Pembrolizumab (Keytruda®) is a human IgG4 monoclonal antibody (mAb) from the group of immunomodulators, which binds to programmed death receptor 1 (PD-1). Given its structural complexity, physical aggregation and chemical degradation can occur throughout its life, and even modest environmental stresses can cause extensive damage which may affect the safety and efficacy of the medicine [1].

Aim and objectives

To assess the impact of agitation on pembrolizumab (Keytruda®, 25 mg/mL) safety and efficacy through the study of aggregation and functionality when mishandling in real hospital conditions.

Materials and methods

1. Agitation stress:
   - 24 h, 25 °C
   - 300 rounds/min

2. Gentle agitation:
   - 1 min, 25 °C
   - Manual agitation

Pembrolizumab control sample (25 mg/mL) showed a single particulate population with hydrodynamic diameter (HD) of 9.5±2.8 nm corresponding to pembrolizumab monomers. SE/UHPLC-UV chromatograms of the control sample revealed a main chromatographic peak assigned to pembrolizumab monomers and a small one assigned to native dimers. DLS and SE/UHPLC-UV showed that agitation stress did not promote increase in aggregation. However, pembrolizumab functionality was affected after applying agitation stress since ELISA revealed a significant loss of functionality. As a consequence, a gentle agitation of pembrolizumab was performed in order to investigate if this loss of functionality could also happen in less stressful conditions. As a result, ELISA also revealed a significant loss of functionality in gently agitated pembrolizumab.

Results

**Particle size distribution by volume graphs**

**Aggregate profile was determined by Size-Exclusion High-Performance Liquid Chromatography (SE/UHPLC-UV)**

**Functionality was evaluated by Enzyme-Linked Immunosorbent Assay (ELISA)**

**Conclusion and relevance**

The exposure to agitation stress did not induce aggregate formation in pembrolizumab. Nevertheless, both agitation stress and gentle agitation led to a loss of its functionality not related to aggregation. Thus, we recommend preventing pembrolizumab from agitation when handling in hospitals.