Development of a Robust Reporter-based T cell Activation Assay for Therapeutic Biologics in Immunotherapy

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1. Assay Principle

- Jurkat T-cells stably expressing luciferase reporter driven by IL2 promoter or NFAT-RE, are used as effector cells.
- Tumor cell lines endogenously expressing cancer antigen are used as antigen presenting cells (APC).
- By co-cultivating the two cell lines in the presence of CD3 bispecific antibody, TCR/CD3 is activated in Jurkat effector cells.
- Luciferase activity is up regulated through IL-2 promoter or NFAT-RE activation.

2. Genetically Engineered Jurkat T Cell Reporter Cell lines as Effector Cells

3. Assay Format and Features

   - Plate antigen-expressing target cells
   - Add test antibody
   - Add Jurkat reporter cells
   - Induction for 5 hours
   - Add Bio-Glo™ detection reagent
   - Read plates

   **Assay Features:**
   - No need for primary effector cells
   - Simple, homogenous
   - Specific and robust assay signal
   - Short assay time, finish in one day

4. Robust Reporter Response upon Stimulation with CD3 Antibody

5. Robust Reporter Response to CD3 x EpCAM Ab Catumaxomab using EpCAM™ Target Cells

6. Abatacept Inhibition of T cell Co-stimulation can be Detected using IL-2 Reporter, But Not with NFAT-RE Reporter Expressed Effector Cells

7. Suitability for antibody stability study

8. Assay Specificity, Ability to Determine Relative Potency and Assay Linearity

9. Summary

- We developed a cell-based T cell activation bioassay using engineered Jurkat reporter cells as effector cells.
- Both Jurkat /IL-2 reporter cells and Jurkat /NFAT-RE reporter cells showed robust reporter response to crosslinked CD3 antibody or CD3xEpCAM antibody catumaxomab coated EpCAM™ target cells.
- Jurkat /IL-2 reporter cells, but not Jurkat /NFAT-RE reporter cells showed dose-dependent inhibition of CD28 mediated reporter response by CTLA4-Fc fusion abatacept.
- Both Jurkat reporter cells are able to detect the loss of biological activity for heat-treated catumaxomab, demonstrating assay suitability in stability study.
- The assay is specific, can be used for relative potency determination and shows good assay linearity.