# Mechanism of Action Assays to Determine the Fc Effector Function of Palivizumab

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## 1 Introduction

### Palivizumab

- Humanized monoclonal IgG1 antibody directed against an epitope in the A antigenic site of the F protein of respiratory syncytial virus (RSV)
- Used for prevention of RSV infection in children

### Assays for Palivizumab

- Even though the Fc effector function potential was assessed to be low for Palivizumab there is a requirement to evaluate these capabilities, especially with regards to glyco-optimized follow-on biologics with potentially altered Fc effector function potential
- The presented model for mechanism of action (MoA) testing of Palivizumab is based on a human lung carcinoma cell line with epithelial-like morphology
- The cells are infected with RSV A Long, a prototype strain for subtype A
- The infected cells are then used as target cells in reporter surrogate approaches to assess the antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) capabilities of Palivizumab

## 2 Palivizumab mediates ADCC via FcγRIIIa and ADCP via FcγRIIa and FcγRI

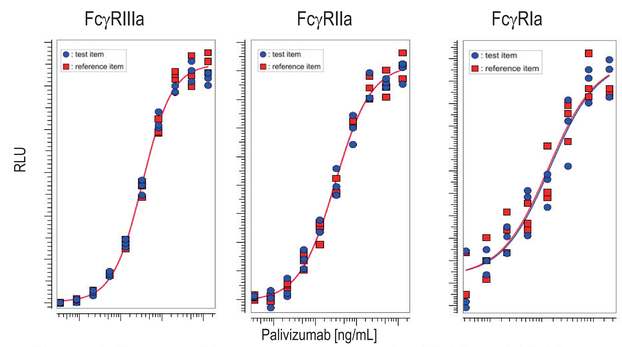


**Figure 1**: Reporter-Bioassays to assess the ADCC and ADCP-responses of Palivizumab

## 3 ADCC and ADCP Reporter Bioassays for Palivizumab are linear for nominal concentrations ranging from 50 to 150%

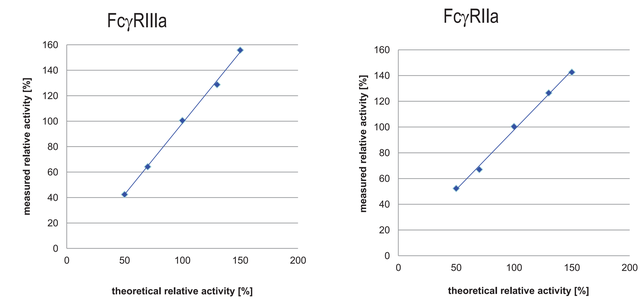


**Figure 2**: Assessment of Linearity covering a range of nominal concentrations from 50 to 150%

## 4 ADCC and ADCP Reporter Bioassays for Palivizumab are specific

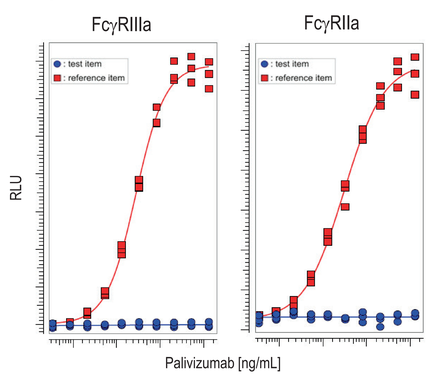


**Figure 3**: Specificity was shown using a non-relevant therapeutic mAb as a test item compared to Palivizumab

## 5 ADCC Reporter Bioassay (FcγRIIIa) for Palivizumab is stability-indicating

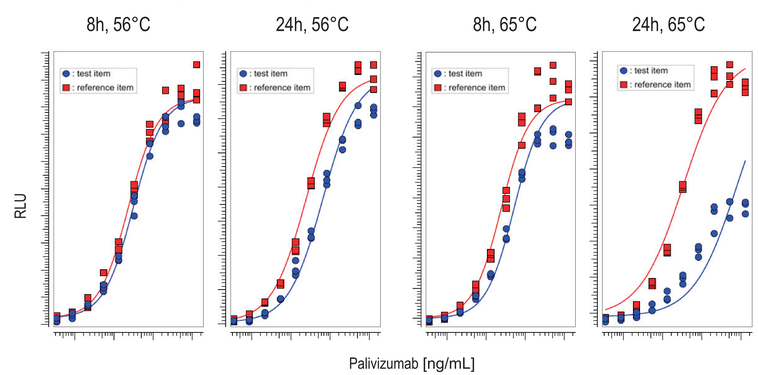


**Figure 4**: ADCC response of Palivizumab at non-denaturing and denaturing heat stress conditions for 8 hours and 24 hours

## 6 Summary

- Palivizumab is capable to induce a significant response for ADCC via FcγRIIIa and ADCP via FcγRIIa, but is a weak inducer of the FcγRI signalling pathway in a Reporter surrogate approach (Figure 1)
- The ADCC Reporter (FcγRIIIa) and the ADCP Reporter (FcγRIIa) are linear for nominal concentrations ranging from 50 to 150% and specific if a non-relevant therapeutic antibody is compared to Palivizumab at the same concentrations (Figures 2 and 3)
- Stability-indicating properties of the reporter assays are shown by using heat stressed samples for ADCC via FcγRIIIa (Figure 4) and ADCP via FcγRIIa (data not shown)
- The reporter assays are reliable, reproducible and precise, which is reflected by the rising use and acceptance of surrogate approaches instead of the tedious, time consuming and sometimes highly variable primary MoA assays

## 7 Acknowledgment

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