

CONFERENCE

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TITLE

Development and Testing of a Cell-based Assay that Detects Reslizumab Neutralizing Anti-drug Antibodies

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ABSTRACT

Purpose: To develop and test a cell-based assay that detects anti-drug antibodies (ADA) that biologically neutralize Reslizumab. The assay is intended to be used for clinical samples that have been shown to be ADA positive in a validated ELISA screening.

Methods: Reslizumab is a humanized anti-human interleukin-5 (IL-5) monoclonal antibody in clinical development for the treatment of eosinophilic esophagitis and asthma. An IL-5 dependent TF-1 cell line was used to detect inhibition by Reslizumab and the neutralization of Reslizumab by ADA. A cell suspension of 100 μ L was seeded onto 96-well assay plates at an approximate density of 1.4×10^5 cells/mL. A solution consisting of IL-5, Reslizumab and ADA were incubated at room temperature for 30 minutes to allow binding. Aliquots of the solution (100 μ L) were added to the plate with cells and incubated at 37°C with 5% CO₂ for 3 days. Cell viability was then examined by Promega CellTiter-Glo luminescent kit for measurement of ATP. In the presence of IL-5, multiple concentrations of Reslizumab and ADA positive controls (rabbit anti-Reslizumab polyclonal antibody) were tested for drug tolerance, assay selectivity, and evaluation of inter-batch precision.

Results: The assay results showed that Reslizumab was neutralized by the ADA positive control within the working range of this cell-based assay at 0.75 – 15 μ g/mL. The inter-assay coefficient of variation (%CV) was less than 20%. The assay sensitivity is approximately 0.75 μ g/mL of positive control in blank human serum. The assay can tolerate at least an additional 1.8 μ g/mL of reslizumab in human serum without an adjustment to the working range. Higher drug concentrations can be tolerated with titration and range adjustment. Ten individual lots of human serum were spiked with 1 μ g/mL of antibody positive controls and all showed positive results.

Conclusions: A cell-based assay has been developed and tested that can detect neutralizing anti-Reslizumab antibodies in human serum samples collected in ongoing clinical studies.