Developability Assessment – A Key Process to Derisk the Selection of ADC Clinical Candidates

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Introduction

While identification of a lead candidate represents an important first step in the development of a novel ADC, broadly validating its design and mode of action, developability assessment is an equally important stage in the process of lead candidate optimization and clinical lead ADC selection.

Developability assessment aims to analyze ADC candidates through a range of assays evaluating their biological functionality, safety and manufacturability properties and detect any liability likely to impact their clinical performance or affect their ability to be manufactured successfully. Identification of liabilities provides areas to focus on for lead optimization and facilitates the selection of an optimal clinical lead.

Abzena applies a series of stage appropriate experiments to systematically evaluate and optimize the properties of ADC candidates, ultimately allowing the selection of the best candidates for development with the greatest chance of clinical success.

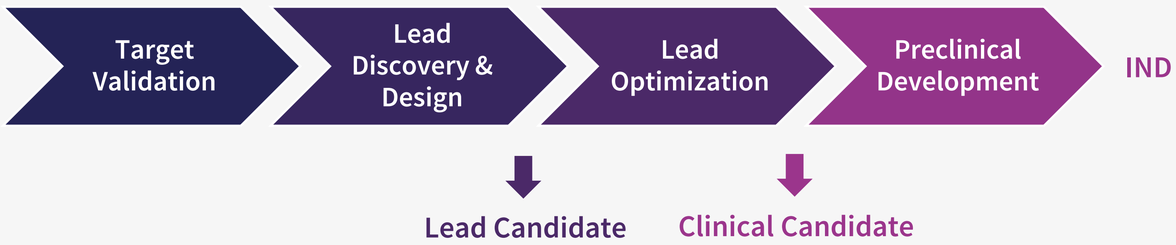

Figure 1. Overview of the Drug Discovery Process. Throughout the lead discovery and design and lead optimisation stages, assays can be deployed to evaluate the developability of the ADC candidates. Developability assessment aims at evaluating the functionality, safety and manufacturability of ADC candidates and guides the selection of the best clinical candidate.

Evaluating Functionality

ADC functionality can be assessed in non-cell-based assays. Abzena uses surface plasmon resonance (SPR) to determine antigen binding of the ADC. Fc function can also be characterized by SPR.

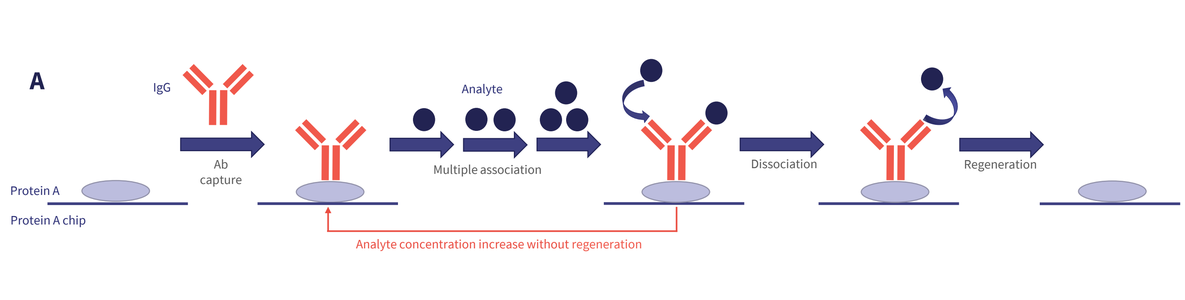

Figure 2. Antigen Binding Characterization by Surface Plasmon Resonance. A. Biacore single-cycle kinetics (SCK) analysis workflow of the binding of an antibody to its cognate antigen. B. Sensorgrams of the HER2 binding SCK analysis of different trastuzumab ADCs and benchmarks.

ADC functionality can also be assessed in cell-based assays. 2D endpoint cell viability assays are key assays to rapidly screen multiple candidates for cell killing activity.

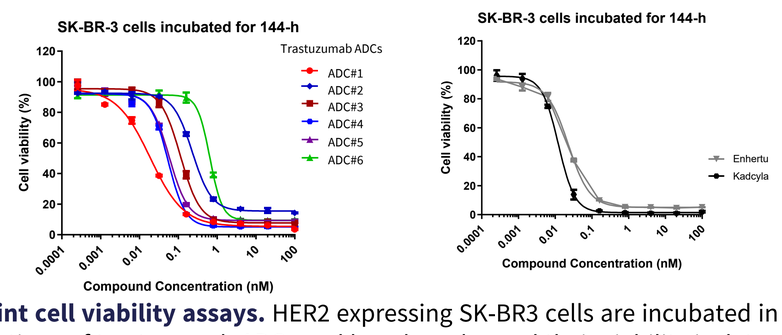

Figure 3. 2D endpoint cell viability assays. HER2 expressing SK-BR3 cells are incubated in the presence of increasing concentrations of trastuzumab ADCs and benchmarks, and their viability is determined after 96 h using the CellTiter-Glo™ luminescence assay. IC50 and minimum cell viability values are compared to benchmarks.

To gain fuller understanding of the different modes of action of ADC candidates beyond their ability to directly kill antigen expressing cells, bystander activity and antibody-directed cell cytotoxicity (ADCC) can be assessed *in vitro* in more complex live cell imaging assays.

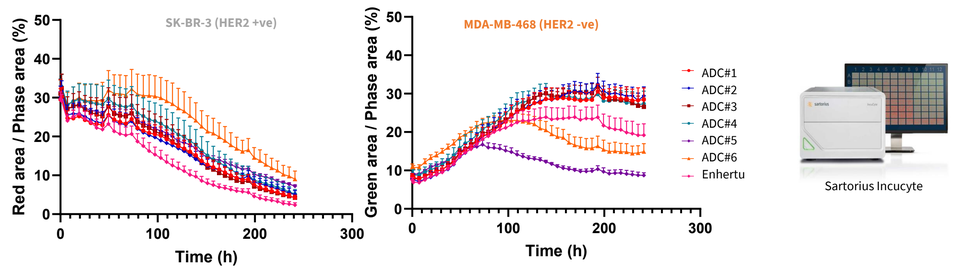

Figure 4. Bystander activity assay with the Incucyte® live cell imaging system. HER2 positive (SK-BR3) and HER2 negative (MDA-MB-468) cells are respectively transduced with Cytolight Red and Green lentivirus reagents and co-cultured in the presence of trastuzumab ADCs and benchmarks. The size of each cell population is monitored by fluorescence imaging. While all trastuzumab ADCs and benchmarks tested demonstrated cytotoxic activity on HER2 positive cells, variable levels of bystander activity were observed on HER2 negative cells.

Evaluating Safety

Toxic side-effects remain a major obstacle to the success of ADCs in the clinic. Dose-limiting toxicities often prevent the administration of an optimal therapeutic dose of ADC to patients. Toxicities can come from ADC uptake by healthy tissue expressing the target antigen, uptake of the whole ADC by healthy tissue through non-specific endocytosis, or uptake of free payload by healthy cells. Serum stability studies provide information on the extent of free payload release in systemic circulation and toxicities associated to it.

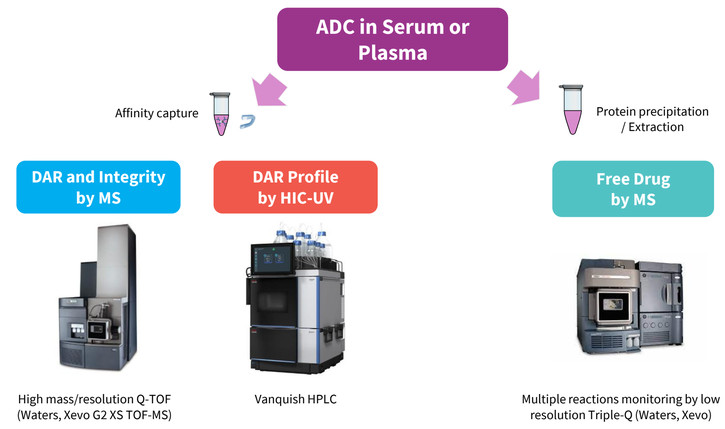

Figure 5. Serum stability studies workflow overview. ADCs are incubated at 37 °C in serum or plasma from human or preclinical species origin and timepoints are collected over up to 7 days. ADCs are affinity captured using anti-Fc antibody / target antigen coated on magnetic beads then eluted and analysed by LC-MS or HIC-UV to monitor changes in DAR. Samples can also be protein precipitated, and small molecules extracted for analysis and quantification by LC-MS/MS to determine free payload concentration.

Evaluating Manufacturability

Emphasis is often placed on functionality and safety for the selection of ADC clinical lead candidates, as these parameters determine the therapeutic activity of an ADC. To ensure successful development of an ADC into the clinic, its manufacturability is also of critical importance. Different assays are used to evaluate the ability of different ADC lead candidates to be manufactured at the scale required to support clinical trials and their ability to be manufactured in a stable form. Different biophysical parameters including the ability of the candidates to withstand thermal, freeze-thaw or chemical stress, or their hydrophobic character or propensity to self-associate or form aggregates can be analysed to provide information on the stability of the candidates.

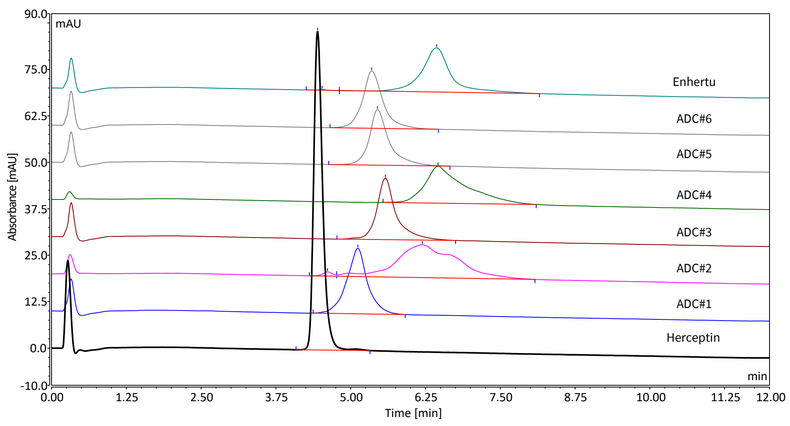

Figure 5. Hydrophobic Interaction Chromatograms. The homogeneity and hydrophobicity of trastuzumab ADCs was analysed by HIC and compared to Herceptin and Enhertu.

Summary

Developability assessment is increasingly integrated into the drug discovery process to guide the selection of ADC clinical candidates, lowering the likelihood that a molecule with poor drug-like properties moves into the CMC and manufacturing stages and de-risking the overall program.

Abzena's ADC developability studies provide multiple readouts capturing the fundamental characteristics of successful drug design: functionality, safety and manufacturability. Identification of liabilities through developability assessment allows to put in place focused optimization cycles, ultimately leading to the progression into clinical studies of an ADC lead candidate with the greatest chances success.