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In vitro Analytical Characterization based Quality and Potency Assessment of mRNA Vaccines

Gautam Sanyal

Robust in vitro potency assays have successfully replaced animal-based (in vivo) tests for lot release of a few recombinant protein-based vaccines after correlation between in vitro and in vivo assays was established. mRNA vaccines differ from protein subunit or virus like particle vaccines in requiring a cell transfection step for translation of mRNA to express the encoded protein antigen. To evaluate in vitro-in vivo correlation, test samples of varying target potencies ranging from 100% to 0% can be created by inducing gradual structural degradation under stress conditions such as thermal stress. These samples can be tested in parallel by an in vitro cell transfection assay and in vivo antibody induction and immune response in vaccinated mice. This technique has been used for recombinant protein-based vaccines, which have the advantage of a single step in vitro assay that does not require cell-based protein expression. Nevertheless, such a systematic evaluation is entirely possible, as it has been demonstrated that changes in structural integrity of mRNA constructs encapsulated in lipid nanoparticles, are correlated with expression of functionally intact proteins in cells. Furthermore, robust analytical characterization assays may be developed with the ability to correlate primary and higher order structures of mRNA constructs with immunologically relevant functionality of the expressed protein. These analytical assays may, in future, eliminate the need for a "potency assay" for well-characterized mRNA vaccines.