Real-Time Live Cell Imaging in Successful Antibody-Drug Conjugate Development

Robert J. Francis* , Natasha Carter, Grant Harradence, Robert Cunningham, Nathan Shaw, Adele Kinsey, Maryam Ahmadi, Erika Kovacs, Robert Holgate, Campbell Bunce
Abzena, Babraham Research Campus, Cambridge, UK
* email: Robert.francis@abzena.com

Introduction

Antibody Drug Conjugates (ADCs) are novel and emerging targeted therapeutics against cancer. ADCs deliver the specificity of mAb and cytotoxicity of small molecule drugs. Developability assessment underpins all aspects of ADC drug discovery, lead selection and optimisation. Abzena uses real- time live cell imaging in the developability assessment of ADCs to select a lead drug candidate with the greatest chance of clinical success.

ADC and related biologic consideration

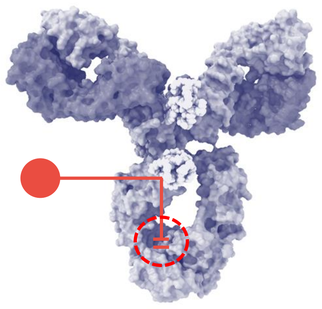
A simple concept but complex products

To Identifying risks early in development helps to reduce cost, quality and time issues at later stages of manufacture and clinical evaluation

FUNCTIONALITY

The candidates show the desired biological function

- Drug attachment does not interfere with Ag binding
- Drug is efficiently internalised
- Drug is released in the target cells/tumor micro-environment
- Drug can induce cellular proliferation arrest in target cells



MANUFACTURABILITY

Assesses the candidate for issues that may affect their ability to be manufactured

- Drug attachment with high efficiency & reproducibility

SAFETY

Likelihood of candidates displaying toxic side-effects

- Antibody does not demonstrate unwanted Fc- effector function
- ADC does not induce off- target cytotoxicity

ADC cellular lifecycle

Internalisation and payload release in target cells

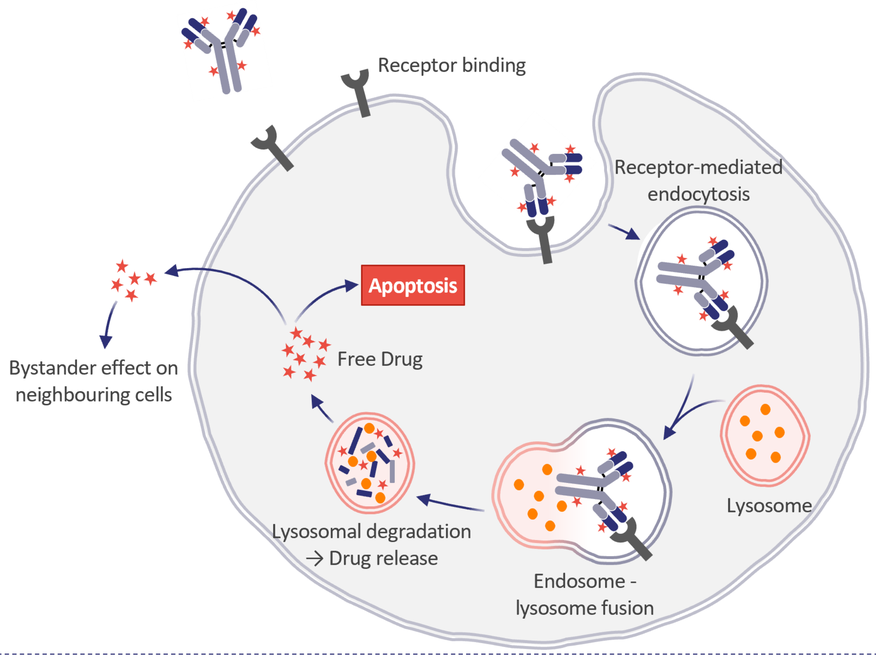


Figure 1: ADCs have a multi-step mechanism of action with specific requirements for each step / component.

Assessment of cell line suitability and antigen binding of ADCs

Cell line selection to ensure suitability for downstream assessments

- Does the target cell line express sufficient target antigen?
- Does the ADC bind the target antigen?
- Is the cell line sensitive to toxic effects of payload?

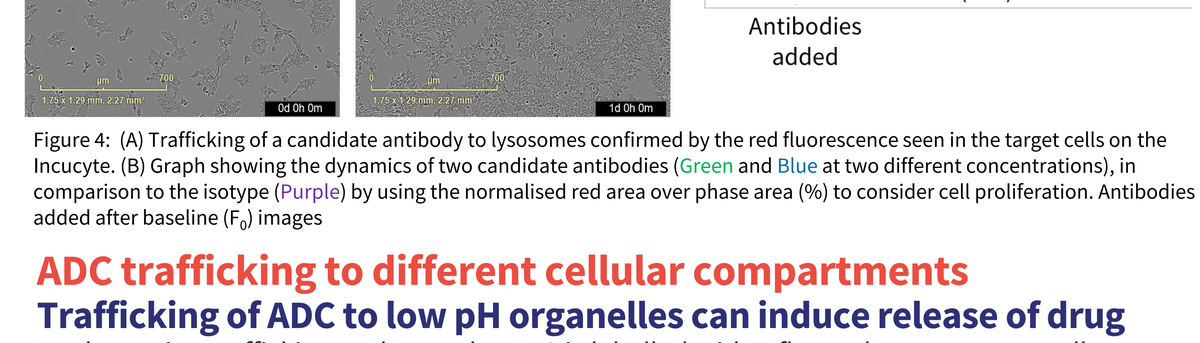


Figure 3: Cell lines are tested for (A) sensitivity to drug and (B) binding to ADC.

Assessment of tumor cell killing by ADCs in 2D cultures

Mode of action of ADC determined in high throughput assays

To test how well an ADC can kill a target cell line, 2D viability assays are used. Live cell imaging of apoptosis and necrosis are assessed.

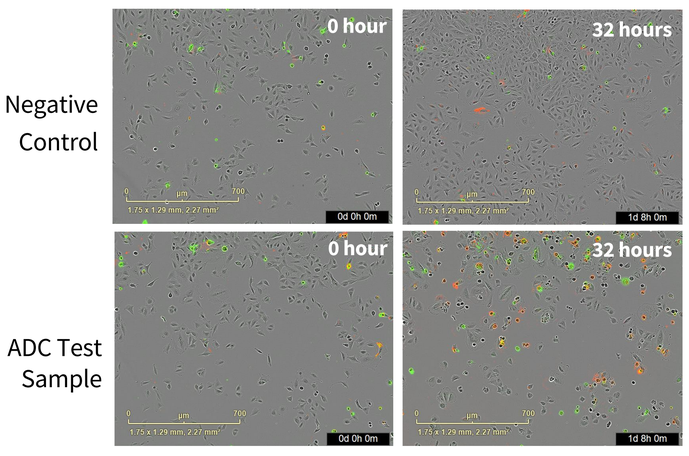


Figure 6: A549 cells incubated with an ADC and a negative control.

Red = AnnexinV-647 and Green = Sytotox Green

Assessment of ADC internalisation

Internalisation of ADC is a key step in mechanism of action of ADC

Using the Fabfluor-pH protocol (Sartorius) we can assess the internalisation of Fc-containing antibodies. As the Fabfluor-pH labelled antibodies enter the acidic lysosomes, the Fabfluor-pH begins to intensely fluoresce red.

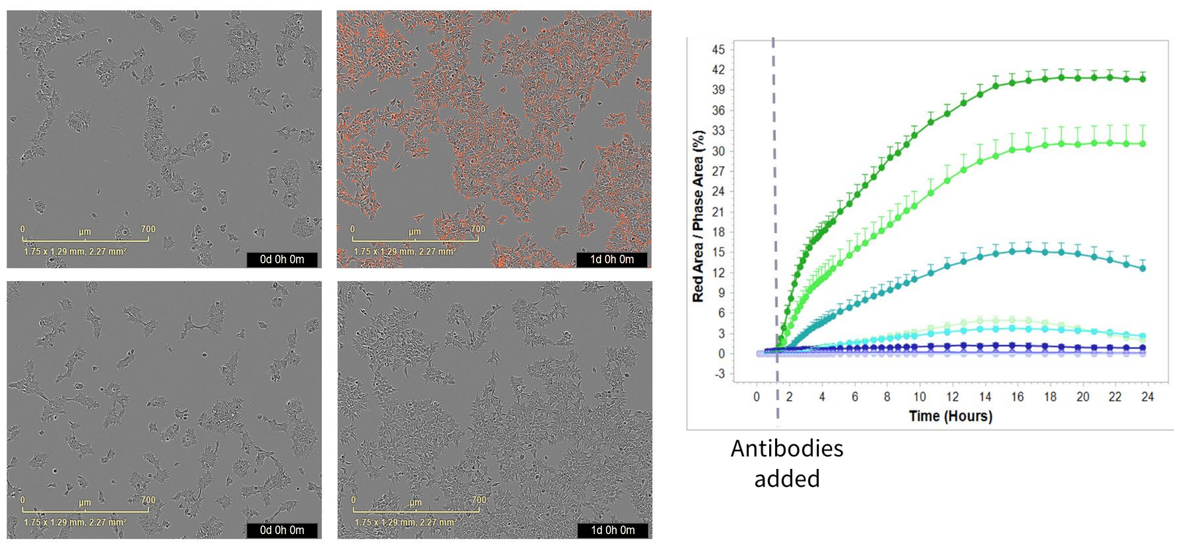


Figure 4: (A) Trafficking of a candidate antibody to lysosomes confirmed by the red fluorescence seen in the target cells on the Incucyte. (B) Graph showing the dynamics of two candidate antibodies (Green and Blue at two different concentrations), in comparison to the isotype (Purple) by using the normalised red area over phase area (%) to consider cell proliferation. Antibodies added after baseline (F_0) images

ADC trafficking to different cellular compartments

Trafficking of ADC to low pH organelles can induce release of drug

To determine trafficking pathway, the ADC is labelled with a fluorochrome. Target cells are modified to express fluorescent protein-tagged receptors, and/or stained for live cells such as lysotracker green DND-26. Trafficking and co-localisation are measured using spinning disk confocal microscope.

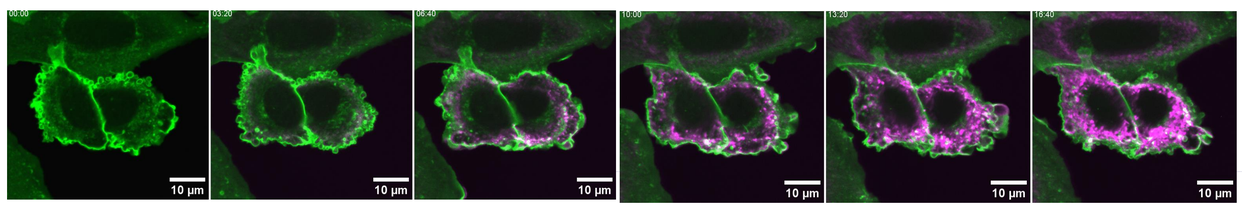


Figure 5 . Fluorochrome labelled biologic is added to GFP expressing CHO cells and imaged over time using spinning disk confocal microscopy. Pink = biologic, Green = GFP tagged receptor, White = co-localisation of both

Efficacy of the ADC in 3D spheroid tumors

Physiologically relevant models used to determine mode of action

Tumour may be grown in spheroid systems to produce more physiologically relevant tumour environment. Spheroid models can highlight the effect of tumour penetration, cellular interaction and resistance on efficacy of ADC.

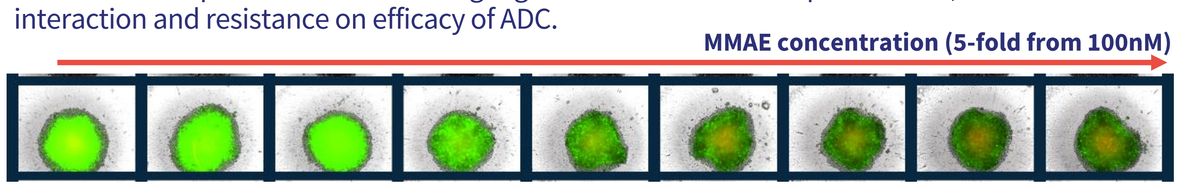


Figure 7: Spheroids of a cancer cell line that have been killed with Monomethyl Auristatin E (MMAE). These images were captured on the Incucyte after 7 days. Green = sytox green Red = live cells

Safety assessment of ADCs

ADCs may pose a risk of inducing off-target toxicity

Fc mediated effector function can enhance off-target toxicity in patients. Conjugation of payloads may result in hydrophobicity and increased aggregation of the ADC, potentially resulting in increased off-target internalisation and Fc effector function.

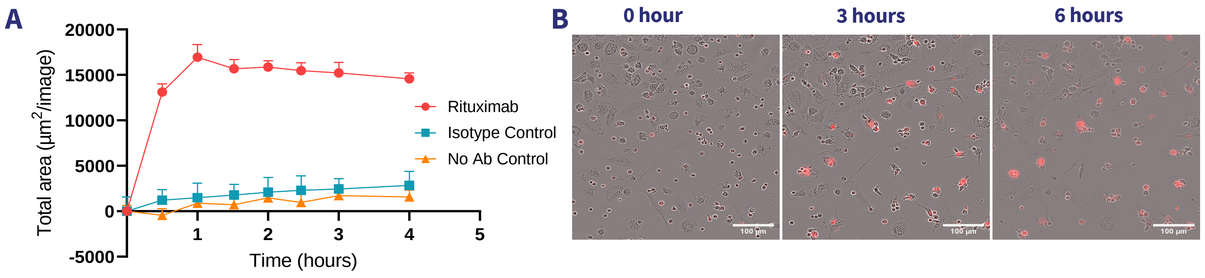


Figure 8: pHrodo labelled target cells (Raji) opsonized with rituximab , are co-incubated with macrophages. Phagocytosed Raji cells fluorescence as they reach the phagosome. (A) Summary data of phagocytosis of Raji cells. Total area over a threshold considers the macrophags as they turn red due to uptake of the pHrodo labelled target cells (B) Selected pictures taken from ADCP time course.

Summary

ADCs are novel therapeutics that deliver precision and efficacy in treatment of cancer. Understanding the mode of action, the antibody activity and the effectiveness of the cytotoxic drug, will allow the generation of new ADCs.

The versatility that live cell imaging provides, either through the Incucyte or higher resolution spinning disk confocal microscopy, can be harnessed to capture the fundamental characteristics of successful drug design and increases the likelihood of obtaining a successful therapeutic candidate.