Antibody Dependent Cell-Mediated Cytotoxicity (ADCC)-Based Reporter Gene Neutralizing Antibodies (NAb) Assay in Support of Benralizumab (MEDI-563) Clinical Development
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Purpose
Immunogenicity is one of the safety measures that require adequate monitoring during clinical studies. Characterization of anti-drug antibodies (ADAs) for neutralizing activity is commonly part of immunogenicity testing package for biopharmaceuticals. To support benralizumab (MEDI-563, afucosylated anti-IL5R mAb) clinical studies for the treatment of asthma, an ADCC reporter gene-based neutralizing antibody (NAb) assay was developed to detect NAb against benralizumab in human serum.

Methods
The assay for detection of NAb against benralizumab is an ADCC-based reporter gene bioassay. Target cells are CTLL-2/IL5 receptor alpha (IL5Rα) cells, a murine cytotoxic cell line stably transfected with IL5Rα. NK92/ Nuclear Factor of Activated T (NFAT) cells (the effector cells of ADCC) are human NK cells that have been dually transfected with both CD16 and luciferase (the reporter gene that is under the control of a NFAT promoter). The relative luminescence signal (RLU) is proportional to the ADCC activity, and inversely proportional to the levels of NAb present in serum samples. The assay was evaluated through the following activities: examining serum matrix effect; establishing the screening assay cut point factor (CPF); assessing assay sensitivity, selectivity (matrix interference) and specificity; and determining the drug tolerance.

Results
The serum tolerance of the cells was determined to be 2.5% (equivalent to 40-fold sample dilution). The cut point factor derived from asthma serum samples was 0.82. The assay sensitivity was 1.6 µg/mL in neat serum. The assay was not susceptible to non-specific matrix effects. Neither benralizumab Fab nor the immunocomplex comprised of benralizumab with polyclonal/monoclonal anti-idiotypic antibodies to benralizumab induced ADCC response. The assay can detect 1.6 µg/mL NAb in the presence of 0.8 µg/mL benralizumab and can detect 5 µg/mL NAb in the presence of 1.56 µg/mL benralizumab in pooled serum.

Conclusion
The ADCC-based NAb assay was successfully developed. The assay performance specifications warrant that the assay is suitable for validation and clinical implementation.