Characterization and Lot Release Assays for Antibody Drug Conjugates (ADCs)

Ulrike Herbrand, Sandra Bauer, Verena Sonnenberg Charles River Biologics Testing Solutions, Erkrath, Germany

1 Introduction

Antibody-drug conjugates (ADCs) add an additional level of challenge to the testing of biotherapeutics. Besides the antibody, which needs to be evaluated for potential and known mechanisms of action (MoA), there is a cytostatic compound conjugated to the antibody that alters the behavior of the antibody-vehicle within the typical assays. Assays to measure proliferation, apoptosis, ADCC, ADCP, internalization and cell cycle arrest for the model ADC Trastuzumab emtansine are addressed in this poster.

2 Trastuzumab emtansine

- Trastuzumab emtansine (Kadcyla®, T-DM1) is an ADC consisting of the mAb Trastuzumab (Herceptin) linked to the cytotoxic agent emtansine (DM1).
- Approved in 2013 by the FDA for the treatment of HER2-positive metastatic breast cancer after prior treatment with trastuzumab (Herceptin®) and a taxane
- Trastuzumab alone stops growth of cancer cells by binding to the HER2/neu receptor, whereas DM1 enters cells and destroys them by binding to tubulin
- Kadcyla® is made to bring chemotherapy inside HER2-positive cancer cells and kill them while causing less harm to normal cells

3 Proliferation

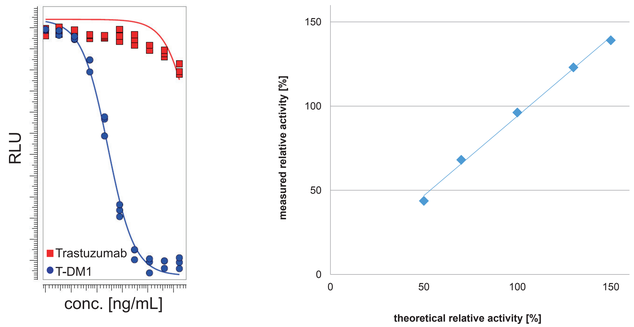

Figure 1: Proliferation assay with luminescence readout based on ATP release; comparison of unconjugated mAb and ADC (left) and limited dilution linearity of ADC (right)

4 Caspase 3/7 Apoptosis

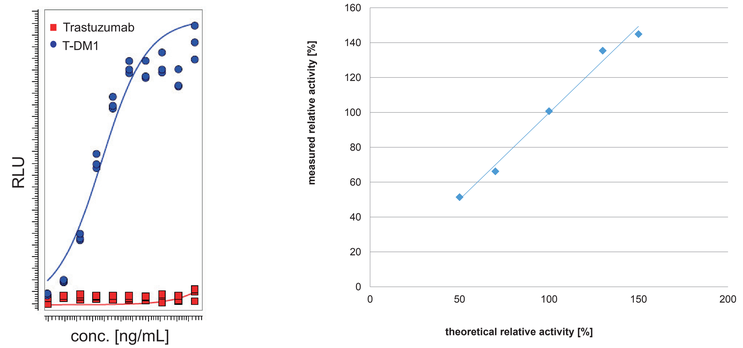

Figure 2: Caspase 3/7 Apoptosis assay with luminescence readout to measure cytotoxicity; comparison of unconjugated mAb and ADC (left) and limited dilution linearity of ADC (right)

5 ADCC & ADCP

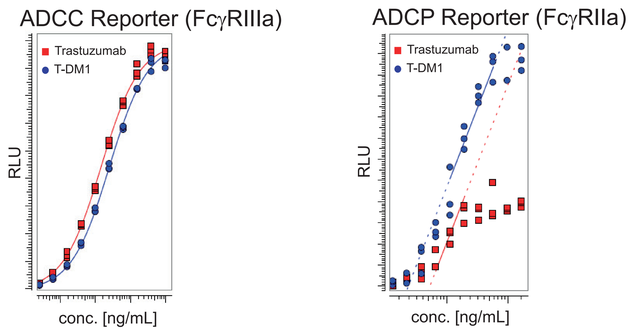

Figure 3: Reporter-based antibody-dependent cellular cytotoxicity (ADCC, left) and antibody-dependent cellular phagocytosis (ADCP, right) assays with luminescence readout; comparison of unconjugated mAb and ADC

6 Cell Cycle Arrest

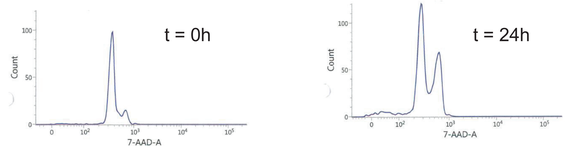

Figure 4: Cell cycle arrest in the presence of T-DM1 with flow-cytometric 7AAD readout, 24 h incubation time

7 Internalization

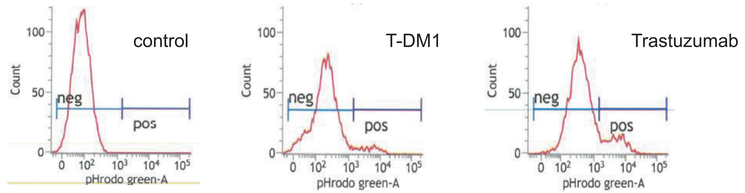

Figure 5: Internalization assay with pHRhodo green labeling and flow-cytometric readout

8 Summary

- T-DM1 inhibits the proliferation of a suitable target cell line dose-dependently within 72h, whereas Herceptin at the same concentration has nearly no impact on the cell proliferation (Figure 1)
- T-DM1 activates the Caspase 3/7 pathway dose-dependently within 48h, whereas Herceptin at the same concentration has nearly no impact on this pathway-specific cytotoxicity (Figure 2)
- Proliferation and Caspase 3/7 Apotosis Assays are suitable for QC-purposes (limited dilution linearity in Figures 1 & 2), ICH-compliant method validation and both assays show stability-indicating properties (data not shown)
- ADCC of the antibody-drug conjugate is reduced compared to Trastuzumab whereas ADCP is enhanced in reporter-based surrogate approaches (Figure 3)
- T-DM1 causes a significant cell cycle arrest at G2/M after 24h incubation (Figure 4)
- Trastuzumab-internalization is more significant than the internalization of T-DM1 at the same concentration after 30h incubation (Figure 5)
- ADCC and ADCP reporter assays as well as flow cytometric cell cycle arrest and internalization assays are suitable for ADC characterization

9 Acknowledgement

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