Evaluation of aggregation propensity and thermal stability in early formulation screening applied to a market-authorized mAb

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Formulation development intends to improve the integrity of a therapeutic protein, which is essential for required efficacy, shelf life and safety. During storage, chemical modifications and structural changes may impair the API’s potency and pharmacodynamics. Introducing thermal stress is a common way to simulate and accelerate storage effects. Hence, the thermal stability is a suitable indicator for a protein’s long-term stability. Moreover, the formation of aggregates activating undesirable immune responses poses a serious threat to patient safety. Therefore, aggregation propensity and thermal stability are crucial critical quality attributes which need to be addressed in early formulation development. Even the formulations of many approved APIs on the market have room for improvement. Due to expiring patent protection, this clears the way for time- and cost-effective development of “biobetters” by improving the formulation without changing the API itself.[1] Here, we present a strategy for fast initial liquid formulation screening using the market-authorized therapeutic antibody Nivolumab (Opdivo®), which is intravenously applied at 10 g/L. Starting out from own stability data of Nivolumab in its marketed formulation, we pursued a rational based approach including the following variations in its formulation: (i) use of three pH buffer components (sodium citrate, sodium phosphate, and histidine); (ii) use of three salt concentrations; (iii) use of three pH values based on Nivolumab’s pI value; (iv) use of three sugars (mannitol, sucrose, and trehalose) acting as structural stabilizers; and (v) inclusion or exclusion of penthetic acid as chelating agent. Polysorbate as surfactant was obligatorily included in each tested formulation. Nivolumab was rebuffered into the different formulations and adjusted to 10 g/L. The critical quality attribute aggregation propensity was assessed as follows: first, samples were visually inspected for turbidity. All formulations without visible precipitation were subjected to elevated temperature stress. Afterwards, the formulations which still showed visually clear appearances were analyzed by DLS and SEC to identify subvisible aggregates. The critical quality attribute thermal stability was assessed by DSC analysis of the differently formulated Nivolumab samples to elucidate their onset of denaturation and melting temperature. The best formulations with the least propensity for aggregation and the highest thermal stability were chosen for formulation improvement in future experiments. The poster provides insight into the correlation between Nivolumab’s stability and the formulation composition. The presented work will be followed by in detail protein characterization utilizing a broad analysis panel (including LC-MS, FTIR, CE, CD or UV/Vis spectroscopy) to elucidate the formulation’s impact on structure, charge variants and amino acid modifications. This iterative approach leads the way to a formulation ensuring long shelf life, high efficacy and highest patient safety.