1. Introduction

Drug developers and regulatory authorities recognize antibody-dependent cell-mediated cytoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) as important mechanisms of action (MOA) of therapeutic antibodies. Traditional ADCC and ADCP bioassays use primary cells, which are labor intensive and highly variable. Less variable, easy-to-use and consistent analysis of these important MOA is needed in drug development programs.

To meet this need, we have developed a suite of functional cell-based FC Effector reporter bioassays for the following receptors:

- Human FCyRlla (V158 and F158 variants)
- Human FCyRllb (H313 and R313 variants)
- Mouse FCyRllb & FCyRllb

Each bioassay is provided in “thaw-and-use” format for a rapid and convenient workflow and further reduction in assay variability. In qualification studies according to ICH guidelines, the bioassays show specificity, accuracy, precision, and linearity enabling their use in antibody screening, characterization, stability and potency studies. *Mouse Fc Effector Bioassay data are not shown here (please inquire).

2. FCyR Reporter Bioassay Concept, Format and Workflow

**Surrogate Measure of In Vivo Biology**

- FCy-mediated ADCC
- Fc EffectorReporter Bioassay

**Bioassay Components:**

- Effector Cells: Jurkat T cells genetically engineered with the appropriate FCy and anti-CD20 response element that drives luciferase expression (NFAF-HElux)
- Target Cells: Customer-defined
- Anti-CD20: Customer-defined

**Bioassay Kit Formats:**

- Core Kit: Effector Cells, Medium, Low IgG Serum, Bio-Git (Luciferase Reagent)
- Complete Kit: Core Kit components listed above, Target Cells (CD20) Control Ab, Anti-CD20

**Rapid & Convenient Workflow**

1. Add mAb
2. Response Induction (24 h)
3. Thaw and add FCy Effector-Expressing Cells
4. Add Bio-Git (Luciferase Assay Reagent)
5. Measure luminescence

3. FCyR Reporter Bioassays are Specific

**Rituximab:** chimeric anti-CD20 mAb (expressed on WL2.S cells)

**Trastuzumab:** anti-Her2/neu mAb (not expressed on WL2.S cells)

ASA assay signal is dependent on:

- Target cells expressing Ab recognized antigen
- Effector cells expressing FCy
- Antibody-specific anti-CD20 antibody

**Variations of the ADCC Reporter Bioassays (FcYRlla-V158) were used to measure the FCy effector function of either Rituximab or Trastuzumab to demonstrate assay variability using all of the FCyR Reporter Bioassays referenced in panel 1 (data not shown).**

4. FCyRlla-V158 and -F158 Reporter Bioassays Measure Expected Potency

**ADCC cytotoxicity bioassay (PBMCs from 15 viable blood donors)**

**ADCC Reporter Bioassay, V Variant**

**Antibody (trastuzumab) potency was measured using a primary cell-based ADCC assay (left panel) and the ADCC Reporter Bioassay (FcYRlla-V158, right panel).**

The cumulative ADCC Reporter Bioassay shows similar potency but much less variability (as indicated by the smaller error bars). *Mause Fc Effector Bioassay data are not shown here (please inquire)."