# Application of Real-Time Live Cell Imaging in the Development of Antibody-Drug Conjugates

Robert J. Francis*, Grant Harradence, Robert Cunningham, Nathan Shaw, Pearce Curran, Adele Kinsey, Erika Kovacs*, Robert Holgate, Campbell Bunce
Abzena, Babraham Research Campus, Cambridge, UK
* email: robert.francis@abzena.com or erika.kovacs@abzena.com

## Abstract

Antibody Drug Conjugates (ADCs) have rapidly become an important class of targeted therapeutics against cancer, delivering both the specificity of mAbs and the cytotoxicity of small molecule drugs.

Continual developability assessment underpins all aspects of ADC drug discovery and development and the appropriate use of real- time live cell imaging as part of the developability assessment of ADCs can play a significant role in the selection of candidates with the greatest chance of clinical success.

## Our global footprint. 450+ Employees. Over 500+ Customers served.

**Cambridge, UK**

Excellence in lead discovery & design through to candidate selection and cell line development

**San Diego, CA**

Excellence in biologics development and manufacturing at our Lusk, Sorrento and Loker facilities

**Bristol, PA**

Excellence in bioconjugation and complex chemistry development and manufacturing

## ADC and related biologic consideration

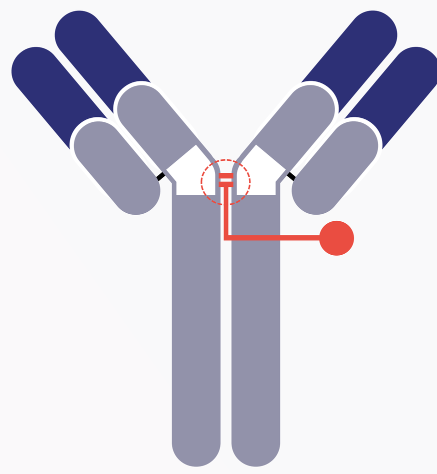
### A simple concept but complex products

Assessing Developability early in development helps to identify risks that in turn reduces cost, quality and time issues at later stages of manufacture and clinical evaluation

#### Functionality

**Assesses target driven, intrinsic (e.g. FcR) and extrinsic (e.g. payload-mediated) activities**

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



#### Manufacturability

**Assesses candidates for issues that may affect their ability to be manufactured**

- Drug attachment with high efficiency & reproducibility
- Appropriate phys/chem properties

#### Safety

**Assesses the likelihood of unwanted side-effects**

- Absence of immunogenicity
- Avoidance of undesirable Fc- effector function
- off- target cytotoxicity

## ADC cellular lifecycle

### Internalisation and payload release in target cells

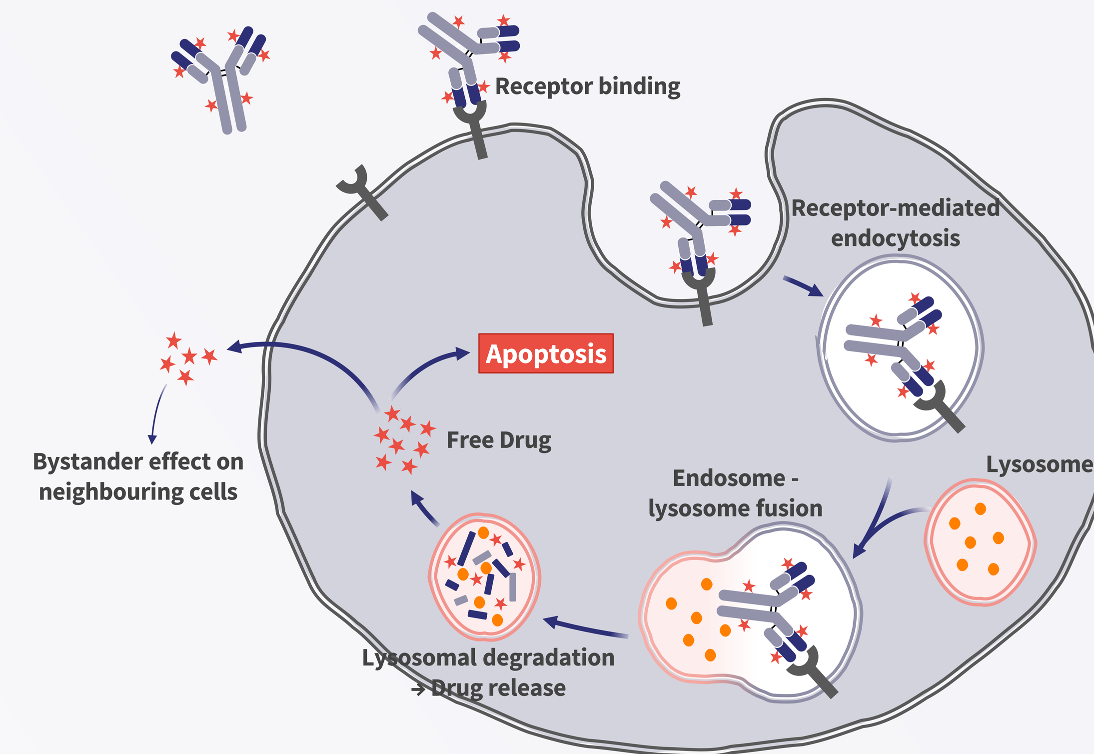



**Figure 1.** ADCs have a multi-step mechanism of action with specific requirements for each step / component.

## 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?

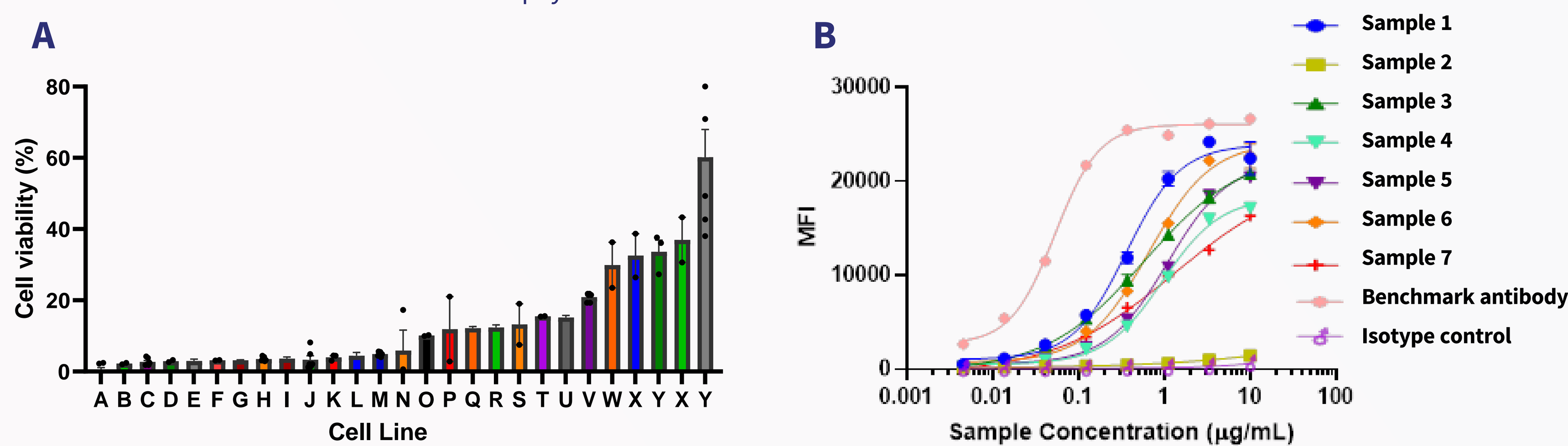



**Figure 2.** Cell lines are tested for: (**A**) sensitivity to drug, and; (**B**) binding to ADC.

## Assessment of tumor cell killing by ADCs in 2D cultures

### Mode of action of ADC determined in high throughput assays

To assess how an ADC can kill a target cell line, 2D viability assays are used. Live cell imaging can determine levels of apoptosis and necrosis as well as proliferation.

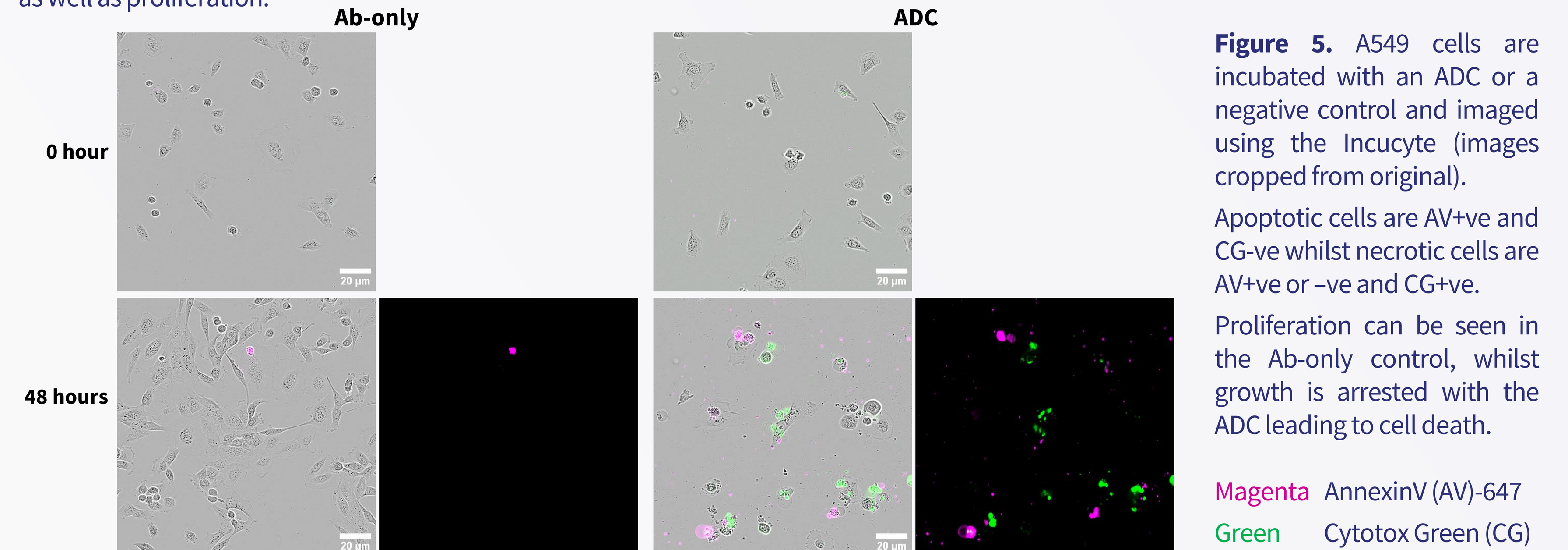



**Figure 5.** A549 cells are incubated with an ADC or a negative control and imaged using the Incucyte (images cropped from original).

Apoptotic cells are AV+ve and CG-ve whilst necrotic cells are AV+ve or –ve and CG+ve.

Proliferation can be seen in the Ab-only control, whilst growth is arrested with the ADC leading to cell death.

Magenta AnnexinV (AV)-647
Green Cytotox Green (CG)

## Assessment of ADC internalisation

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

Using the Fabfluor-pH protocol (Sartorius) the internalisation of Fc-containing molecules can be assessed. As the Fabfluor-pH labelled antibodies enter the acidic lysosomes, the Fabfluor-pH begins to intensely fluoresce red. For non-Fc containing antibodies or biologics, direct conjugation of a pH sensitive dye to the molecule can be performed.

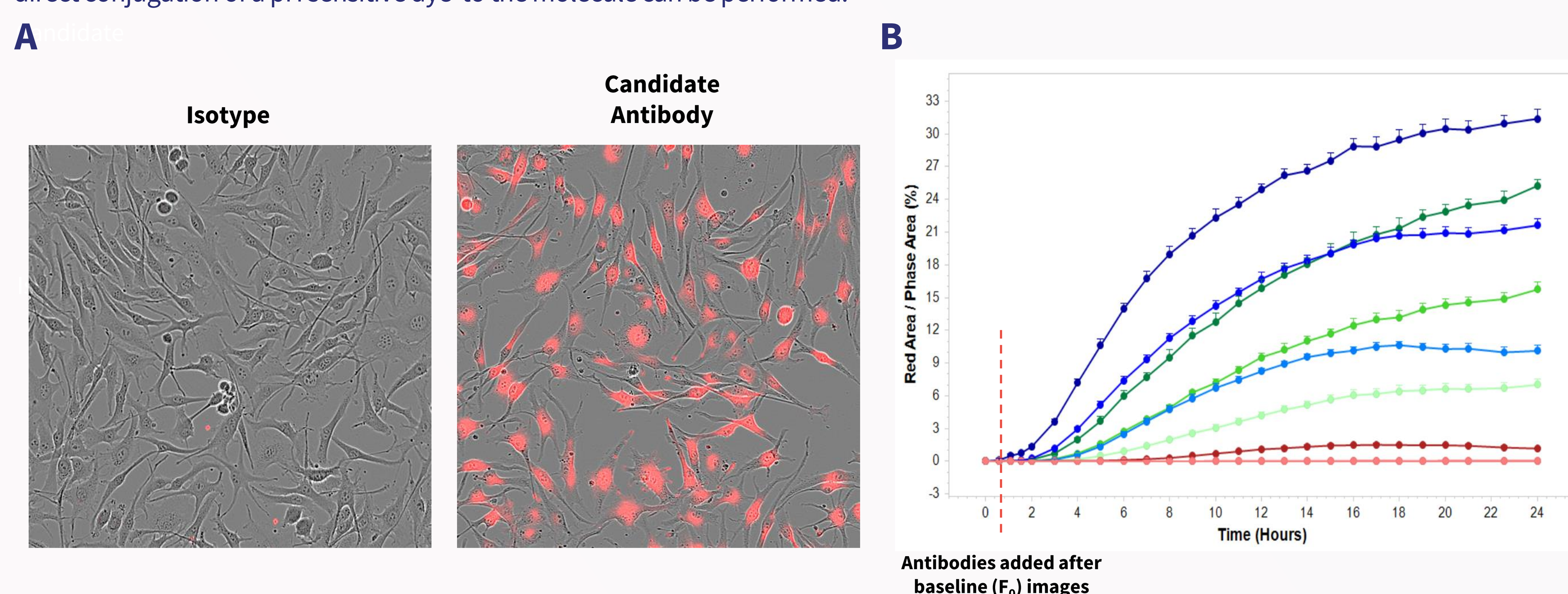



**Figure 3.** (**A**) Trafficking of a candidate antibody to lysosomes is confirmed by the red fluorescence observed in the target cells using the Incucyte; images representative of data produced after 24 hours. (**B**) Graph showing the dynamics of two candidate antibodies (Green and Blue) at three different concentrations (lighter tone = lower the concentration) compared to the isotype (Red) by using the normalised red area over phase area (%) to consider cell proliferation.

## Efficacy of the ADC in 3D spheroid tumors

### Physiologically relevant models used to determine mode of action

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

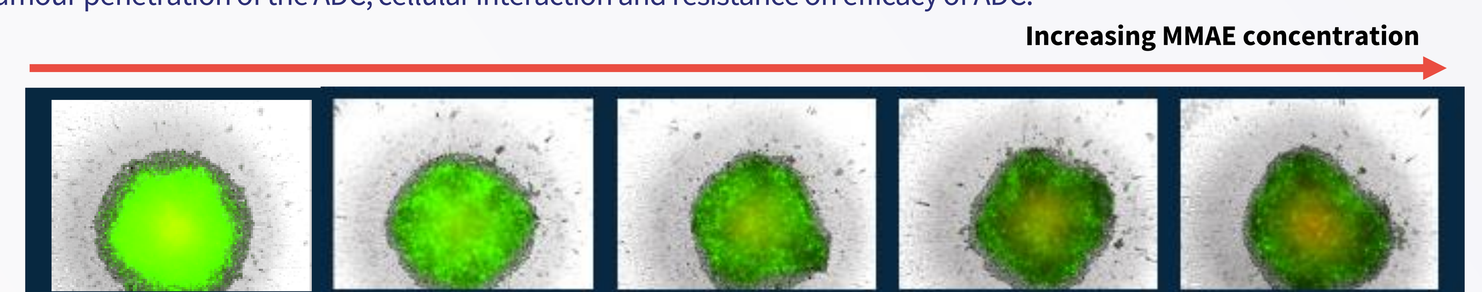



**Figure 6.** Spheroids of a cancer cell line that have been killed with Monomethyl Auristatin E (MMAE). These images were captured on the Incucyte after 7 days. Green = Cytotox green Red = live cells

## ADC trafficking to different cellular compartments

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

To determine trafficking pathway, the antibody backbone 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.

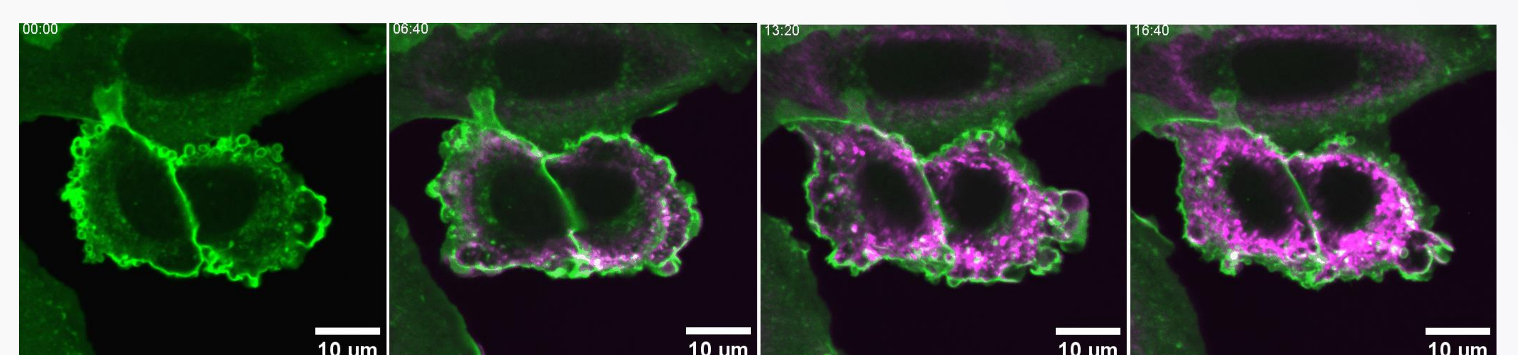



**Figure 7.** Fluorochrome labelled biologic is added to GFP expressing CHO cells and imaged over time using spinning disk confocal microscopy. Pink = biologic, Green = GFP tagged receptor, White = co-localisation of both

## 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.

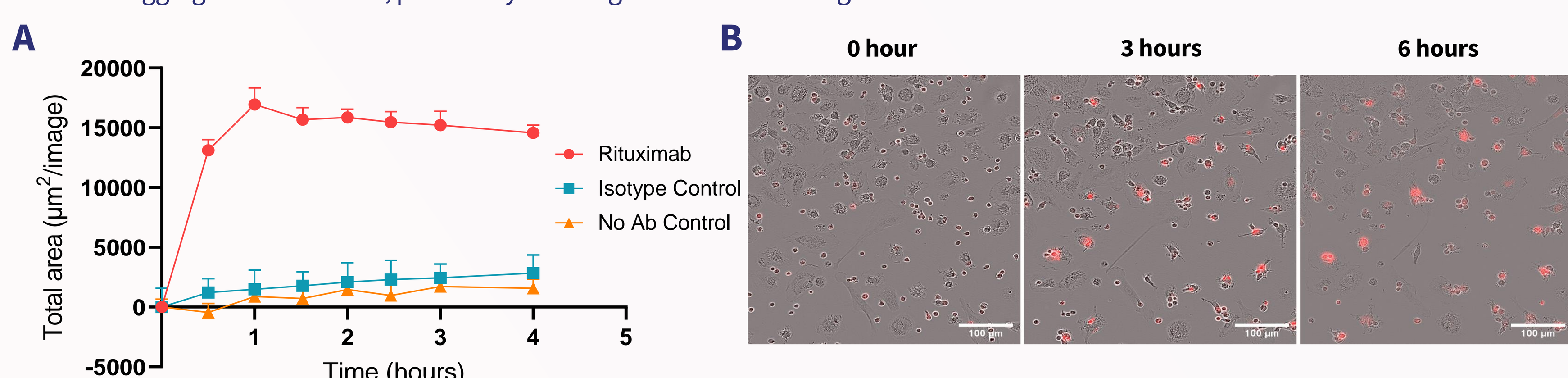



**Figure 4.** pHrodo labelled target cells (Raji) are opsonized with rituximab and co-incubated with macrophages. Phagocytosed Raji cells fluorescence as they reach the phagosome. (**A**) Summary data of phagocytosis of Raji cells. Total area over a threshold considers the macrophages as they turn red 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.