PROCESS OPTIMIZATION, MANUFACTURING CHANGES FROM EARLY TO LATE PHASE DEVELOPMENT, AND COMPARABILITY OF RESOLARIS

Ying Buechler, aTyr Pharma
ybuechler@atyrpharma.com
Darin Lee, aTyr Pharma
Mysore Ramprasad, aTyr Pharma
Chris Chen, aTyr Pharma
Johnny Li, aTyr Pharma
Kristi Haskins, aTyr Pharma
Andrea Cubitt, aTyr Pharma
Ashraf Amanullah, aTyr Pharma

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Resolaris is an *E.coli* expressed recombinant protein derived from human histidyl-tRNA synthetase, and is currently in clinical development for the treatment of severe, rare myopathies with an immune component. The manufacturing process initially developed at laboratory scale resulted in a high degree of product purity. After process transfer to CMO#1 and scale-up to 225L, the purified material unexpectedly had significantly high levels of product related impurities. Two major impurity species were isolated and identified by peptide mapping via LC-MS/MS as norleucine for methionine misincorporation and a clipped form. The norleucine misincorporation was eliminated by amino acid supplementation during fermentation, but supplementation also increased levels of process impurities, later identified as RNA. The clipped form and increased RNA levels were minimized and controlled through modification in both fermentation and downstream procedures. Multiple GMP batches were manufactured (Process1.0) and product used in Phase 1/2a clinical studies. In order to support late phase clinical development and commercialization, the expression vector and cell line were changed to remove the protease and increase productivity. In addition to the improvement in the manufacturing process, the manufacturing site was changed (CMO#2), and further, the drug substance and drug product formulation was also changed to enhance stability. Multiple batches of Resolaris have been manufactured at 2000L scale at CMO#2 (Process 2.0), with product output per batch increased approximately 30-fold *vs.* Process 1.0. In accordance with ICH Q5E, side-by-side analytical comparability and forced degradation studies on representative batches from both processes demonstrated that products from both processes are comparable and acceptable for late phase clinical development.