# Utilizing Live Cell Imaging for Better Lead Selection of Biologics and Bioconjugates

Erika Kovacs
Senior Director, Bioassay

ABZENA

17th September 2025

UK Incucyte® User Group Meeting

# Programs tailored around your molecule

## Flexible solutions to fit your requirements

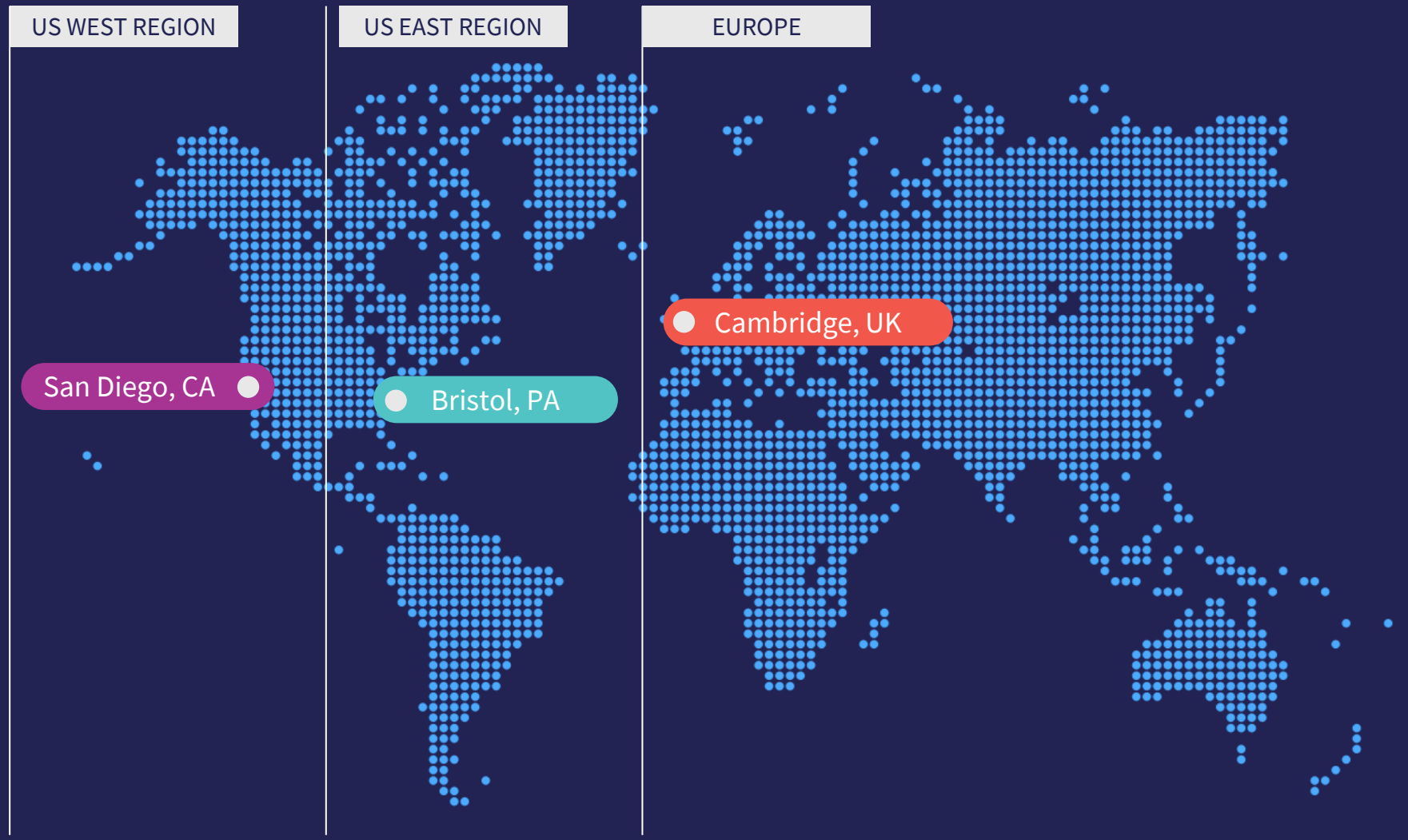
We appreciate that every program is different

By leveraging over two decades of expertise in R&D, our team is committed to providing high quality science to every client

- From the beginning, our team works with you to determine the best strategy to **reduce timelines without compromising quality.**

ABZENA | Moving medicine forward.

# Comprehensive global coverage




**5**
Facilities

**450+**
Employees

**65,000+ sq ft**
Combined cGMP Manufacturing + Laboratory Space

**500+**
Customers Globally

**150**
Programs Delivered Across R&D and CMC Per Year

# Derisking bispecific drug development through developability

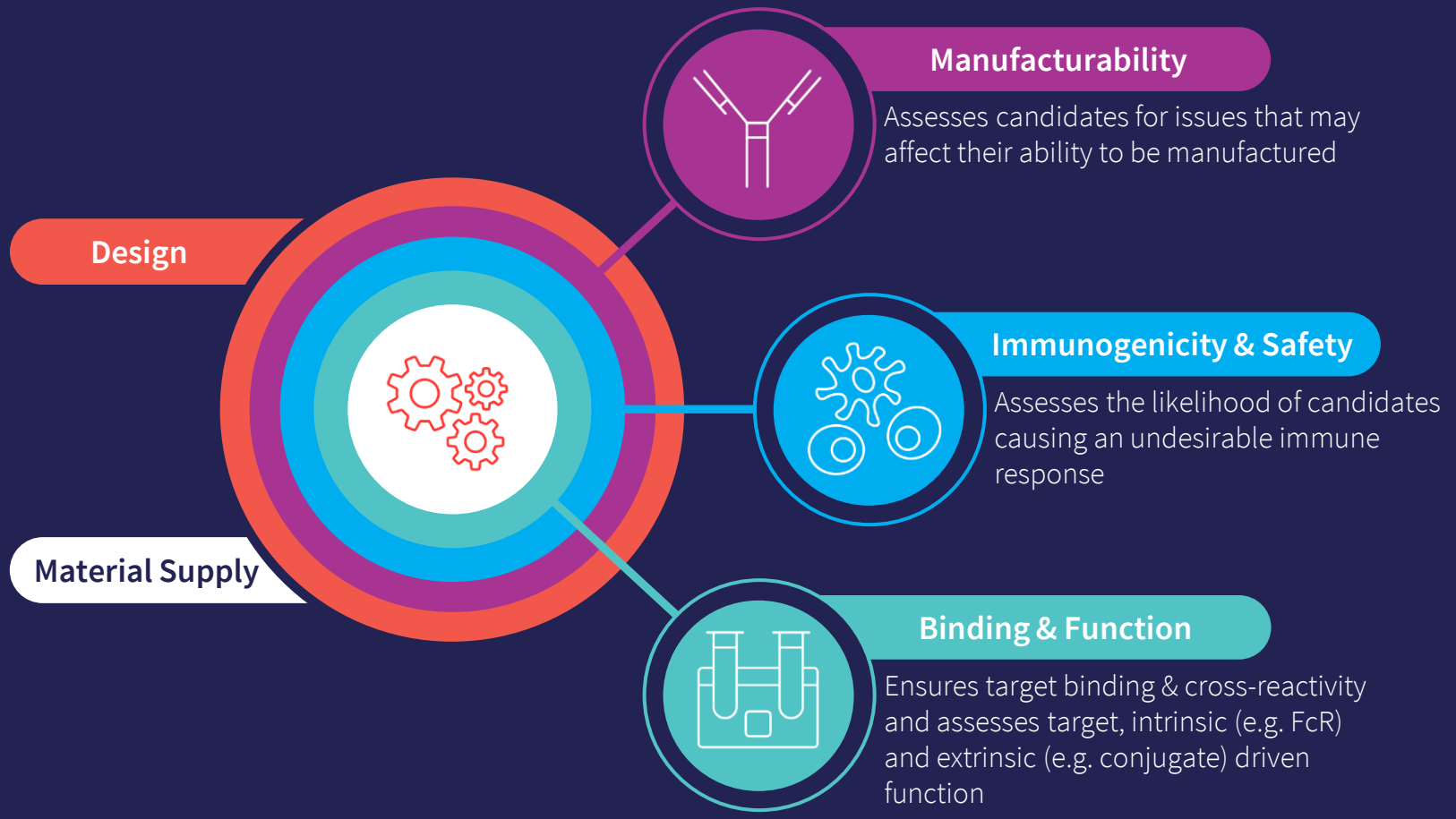
## Candidate selection requires a thorough understanding of your candidate molecule

### Developability asks two fundamental questions

➔ **Can we make it?**

➔ **Does it work?**

Developability takes a **holistic view** to select the best candidate to progress into preclinical development



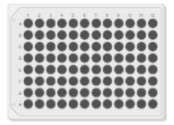
# Stage-appropriate assays for lead selection

## Building the most suitable assay cascade

- Selecting only the **best candidates** for testing in preclinical animal models
- Choose from:
  - ✓ Panel of Abzena's off-the-shelf assays
  - ✓ Custom developed new assay
  - ✓ Assay transferred from Client
- Assays tailored for mode of action and stage

**Relative accuracy, precision and throughput increases**

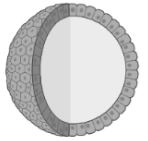
**Biochemical assays**



**Cell-based assays using cell lines**

**Primary cell-based assays**

**Kinetic assays, 3D models and co-cultures**



**Organoids / organ on a chip**

**Animal models**

(Syngeneic, CDX, PDX, Humanised)

**Complexity, variability & biological relevance increases**

# Assay requirements at different stages

Target ID/ Validation → Discovery → Lead Optimization & Candidate Selection → Pre-Clinical Development → IND → Clinical Trials

### Proof of Concept

- ✓ MoA-reflective
- ✓ Cost-effective

### Candidate Screening

- ✓ High-throughput
- ✓ Sensitive
- ✓ Robust
- ✓ Reproducible

### Lead Selection & Characterization

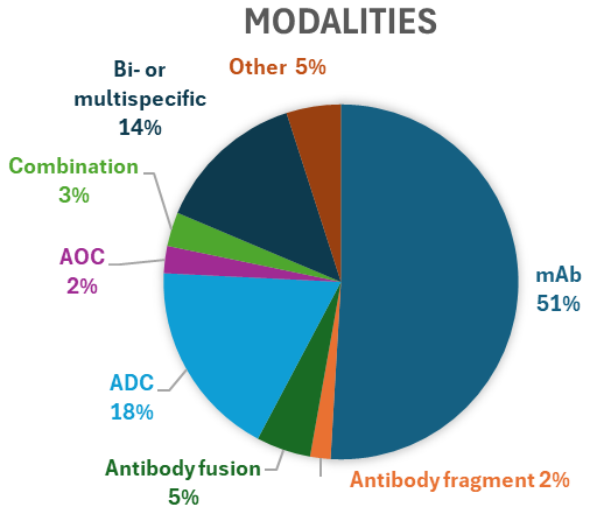
- ✓ MoA-reflective
- ✓ Inform of clinical success

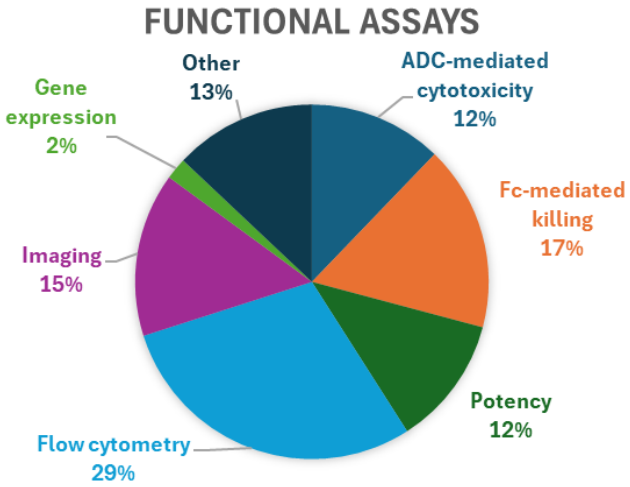
### Lot Release

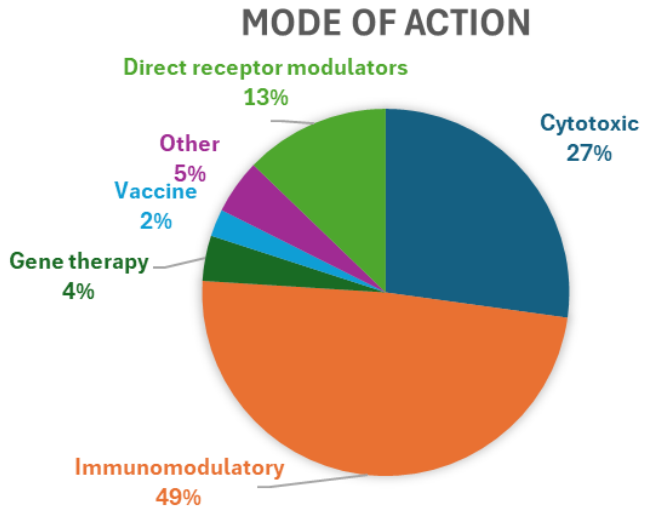
- ✓ Accurate
- ✓ Precise
- ✓ Sensitive
- ✓ Robust
- ✓ Reproducible

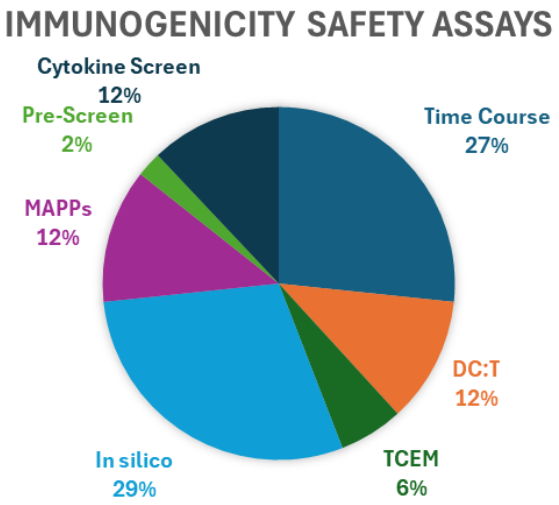
# One size does not fit all

## Impact of Mode of Action (MoA) on assay design




**Department Metrics**

| | |
|---|---|
| Off-the-shelf assays: | 30 |
| 100+ | Custom projects delivered |
| Service lines: | 14 |
| >110 | Targets assessed |

ABZENA

# Case Study #1

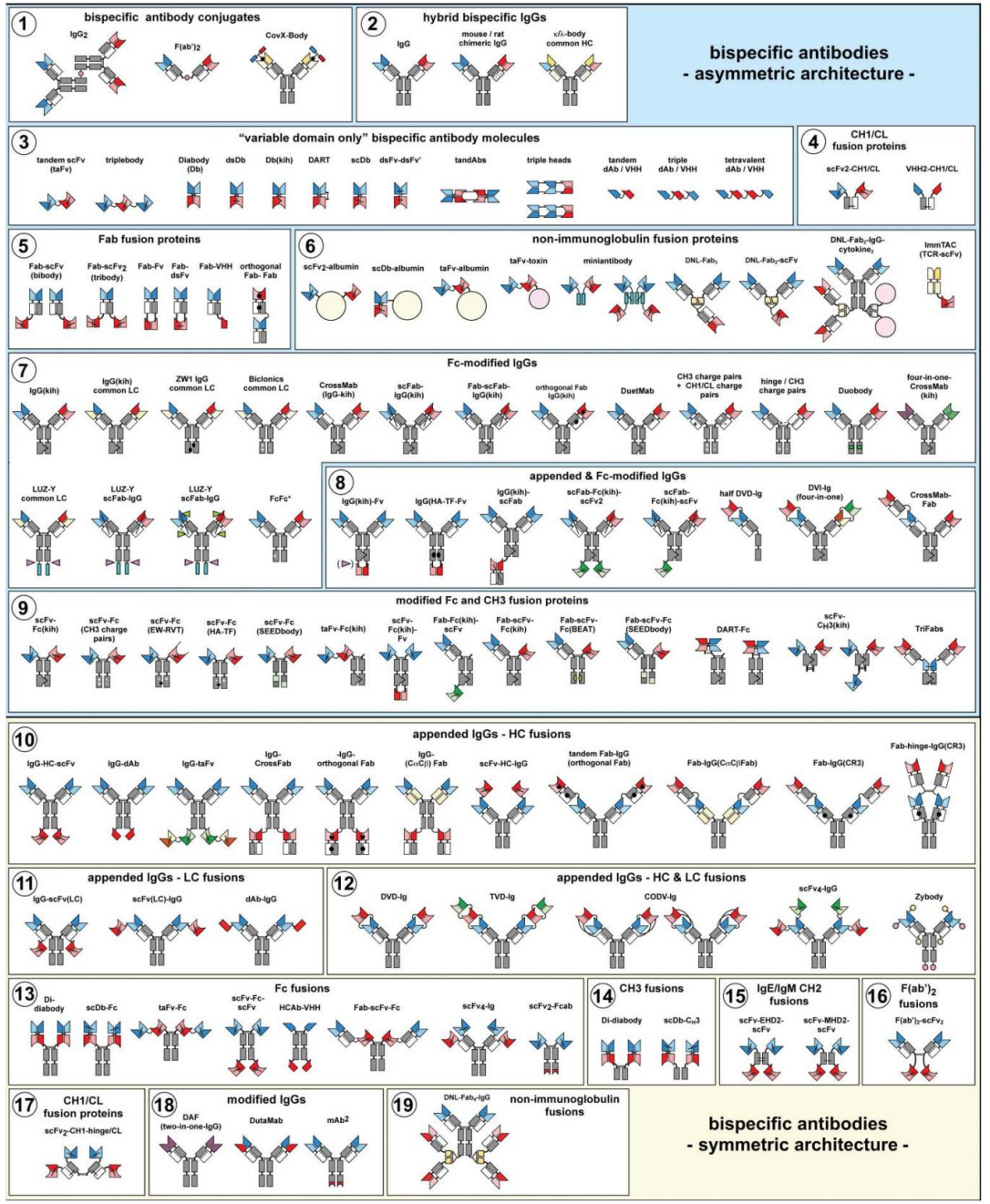
Assessing the Functionality and Safety of Bispecifics

# The “zoo” of bi- and multi-specific antibodies

## There are many ways to combine different specificities

**Multiple factors need to be considered:**

- **Size** – impacts tissue penetration (e.g. solid tumours)
- **Valency** (1:1, 1:2,2:2 etc)
- **Geometry**
- **Half-life**
- Relative **affinities** of different specificities – affinity balancing
- **Protein A binding** to leverage mAb platform
- **IP / FTO**
- Requirement to retain or remove **effector function**




Brinkmann U, Kontermann RE. The making of bispecific antibodies. MAbs. 2017 Feb/Mar;9(2):182-212

# Case study on bispecifics

## Format design

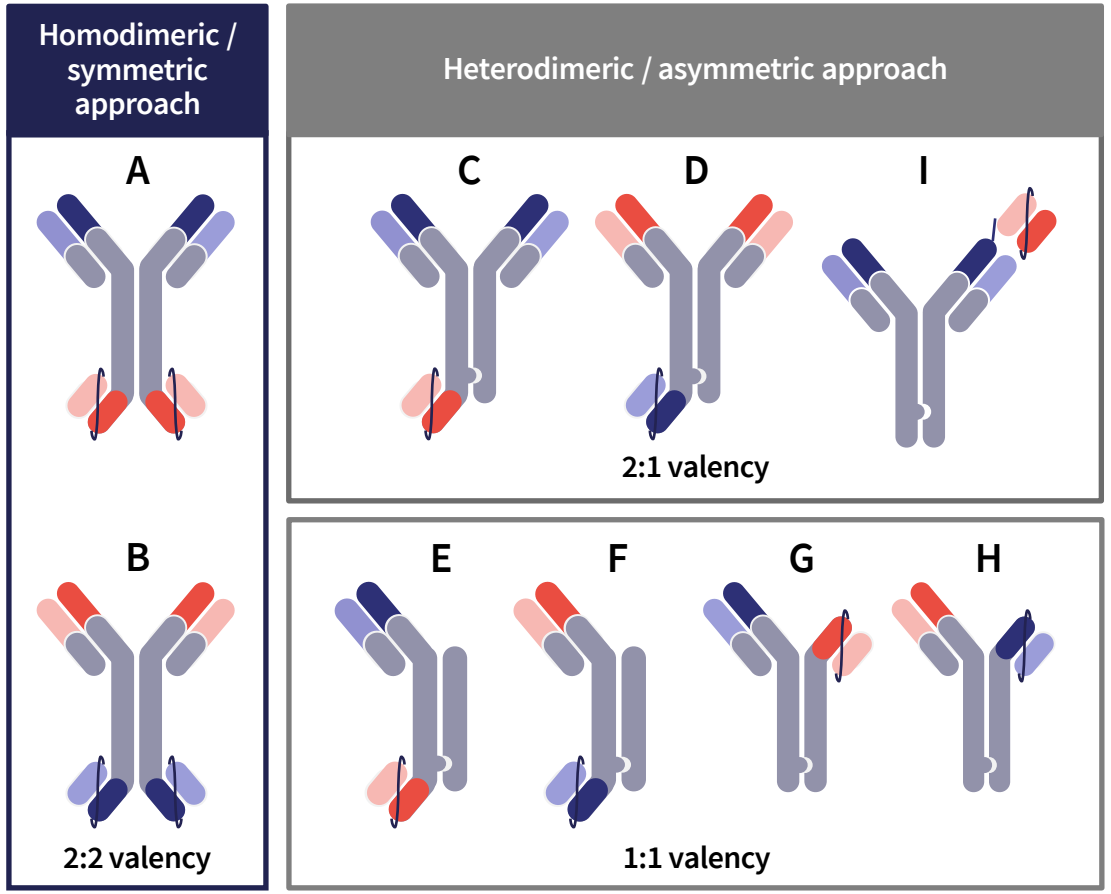
Aim: evaluate a platform for optimal bispecific design and selection.

Cancer target – anti-CD19

T cell recruiter – anti-CD3

9 bispecific constructs were designed

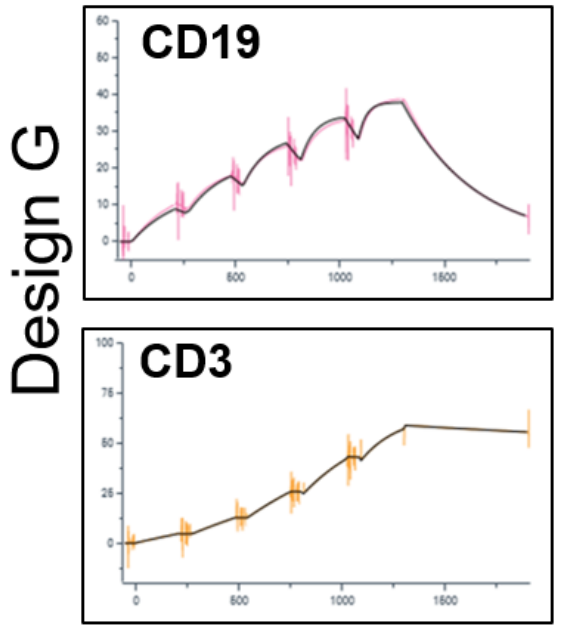
Aiming for the below attributes

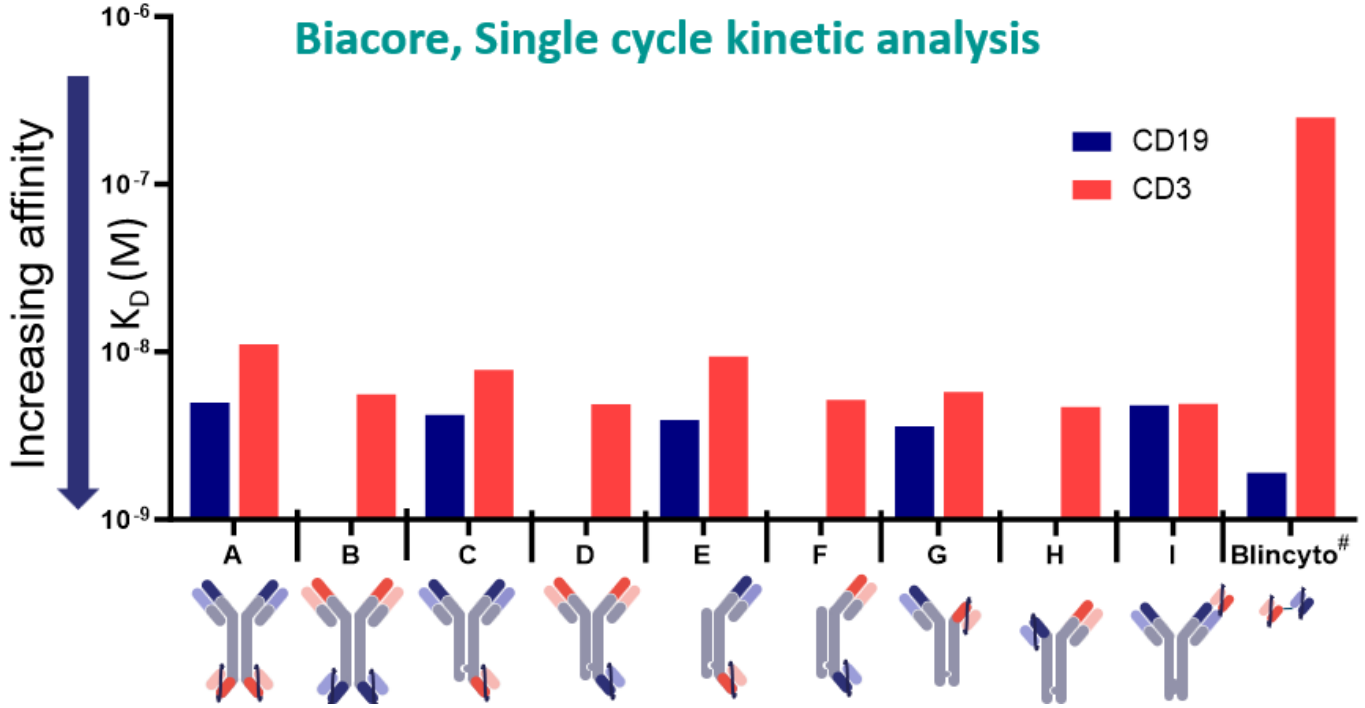



| Attribute | Aim | Attribute | Aim | Attribute | Aim |
|---|---|---|---|---|---|
| **Purity** | High, after simple purification | **Target Affinity** | High affinity to both targets | **Biological function** | T cell recruitment and killing |
| **Yield** | High from standard CHO expression | **Stability** | Limited chain mispairing | **Safety** | Limited cytokine activation |

# Assessing the functionality and safety of bispecifics

## SPR binding assay



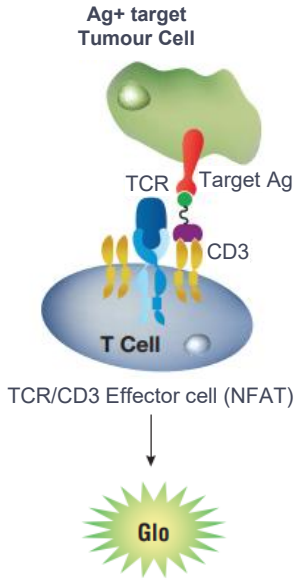




**Observations**

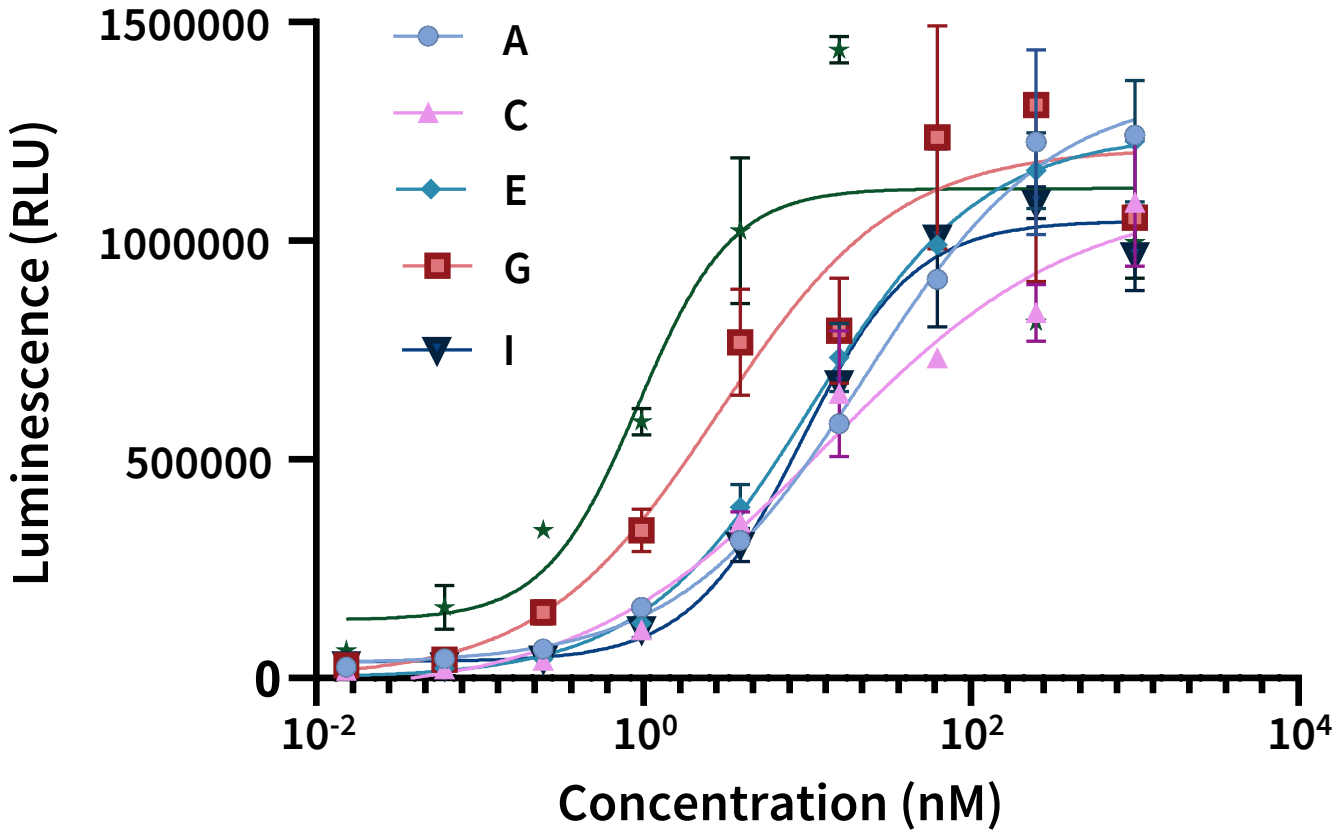
- SPR analysis shows a clear distinction between designs. All anti-CD19 scFv designs showed no detectable binding, suggesting reformatting to scFv has been detrimental.
- All other designs showed strong binding to both antigens. All anti-CD19 scFv designs were eliminated from further study.

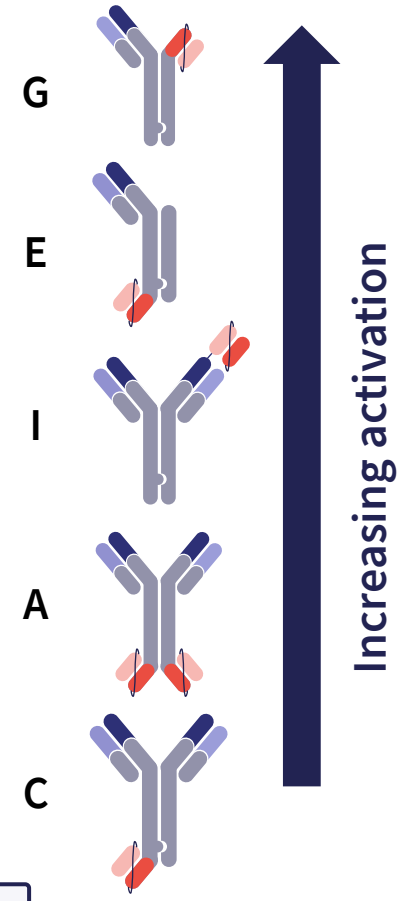
# Assessing the functionality and safety of bispecifics

## T cell activation assay to determine bispecific functionality




A T cell Activation Bioassay using Tumour antigen positive target cells and TCR/CD3 effector cells (NFAT) was used to measure the activity of all bispecific designs.




**Observations**

- Of the five designs tested, one construct (G) shows a strong reporter activity with the remaining four showing slightly weaker, but still significant activity.

# Assessing the functionality and safety of bispecifics

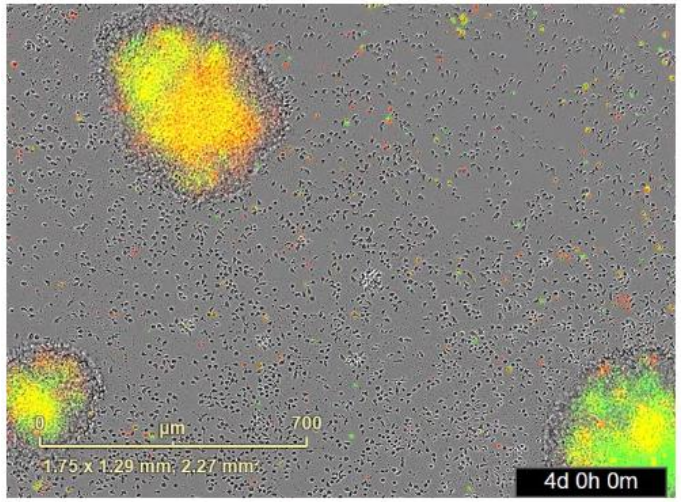
## Assessing cancer cell killing by PBMCs using Incucyte® live cell imaging

### Assay setup

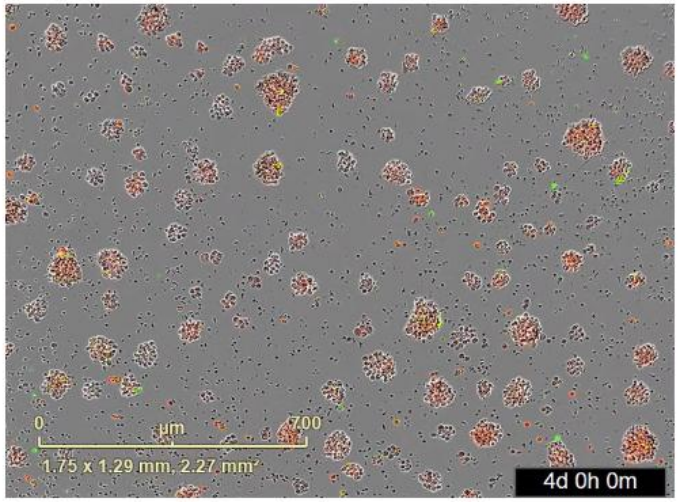
- Cytolight Red-stained target cells are seeded in the presence of purified human PBMCs, Cytotox Green reagent (indicator of dead cells) and test antibodies.
- Plate is monitored using the Incucyte® live cell imaging system for 96 hours.

### Results

- The positive control shows cancer cell death (red turning yellow) and immune cell clustering/proliferation
- Negative control shows healthy proliferation of the red cancer cells.




Positive Control




Negative Control

**Imaging of Cytolight Red stained target cells in the presence of PBMC, Cytotox Green reagent and test antibodies 10nM.**

# Assessing the functionality and safety of bispecifics

## Assessing cancer cell killing by PBMCs using Incucyte® live cell imaging

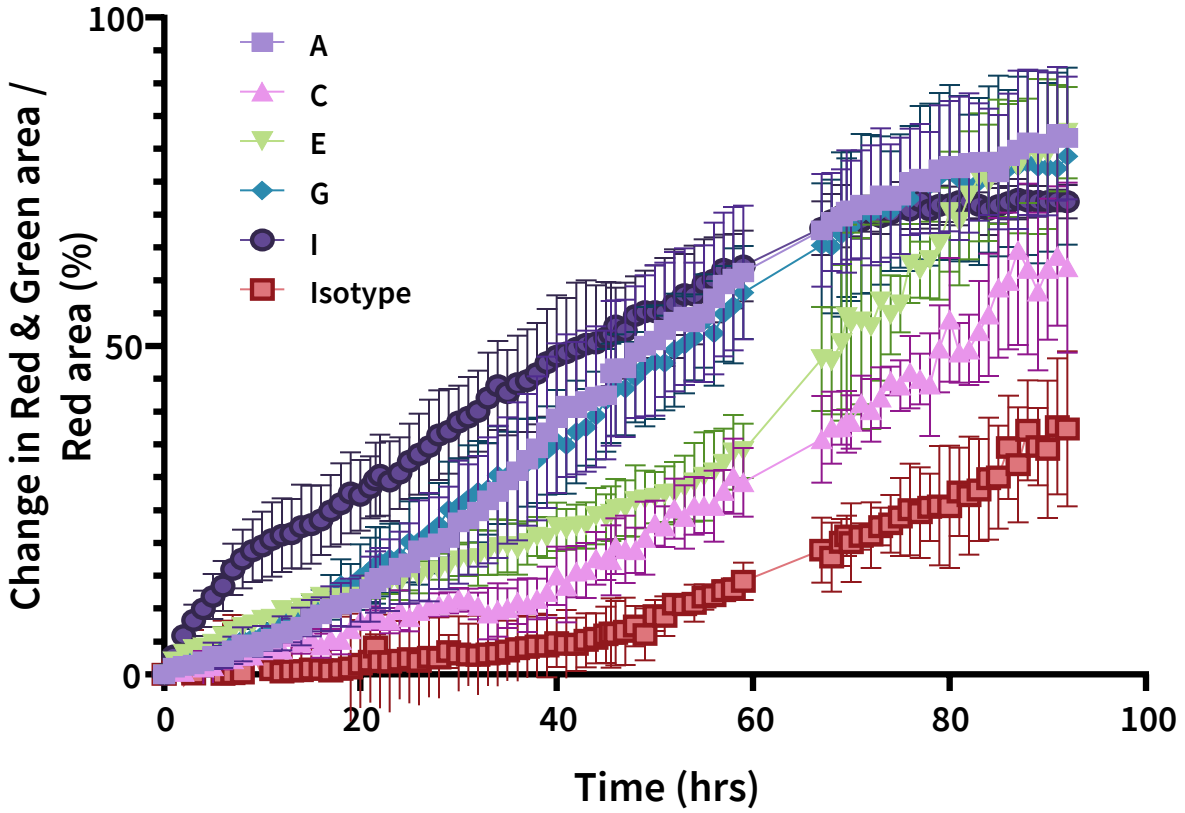
### Method

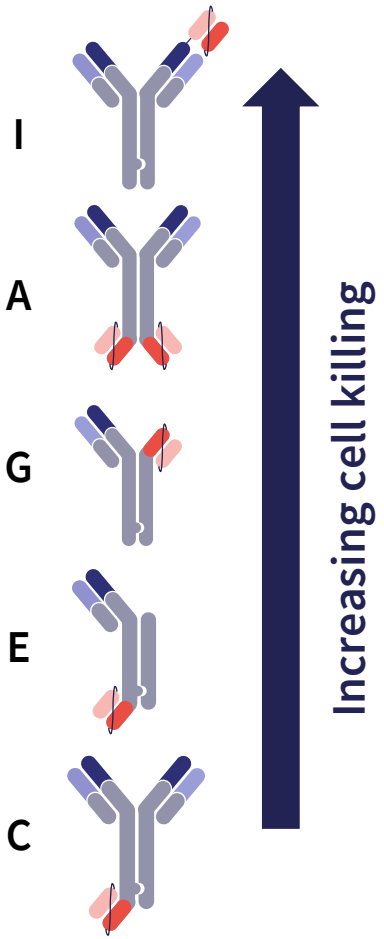
Purified human PMBCs, Cytolight Red-stained target cells, Cytotox Green reagent (indicator for dead cells) and test bispecifics were incubated for 96 hours and monitored using an Incucyte® live cell imaging system.

As T cells are recruited and cancer cells killed, the red and green stains mix, the production of a yellow colour is monitored and is shown in the figure above.

### Observations

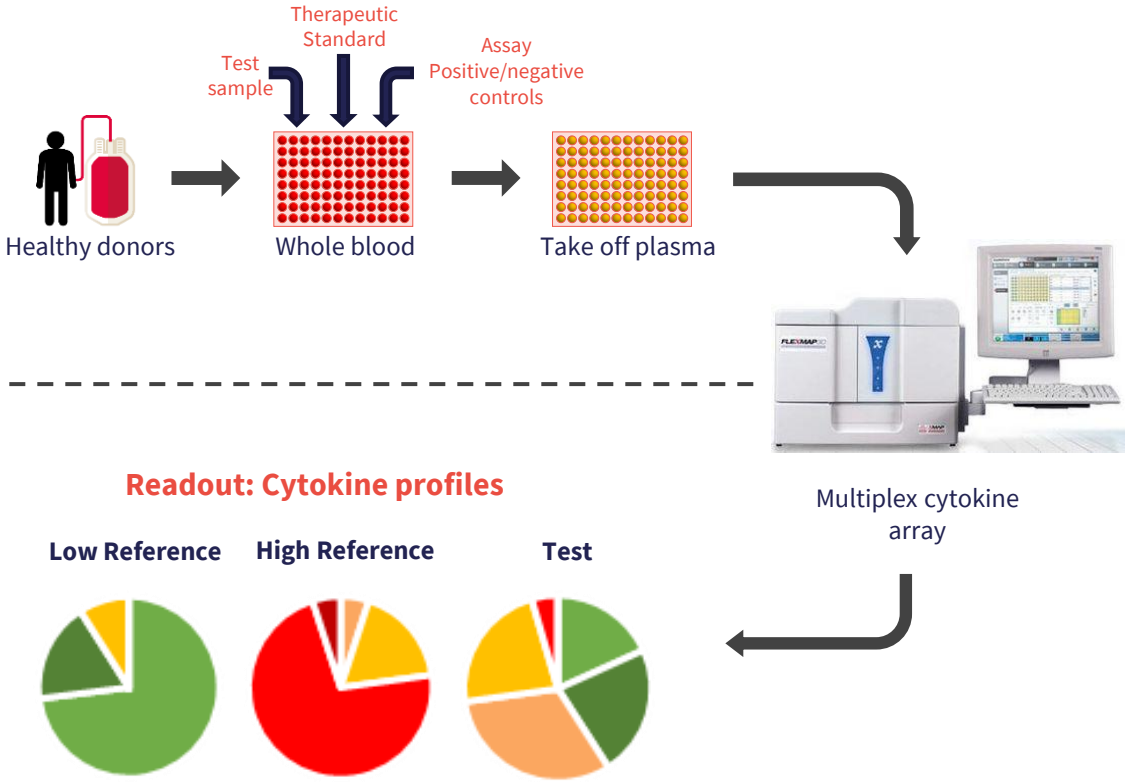
- Of the five designs tested, three show a strong ability to recruit and kill cancer cells (Designs I, A and G).
- Two designs showed limited recruitment activity (E and C).



# Assessing the functionality and safety of bispecifics

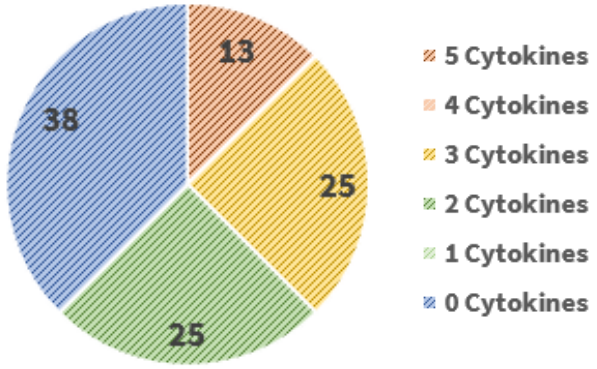
## Assessing the risk of CRS using Cytokine Screen® assay




**Whole blood cultured with samples at 50, 5 and 0.5 nM**

**After 24 hours, supernatants collected, and cytokine concentration determined by Luminex**

**Cytokine panel: IL-6, IL-8, IL-10, IFNγ and TNFα**

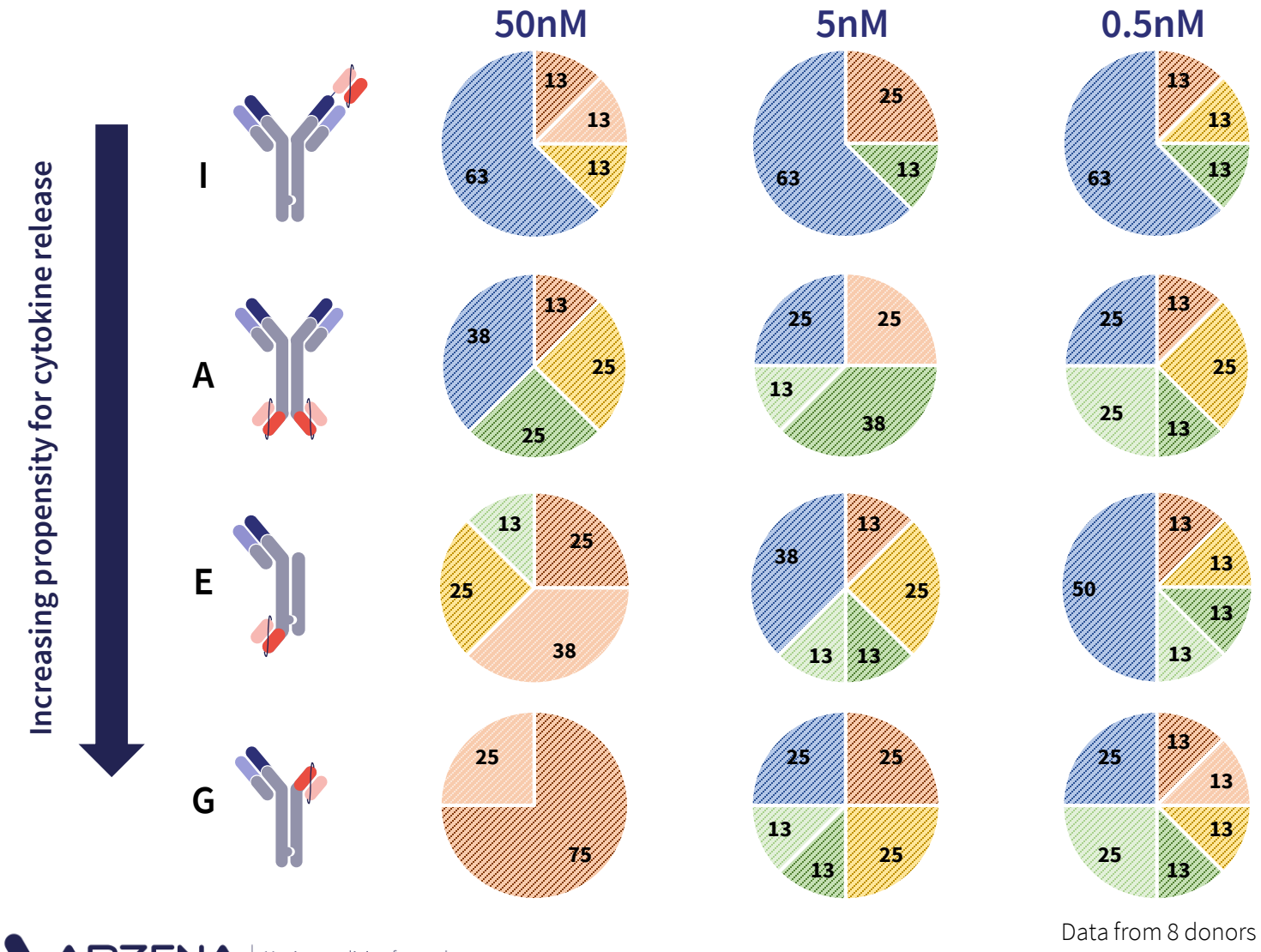



**Example result:**

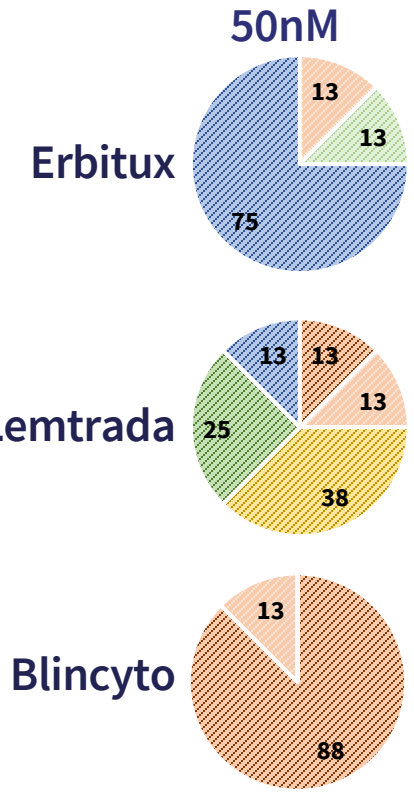
Summary of the number of cytokines detected from each donor expressed as a percentage

# Assessing the functionality and safety of bispecifics

## Whole blood Cytokine Screen® assay




Data from 8 donors




**Summary**

From *in-vitro* assessment and characterization, two formats (**Designs A** and **I**) were identified as strong T cell engagers with low propensity for cytokine release and are suggested as suitable candidates for further *in-vivo* studies

ABZENA

# Case Study #2

Modelling Complex Cell Environments *In vitro* for ADC Lead Selection

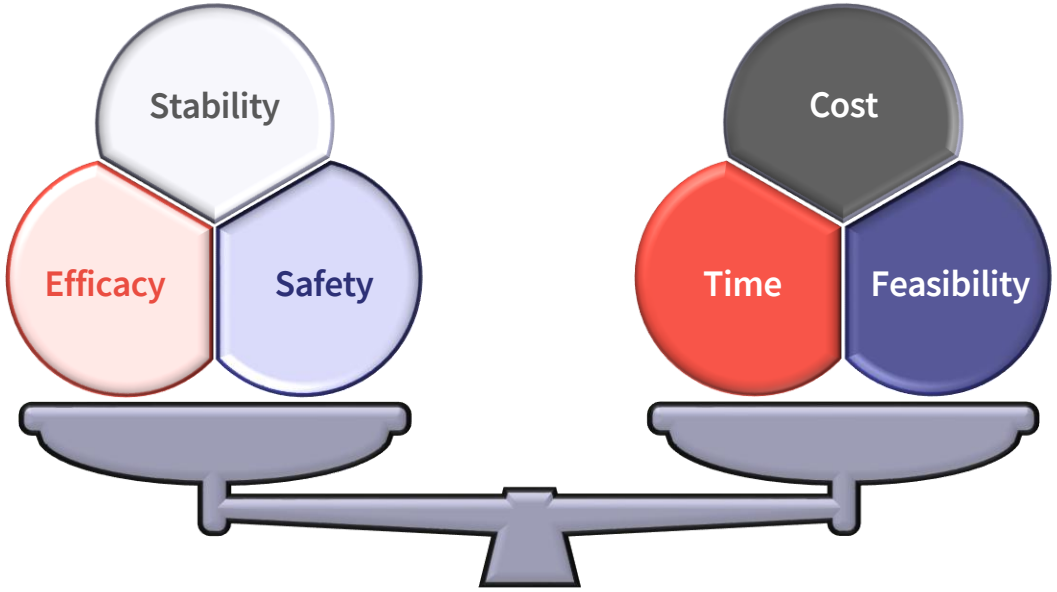
# How can *in vitro* bioassays inform clinical success?

## Benefits of early testing

Bioassays have evolved greatly over the years, but there is still some skepticism - do these results correlate with clinical outcomes?

### Benefits of early *in vitro* testing are multiple:

- ✓ Quicker timelines
- ✓ Cheaper solutions
- ✓ Reducing the need for animal studies
- ✓ Better decision making early on to increase chance of success in the clinic



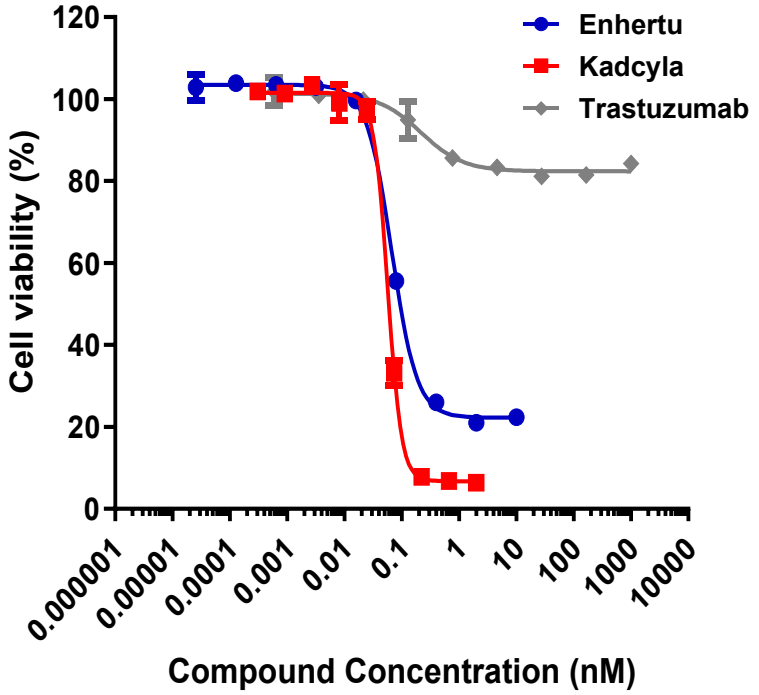

Our case study on Kadcyla and Enhertu demonstrates the amount of valuable information that can be obtained *in vitro,* and how this allows you to start smart and finish fast

# Modelling complex cell environments *in vitro* for ADC lead selection

## Case Study - The story of two Her2-targeting ADCs, Kadcyla and Enhertu

| Kadcyla® ado-trastuzumab emtansine 20 mg/mL INJECTION FOR INTRAVENOUS USE  | |  ENHERTU® fam-trastuzumab deruxtecan-nxki 20 mg/mL INJECTION FOR INTRAVENOUS USE |
|---|---|---|
| **2013** | | **2019** |
| Trastuzumab | **mAb** | Trastuzumab |
| DM1 (tubulin polymerization inhibitor) | **Toxin** | DXd (topoisomerase I inhibitor) |
| Non-cleavable | **Release mechanism** | Enzymatic |
| av. 3.5 | **DAR** | 8 |
| No | **Clinical efficacy on heterogenous tumours** | Yes |

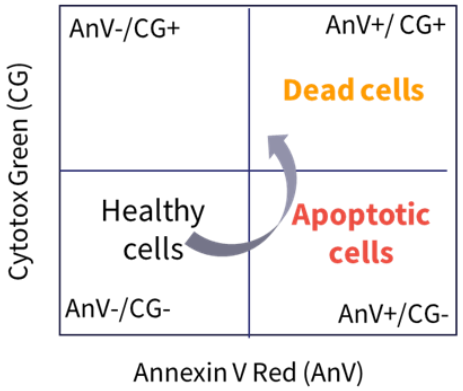
CellTiterGlo® assay using a target positive cell line, after 96h co-incubation

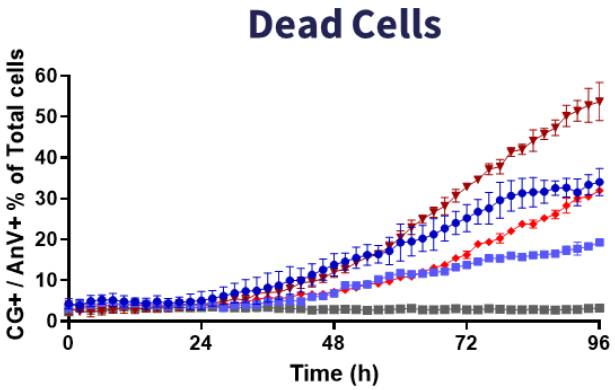


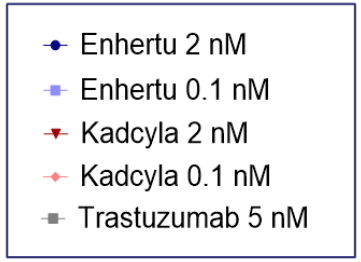

- Potencies are similar, slightly better max. cell killing for Kadcyla

# Modelling complex cell environments *in vitro* for ADC lead selection

## Assessing Kinetics – Real-Time Imaging



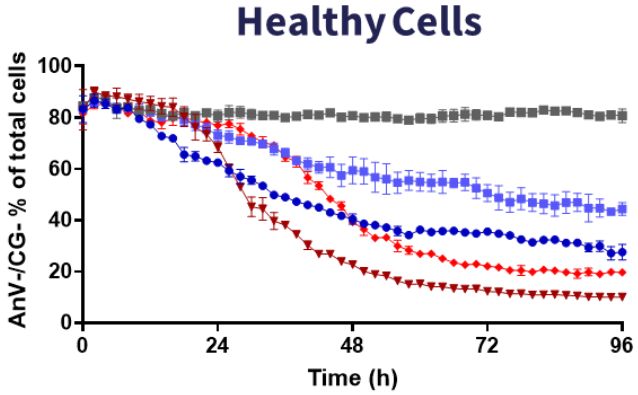



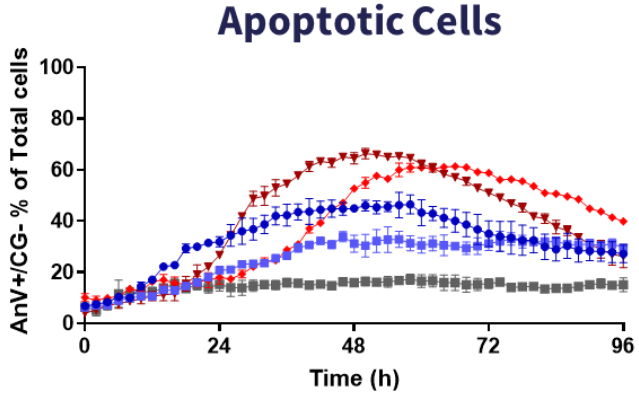




**Readout**

**Annexin V Red -** Apoptosis
**Cytotox Green –** Cytotoxicity

Incucyte® S3 Live cell Imaging System (Sartorius)



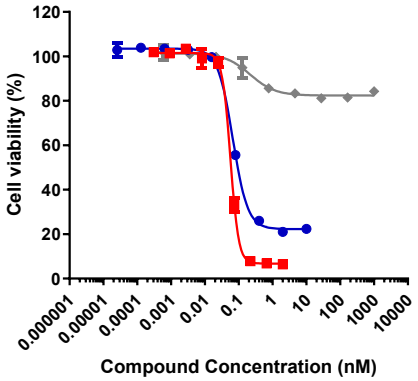




- Cells treated with Enhertu enter apoptosis earlier.
- While the response is delayed for Kadcyla, it displays an increased level of cell killing.
- Results are **consistent with learnings from the endpoint readout, but add additional insight into the MoA**.

# Modelling complex cell environments *in vitro* for ADC lead selection

## Which ADC is the more promising lead?

### Endpoint single cultures



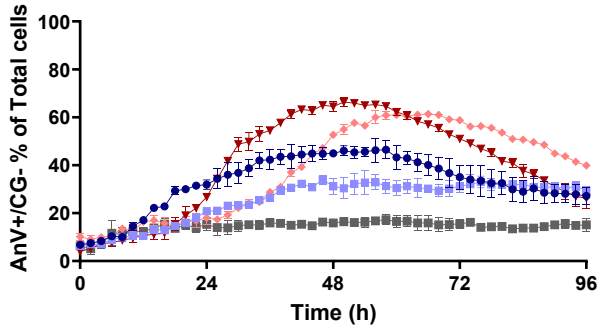

Kadcyla ≥ Enhertu

(+) Higher max. cell kill

Kadcyla ≥ Enhertu

(+) Increased cell kill

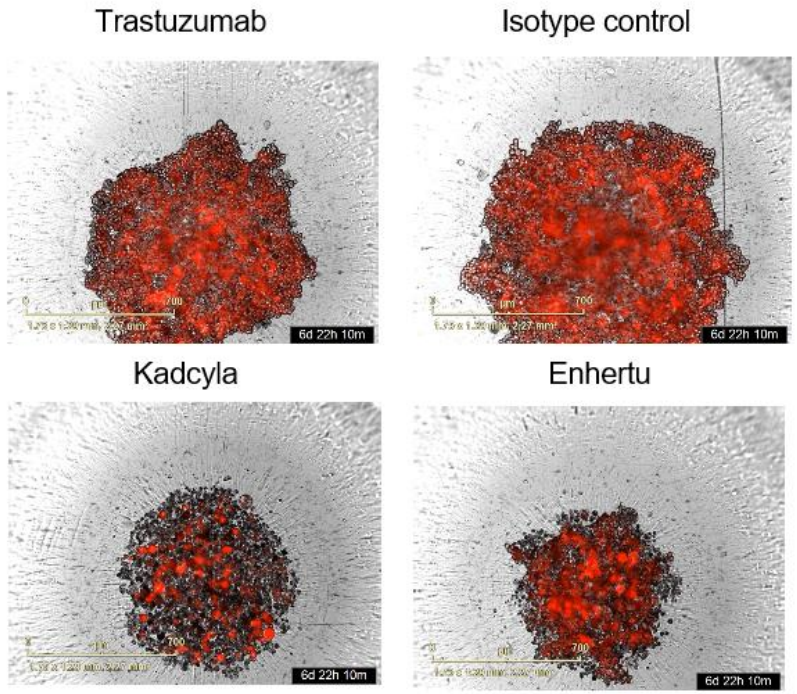
### Real-time single cultures



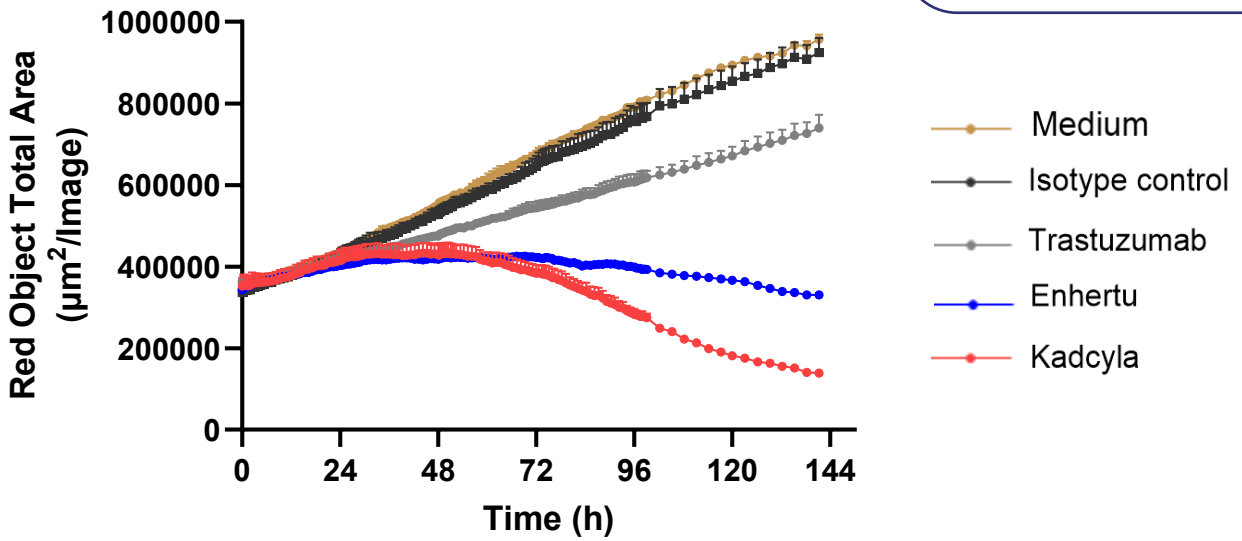

What happens in a model mimicking solid tumours?

# Modelling complex cell environments *in vitro* for ADC lead selection

## Modelling Solid Tumors – 3D Spheroid Assays




Her2+ cells – Controls and ADC 6.8 nM




**Readout**

**CytoLight Red**

Incucyte® S3 Live cell Imaging System (Sartorius)

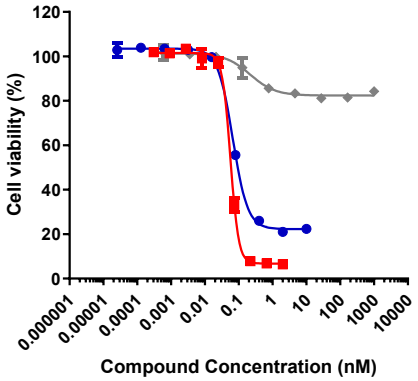
- **Her2+ spheroids are killed** by both Kadcyla and Enhertu after 48 h. Kadcyla results in more pronounced spheroid shrinkage.

$IC_{50}$ and kinetics are shifted compared to 2D cultures, spheroids are 'harder' to kill

# Modelling complex cell environments *in vitro* for ADC lead selection

## Which ADC is the more promising lead?

### Endpoint single cultures




Kadcyla ≥ Enhertu

(+) Higher max. cell kill

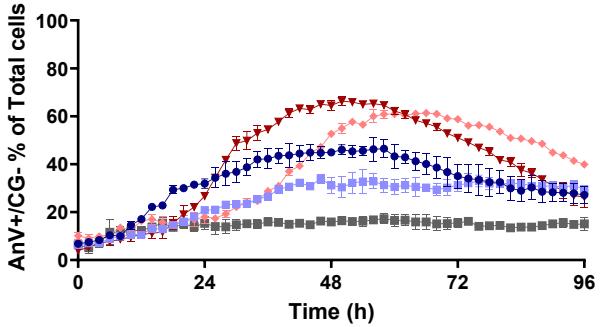
Kadcyla ≥ Enhertu

(+) Increased cells kill

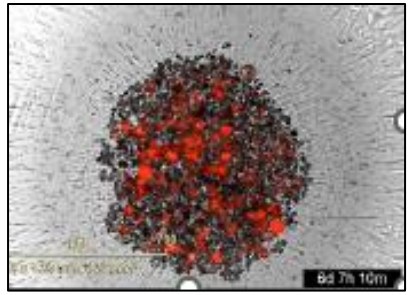
Kadcyla > Enhertu

(+) More pronounced spheroids shrinkage

### Real-time single cultures



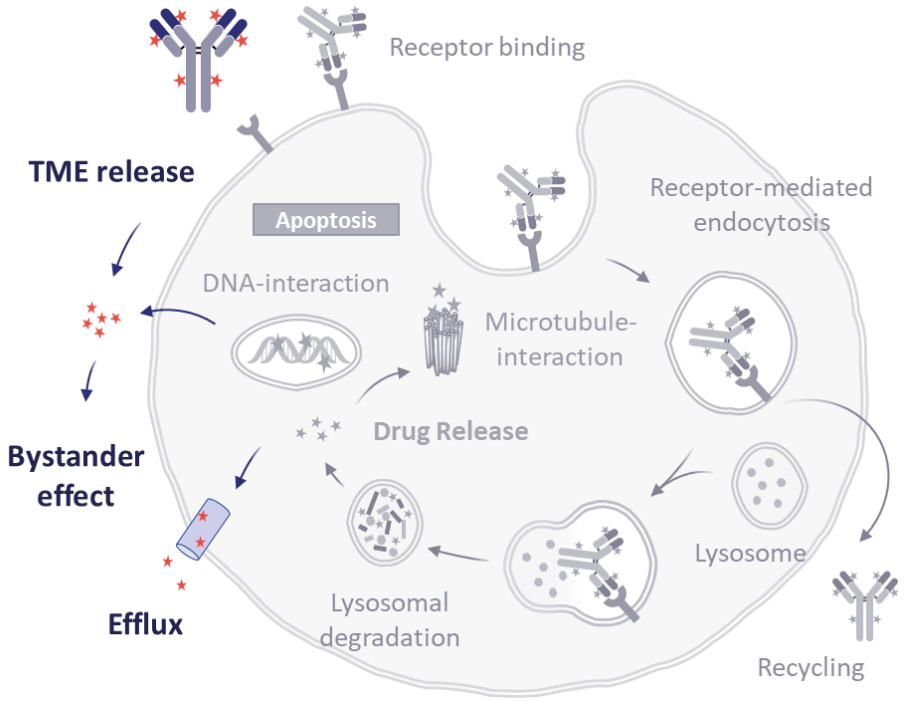

### 3D spheroid cultures



What happens in a model mimicking a heterogenous tumour?

# Modelling complex cell environments *in vitro* for ADC lead selection

## Modelling Heterogenous Tumors and the Bystander Effect




The ADC impacting neighboring antigen-negative cells through payload release from the antigen-positive cells

### Abzena's bystander assay

- ✓ Real co-culture
- ✓ Real-time
- ✓ Monitors both Target$^{POS}$ and Target$^{NEG}$ cell line

### Readout

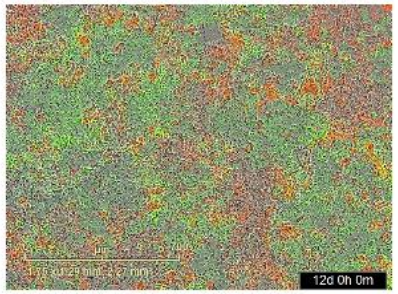
Cytolight Red (Her2$^{POS}$) or Green (Her2$^{NEG}$)

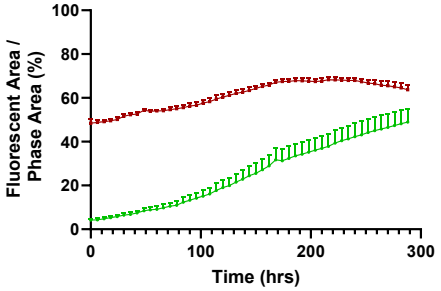
Incucyte® S3 Live cell Imaging System (Sartorius)

# Modelling Complex Cell Environments *In vitro* for ADC Lead Selection

## Assessment of Cell Killing in Co-Cultures - Bystander Assay

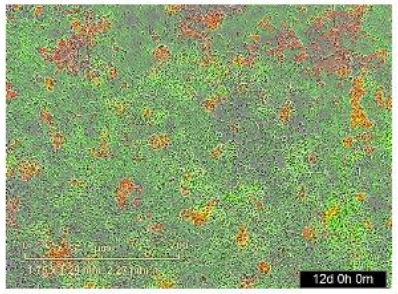
### No treatment

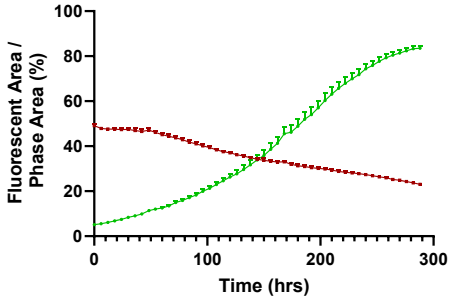



Both cell lines proliferate

### Trastuzumab

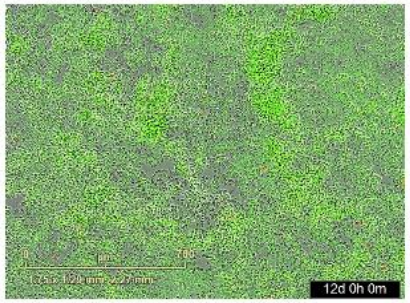


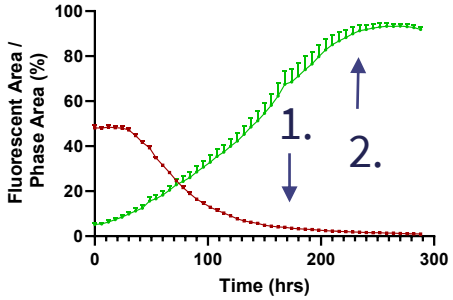




Her2$^{POS}$ proliferate at a slower rate

Her2$^{NEG}$ proliferate as normal

### Kadcyla

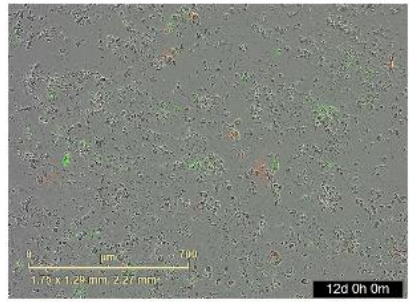


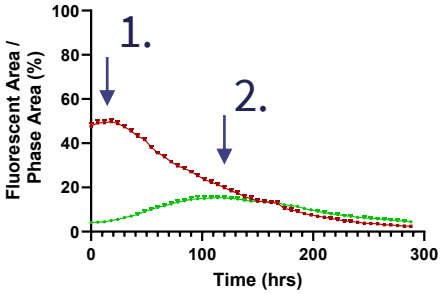




Her2$^{POS}$ die (1)

Her2$^{NEG}$ proliferate into the free space (2)

### Enhertu




Her2$^{POS}$ die (1)

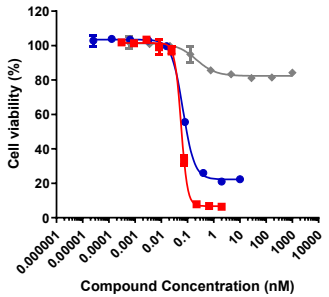
then Her2$^{NEG}$ die (2)

**Bystander Effect**

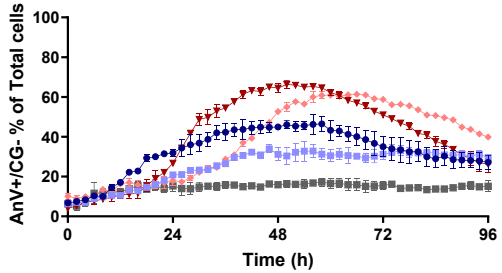
# Modelling Complex Cell Environments *In vitro* for ADC Lead Selection

## Which ADC is the More Promising Lead?

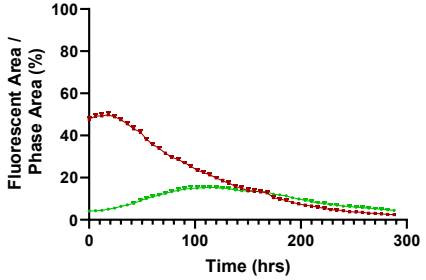
### Endpoint single cultures



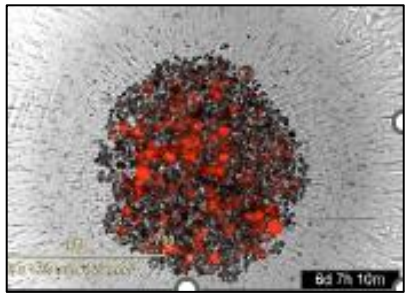

### Real-time single cultures




### Co-cultures




### 3D spheroid cultures



Kadcyla ≥ Enhertu

(+) Higher max. cell kill

Kadcyla ≥ Enhertu

(+) Increased cells kill

Enhertu > Kadcyla for heterogenous tumours

(+) Bystander effect

**Lead ADC candidate**

Kadcyla > Enhertu

(+) More pronounced spheroids shrinkage

Adding the immune component

ABZENA

# Case Study #3

Assessment of Payload and Immune-Mediated Killing in a Single Assay

# Assessment of payload and immune-mediated killing in a single assay

## Kadcyla and Enhertu have complex MoAs:

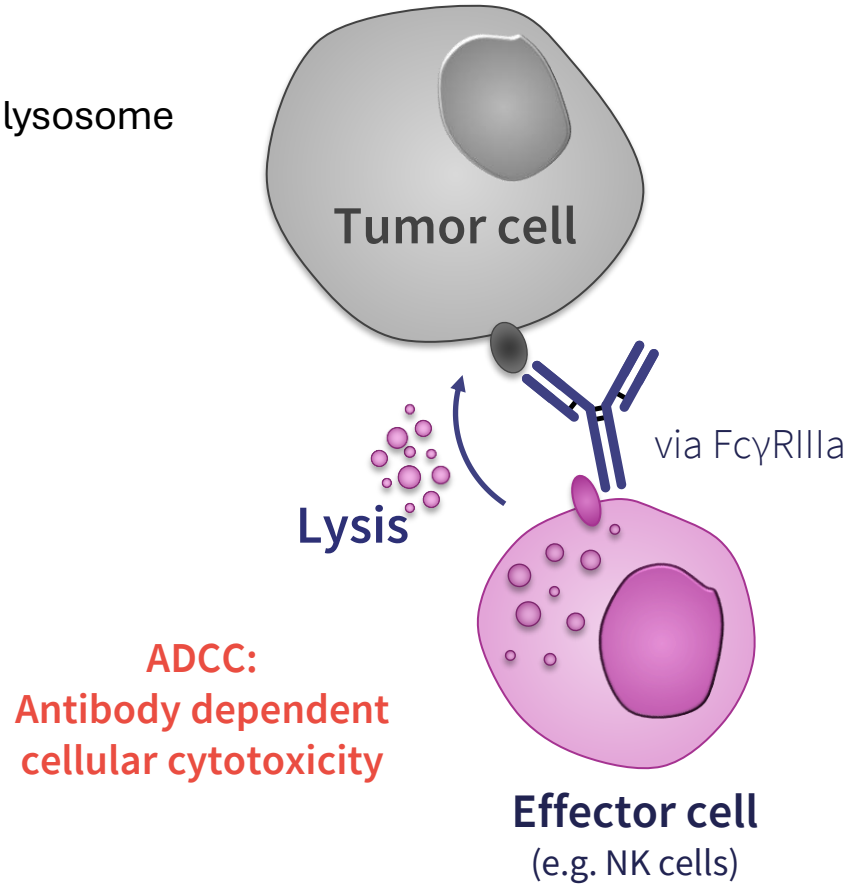
- ✓ Within hours, they elicit ADCC on target cells if immune effectors are present
- ✓ Within days, the payload exerts its killing activity via internalization through the lysosome

## Standard *in vitro* assays will assess these processes individually:

- ✓ E.g., ADCC assay with effector cells up to 4-6 hours
- ✓ Endpoint cytotoxicity readout after 96 hours

## They also fail to capture complexities such as:

- ✓ Kinetics of cell killing
- ✓ Solid tumours
- ✓ Heterogenous tumours
- ✓ Presence or absence of immune cells within these environments



# Assessment of payload and immune-mediated killing in a single assay

## Experimental workflow

**Day 1**

**Spheroid formation**

**Cytolight Red Her2$^{POS}$** and **Green Her2$^{NEG}$** cells seeded in 384w ULA spheroid plates and incubated @ 5% $CO_2$, 37°C

**Day 4**

**Target opsonisation**

Spheroids treated with a titration of test samples

**Day 4**

**Effector Addition**

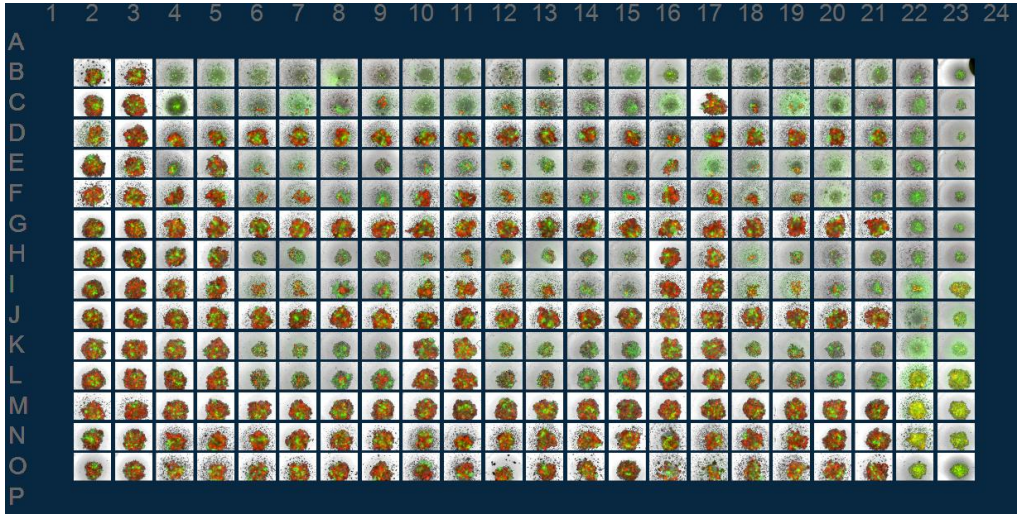
PBMC effectors were added to the opsonised cells at various E:T ratios and incubated @ 5% $CO_2$, 37°C

**Days 4-12**

**Data Acquisition**

Data acquired using Incucyte Live Cell Imaging system

Images collected every 3 hours




Endpoint images

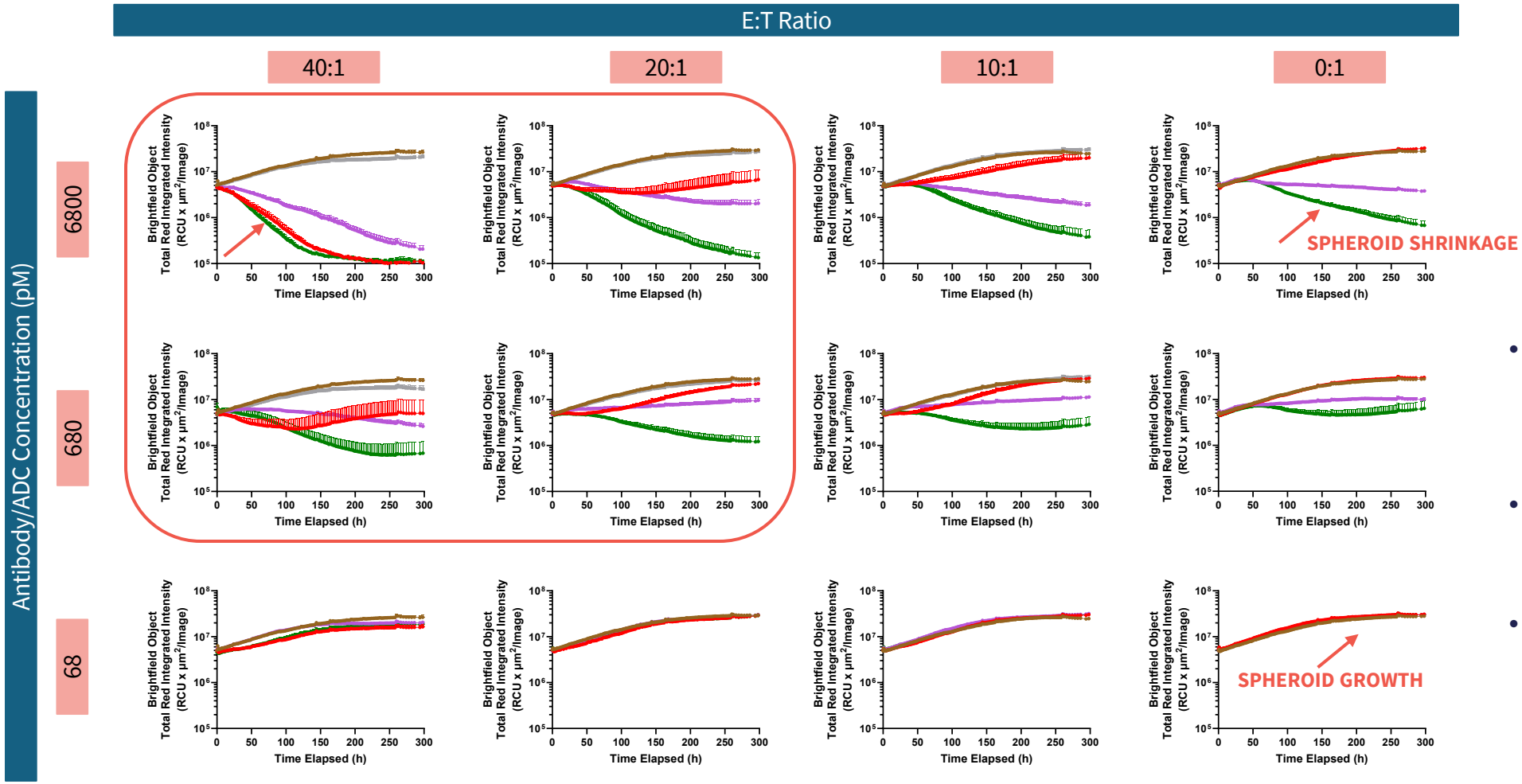
- 2 cell types
- 5 treatments
- 3 concentrations
- 3 healthy PBMC donors
- 4 Effector-to-Target ratios
- 146 scans/timepoints

↓

**Huge dataset from just one plate, resembling various tumour/treatment scenarios**

# Assessment of payload and immune-mediated killing in a single assay

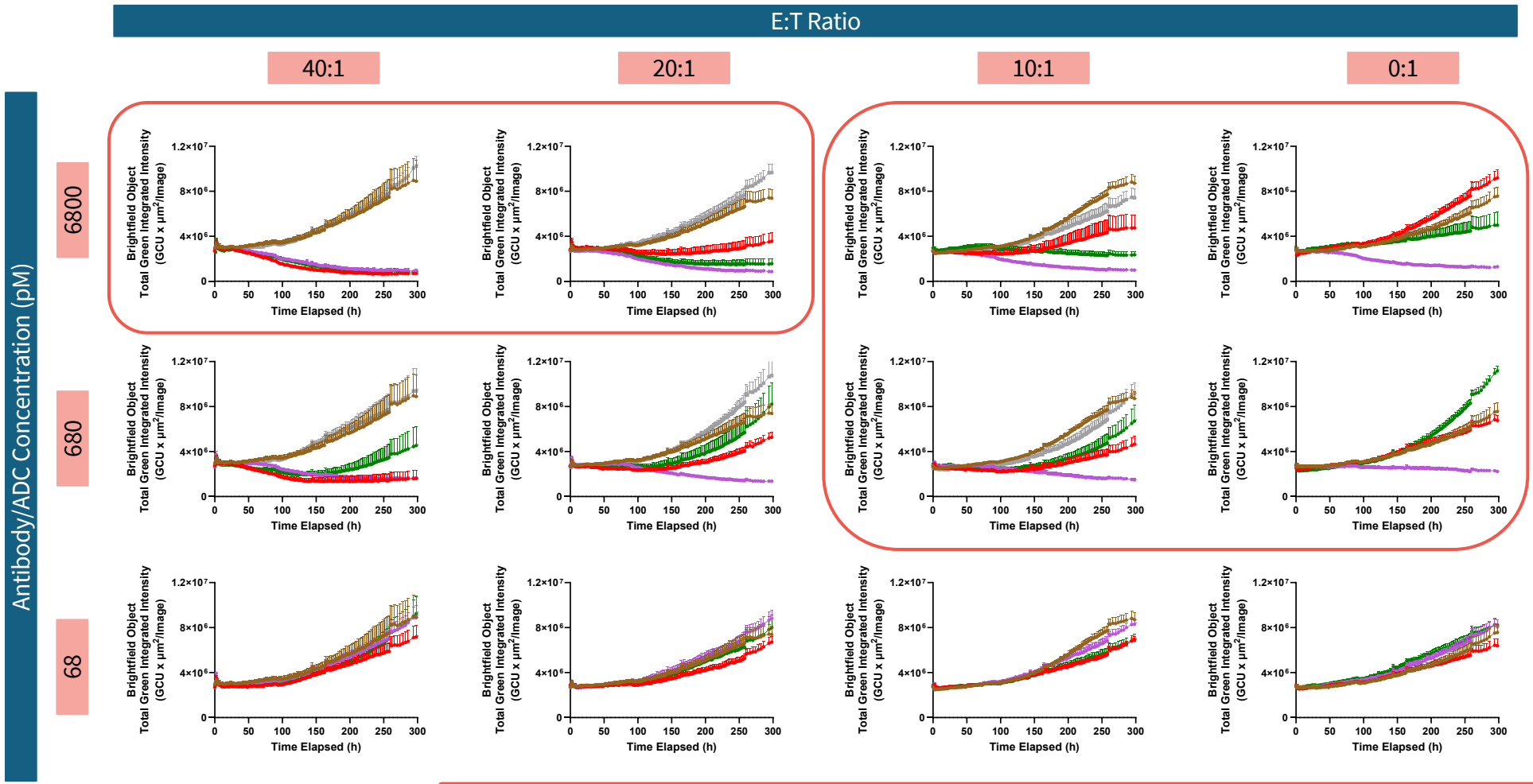
## Summary Data: Her2+ cells




- Dose-dependent killing observed with both ADCs, Kadcyla being more effective
- Trastuzumab only active in the presence of immune cells
- Immune-mediated killing is a quick and effective way of spheroid shrinkage

# Assessment of payload and immune-mediated killing in a single assay

## Summary Data: Her2- cells

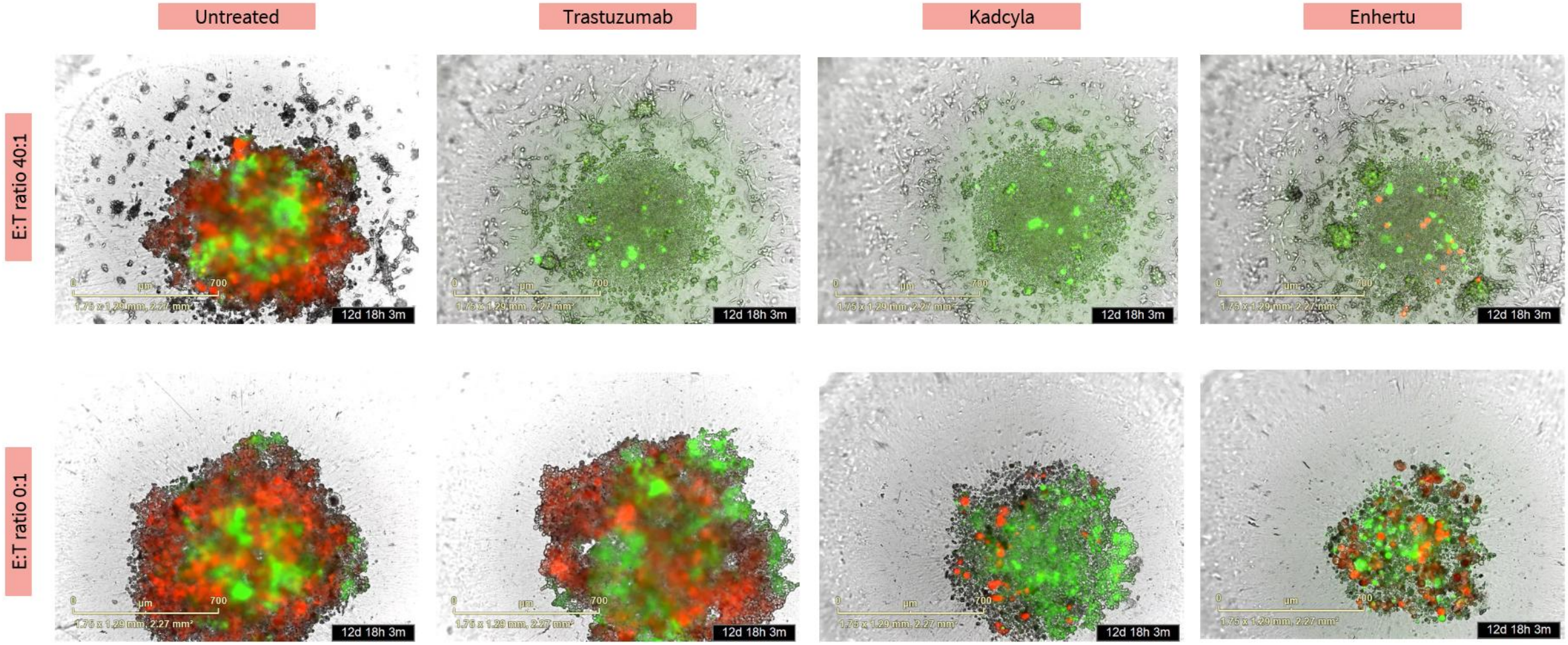



- Dose- and E:T ratio-dependent effects observed, but different as in case of the Her2+ population
- Enhertu demonstrates a clear bystander effect at lower E:T ratios
- At higher E:T ratios, where significant immune activation is observed, all targeted biologics lead to effective elimination of the Her2- cell population

**Predictions can be made on performance in various tumour environments (including immunologically 'hot' and 'cold' tumours)**

# Assessment of payload and immune-mediated killing in a single assay

## Representative endpoint images at 6800 pM



# Summary

- Holistic assessment of efficacy, safety and manufacturability is crucial to maximize success before progressing a drug into the clinic.
- Selecting the right assay for every stage tailored to specific modality and MoA is key.
- Imaging-based techniques are particularly valuable in the lead selection & characterization stage.
- Using *in vitro* assays to model complex environments allows for better lead selection with greater chance of clinical success.

ABZENA

# Thank you to the Bioassay and whole Abzena team

Thomas Cornell
Timothy Wood
Katie Welch
Rob Holgate
Grant Harradence
Robert J. Francis
Johanna Midelet
Yasmin Noble
Rosa Gonzalez-Serrano

ABZENA

Let's move medicines forward together!