Nivolumab (Opdivo®) is a human immunoglobulin G4 (IgG4) monoclonal antibody (mAb) that binds to programmed death receptor 1 (PD-1) and blocks its interaction with PD-L1 and PD-L2. As a complex protein, routine handling or unintentional mishandling of its solutions may cause degradation that could remain unnoticed but could potentially compromise the clinical safety and efficacy of the drug product [1].

To assess the impact on nivolumab (Opdivo®) aggregation process promoted by slight modification in the concentration of the compound (NaCl 0.9% or glucose 5%) used to prepare the clinical diluted solution of nivolumab at 1.0 mg/mL. Also, to assess the impact on the aggregation on nivolumab clinical diluted solutions (1.0 mg/mL, in NaCl 0.9% and glucose 5%) promoted by agitation stress.

Materials and methods

Impact on the aggregation on nivolumab promoted by the variation in the diluent concentration

<table>
<thead>
<tr>
<th>NaCl (%)</th>
<th>Glucose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>0.7</td>
<td>2</td>
</tr>
<tr>
<td>0.8</td>
<td>3</td>
</tr>
<tr>
<td>0.9</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>1.3</td>
<td>9</td>
</tr>
<tr>
<td>1.5</td>
<td>10</td>
</tr>
</tbody>
</table>

Impact on the aggregation on nivolumab promoted by agitation stress

DLS parameters
- Cell description: Plastic cuvette
- Temperature: 20 °C
- Material: Protein
- Dispersant: Water
- Dispersion optics: 90°
- Number of records: 100
- Duration of every record: 10 sec

Results

DLS size distribution by volume graphs: variation of the diluent concentration

NaCl (0.5-1.5%)

Glucose (1-10%)

Reference samples of diluted nivolumab at 1.0 mg/mL in NaCl 0.9% and 5% glucose showed a single particulate population with hydrodynamic diameter (HD) of 9.66 ± 2.96 nm and 10.67 ± 3.68 nm respectively, attributed to nivolumab monomers. No significant changes were observed in the HD when concentration of the diluents was changed. Also, no significant changes were observed after the stress by agitation, with the HR values within the interval corresponding to the size of the monomers of the nivolumab standard solution (reference samples).

DLS size distribution by volume graphs: manual and vortex agitation on 0.9% NaCl samples

DLS size distribution by volume graphs: manual and vortex agitation on 5% glucose samples

Conclusion and relevance

Variation on NaCl and glucose concentration around that indicated for clinical use, i.e. 0.9% and 5% respectively, does not promote aggregation or increase on the particulate size on 1 mg/mL nivolumab solution that could be detected by DLS (0.1 nm - 10 µm). Also, gentle hand shaking and vortex shaking have no impact on aggregation on these clinical nivolumab solutions by increasing the particulate size, similarly measured by DLS.