in 20 mL glass vial	20 mg/mL	30°C	0, 6 hours
Compatibility with PP and PE IV bag	1.5, 5 mg/mL	20°C	0, 24, 48 hours[a]
Compatibility with infusion tubing			0, 4 hours[b]
Compatibility with PE IV bag		30°C	0, 16, 40 hours[c]
Compatibility with EVA IV bag, PB infusion tubing and PS in-line filter membrane		2 - 8°C, 30°C	0, 16, 40 hours (2 - 8°C)[c] 0, 16 hours (30°C)[c]
Compatibility with glass container			
Compatibility with ethylene propylene copolymer IV bag, PE infusion tubing and PES in-line filter membrane			
Compatibility with PVC (TOTM) IV bag			
Compatibility with PVC (DEHP) IV bag			
Compatibility with PP and SEBS copolymer IV bag			
Compatibility with CSTD A[d]			
Compatibility with CSTD B[e]			
Compatibility with CSTD C[f]			
Compatibility with CSTD D[g]			
Compatibility with central port[h]			

[a] Stored in a vial for 3 hours before dilution, stored in an IV bag for 0, 24, or 48 hours after dilution.
[b] Stored in a vial for 3 hours before dilution, stored in an IV bag for 24 hours after dilution, expelled from an IV bag through infusion tubing with in-line filter membrane at 25 mL/h for 2 hours and at 250 mL/h for 2 hours.
[c] Stored in a vial for 3 hours before dilution, stored in an IV bag/container for 0, 16, or 40 hours after dilution, expelled from an IV bag/container through infusion tubing with in-line filter membrane.
[d] Materials of fluid contact parts: PP, SS, silicone rubber/oil, PI, PVC, ABS copolymer
[e] Materials of fluid contact parts: PP, SS, PB, silicone rubber/oil, PI, PVC(TOTM), PES
[f] Materials of fluid contact parts: PE, ABS copolymer, acrylic copolymer, PB, PP, TPE, PC, MABS copolymer, SS, PVC(TOTM)
[g] Materials of fluid contact parts: silicone resin/oil, acrylic copolymer, PBT, PC, ABS copolymer, PTFE, PVC, EVA copolymer
[h] Materials of fluid contact parts: silicone rubber, titanium alloy, PVC(TOTM)

- The quality attributes assessed were pH, Osmolality (mOsmol/kg), protein concentration (mg/mL), visible particles, sub-vis particles/mL (≥ 10 µm, ≥ 25 µm), charge variants (cIEF [comparable to reference, isoelectric point range of detected isoforms, number of detected isoforms]), reduced

SDS-PAGE (%), size variants (SE-HPLC [Monomer (%), aggregates (%), fragments (%)]), FBI (bioassay) (%), CDC (bioassay) (%, ADCC (bioassay) (%).

- For the evaluation of compatibility with PVC containing DEHP as plasticizer, the level of DEHP leaching from PVC administration bag was also assessed.
- The risk assessment was conducted based on the leachable study data generated by considering the actual preparation and administration condition of zolbetuximab infusion solution. The study design is shown in Table 3.

Table 3. Study design of leachable study of PVC Bag with DEHP[1]

Diluent	Sterile 0.9% w/v sodium chloride injection
Infusion solution concentration	2 mg/mL
Infusion solution volume	1000 mL
Storage condition	2 - 8°C for 24 hours and 40 hours (light protected) 40°C for 9 hours and 16 hours (light protected)

- The infusion solutions were prepared in PVC bag with DEHP and stored under specified conditions. The infusion solutions were sampled at each timepoint and subjected to analysis for DEHP quantification according to the test method developed by West Pharmaceutical Services.

Microbial challenge study[1]

- Microbial challenge study was performed for reconstituted DP and infusion solutions of DP to evaluate the microbial growth potential during the preparation and administration.
- The microbial challenge study was executed for reconstituted DP at the concentration of 20 mg/mL and for infusion solution at the concentration of 1.5 mg/mL and 5 mg/mL diluted by sodium chloride 0.9% solution for injection at a temperature range of 2 - 8°C and 30 ± 2°C.
- The study was performed by challenging the product with low levels of microorganisms (≤ 100 CFU/mL of five standard USP organisms as well as typical skin microflora) simulating the level of possible contamination during preparation in a hospital pharmacy.
- The capability of the product to resist microbial proliferation in these conditions was confirmed by enumerating viable organisms over the duration of the study.
- At designated time intervals up to 72 hours, the samples were plated and the number of viable colony forming units was determined.
- Prior to performing the microbial challenge study, a recovery method validation was performed referring to USP to demonstrate that the test article itself does not inhibit the multiplication of microorganisms that may be present.
- 'No increase' in counts was defined as not more than 0.5 $\log_{10}$ from the inoculum level.
- The first study was performed for all microorganisms (*S. aureus, P. aeruginosa, E. coli, S. epidermidis, C. albicans, and A. brasiliensis*) at designated time intervals up to 72 hours.
- Additional testing was performed to include intermediate additional time point for *P. aeruginosa* and *E. coli* based on the result of previous study in which no increase in microbial growth was observed through the 8-hour time point, but increased by more than 0.5 $\log_{10}$ from the inoculum level at the 24-hour time point for *P. aeruginosa* and *E. coli* at 30 ± 2°C.

Abbreviations

ADCC: antibody dependent cellular cytotoxicity; **°C**: degrees Celsius; **CDC**: complement dependent cytotoxicity; **CFU**: colony forming units; **cIEF**: capillary isoelectric focusing; **CSTD**: closed system transfer device; **DEHT**: di(2-ethylhexyl) terephthalate; **DEHP**: di-(2-ethylhexyl) phthalate; **DP**: drug product **EVA**: ethylene-vinyl acetate; **FBI**: flowcytometric binding immunoassay; **IV**: intravenous injection; **MABS**: methyl methacrylate-ABS; **mg**: milligram; **mL**: milliliter; **mOsmol**: milliosmole; **MQL**: minimum quantification limit; **PB**: polybutadiene; **PBT**: polybutylene terephthalate; **PC**:

polycarbonate; **PE**: polyethylene; **PES**: polyethersulfone; **pH**: potential hydrogen; **PI**: polyisoprene; **PP:** polypropylene; **PS**: polysulfone; **PTFE**: polytetrafluoroethylene; **PVC**: polyvinyl chloride; **SDS-PAGE:** sodium dodecyl sulfate poly-acrylamide gel electrophoresis; **SEBS**: styrene-ethylene-butylene-styrene; **SE-HPLC:** size exclusion high performance liquid chromatography; **SS**: stainless steel; **TEHTM**: tris(2-ethylhexyl) trimellitate; **TOTM**: trioctyl trimellitate; **TPE**: thermoplastic elastomer; **w/v**: weight/volume; **µm**: micrometer.

References:

1. Data on File.