Development of a new cell adhesion bioassay format

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Introduction

Biologic drug with MoA = inhibition of cell-cell adhesion; specific target on specific cell type.

Classic cell adhesion assays require separation of non-adherent cells by manual plate washing / inversion / tapping.

This causes assay variability as operator dependent and difficult to define / control, especially when adhesion is weak and / or involves cell rolling.
V-bottom plates centrifuged to separate adherent from non-adherent cells.

Centrifugation can be standardized and is not operator dependent.

Objective: convert HTS assay (designed to qualitatively identify “hits”) into a quantitative relative potency bioassay for QC testing.
Evaluation of three different formats

1) Drug Target Coated to the plate
   Ligand of drug target Endogenously expressed by cells

2) Recombinantly expressed by cells
   Coated to the plate

3) Recombinantly expressed by cells “coated” to the plate
   Endogenously expressed by cells
Evaluation of three different formats

In all three setups a dose-response was obtained, but:

1) Specific cell line expressing ligand needed since ligand requires a specific post-translational modification; variable expression of modified ligand led to low robustness.

2) Better robustness than 1) due to coating of recombinant ligand with controllable quality and transfected cells stably expressing target.

3) Not developed further due to complexity (2 cell lines) and since format 2) was found suitable.
Coating, blocking, sample & cell prep.

1. Ligand of drug target coated on V-bottom plate, subsequently blocked with BSA.

2. Samples diluted in assay medium, added to the plate and temperature equilibrated. Samples (S1, S2), reference, positive-, negative-, blank-control added.

3. Cells stably transfected with drug target are fluorescently labeled using calcein and added to plate.
Adhesion, centrifugation, readout

4. Cells incubated with samples on plate and centrifuged at (300 g, 10 min).

5. Fluorescence measurement from top focused on the cells accumulated in the bottom / center of V-bottom plate wells.

Note: the assay (w/o coating) is performed in ~ half a day.
Data analysis & validation

- Serial dilutions with extended asymptotes (large first and last dilution step).
- Relative potency of the sample is determined against the reference standard.
- Evaluated 4P fit and parallel line analysis (PLA) – PLA gave better accuracy.
- SSTs on regression / fit, parallelism, signal:noise and relative confidence limits of potency estimate.

Method fully validated for routine QC testing based on ICH Q2B.

E.g.: Range 50-150%, accuracy 89-98%, repeatability GRSD = 5%, precision GRSD = 10%, reproducible between labs, selective vs. unrelated drugs.
Assay robustness, critical parameters

- Critical parameters identified during method development and robustness DoE.

- Parameters critical to cell-based bioassays in general, e.g. cell cultivation (as split schedule, max passage number,...), critical reagents,...

- Critical parameters specific to the assay format:
  - V-bottom plates
  - Centrifuge type
  - Fluorescence reader & settings
Coated ligand

Activity of coated ligand strongly dependent on a post translational modification:

- Activity of each new lot is controlled (titration of coated drug target ligand).
- Since limited sources exist, extra measures taken to ensure business continuity during product life cycle (supplier QAA, stocks, long-term stability,...).
V-bottom plate

- V-plate surface and dimensions (e.g. V-angle) may vary between manufacturers and plate types.
- Manufacturer 1 and 2 interchangeable; manufacturer 3 not suitable.
- **Experiment:** Positive control in all plate positions.

- **Large vertical 4 slot rotor:** centrifugal force angle only varies minimally across the plate.

- **Small horizontal 2-slot rotor:** centrifugal force angle varies strongly across the plate.
Reader settings

Reader with properly calibrated X / Y axis (signal intensity per well)

Reader position & plate dimensions have to be accurately defined.

X/Y-axis of the reader might need re-calibration if a signal drift is recognized.
New assay format - implementation

New adhesion assay format compared to target binding ELISA

- DS / DP release and stability samples, drug variants (charge, size, etc.) and degradation products analyzed in both assays
Comparison to ELISA

- DS / DP release and stability samples were analyzed in both assays.
- Correlation observed - slope and y-intercept indicate that decrease in potency is recognized more sensitively by the cell adhesion bioassay.
- ELISA was replaced by cell adhesion bioassay.

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y = 1.35x - 36 \\
R = 0.83
\]
Summary

- Cell adhesion assay originally developed for HTS was repurposed and redeveloped as a CMC relative potency bioassay.

- New format successfully validated and implemented for routine testing replacing a target binding ELISA, as superior in detecting potency changes.

- New format was robust. Specific parameters (e.g. centrifuge, V-bottom plates reader settings) identified and controlled.
  - Main sources of variability in classic adhesion assays not present in new format, resulting in better assay control & robustness.

- May be generally applicable to drugs with cell adhesion as MoA.
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