

# In vitro real-time evaluation of bystander effects of antibody-drug conjugates

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## Introduction

The bystander effect is a critical phenomenon associated with antibody-drug conjugates (ADCs), where cytotoxic drugs released from lysed cancer cells diffuse into neighboring tumor cells, enhancing therapeutic efficacy—particularly in tumors with heterogeneous antigen expression.<sup>1</sup> Despite its significance, methods for detecting and evaluating the bystander effect of ADCs remain under development.

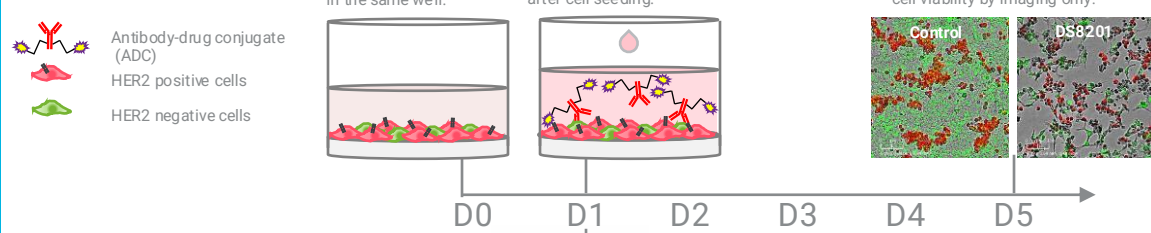
Here, we present two effective methodologies, the in vitro coculture assay and the conditioned medium transfer assay,<sup>2</sup> for monitoring and quantifying the bystander effect of ADCs in real-time using live-cell imaging and impedance measurements with the Agilent xCELLigence RTCA eSight system.

These approaches provide valuable tools for optimizing the efficacy, safety, and pharmacokinetics of therapeutic ADCs during biopharmaceutical development.

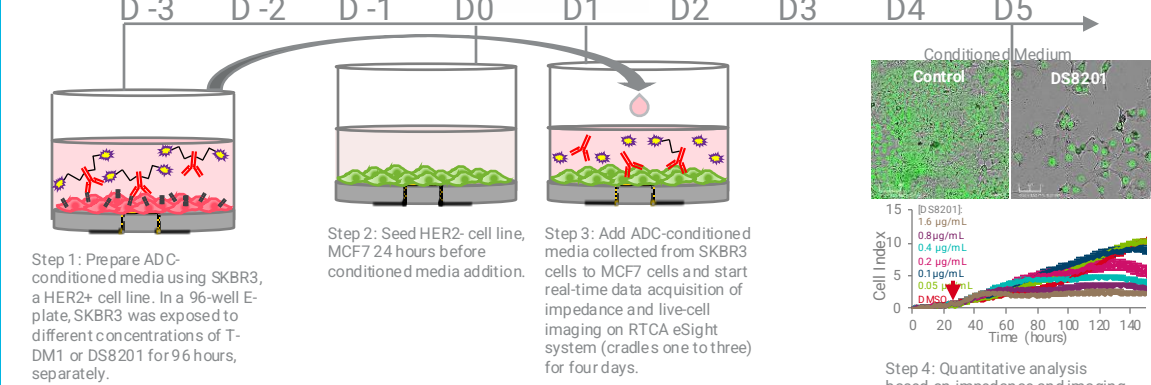
## Experimental

### Workflow for the assessment of bystander effects of ADC using the xCELLigence RTCA eSight system (RTCA eSight)

#### Coculture assay



#### Conditioned media transfer assay



**Coculture assay:** HER2-positive SKBR3-RFP cells and HER2-negative MCF7-GFP cells were co-seeded in a 96-well plate (Corning, #3599) on the day before testing. Following the addition of DS8201 and T-DM1 to the cells the next day, live-cell imaging was acquired on the RTCA eSight for 96 hours.

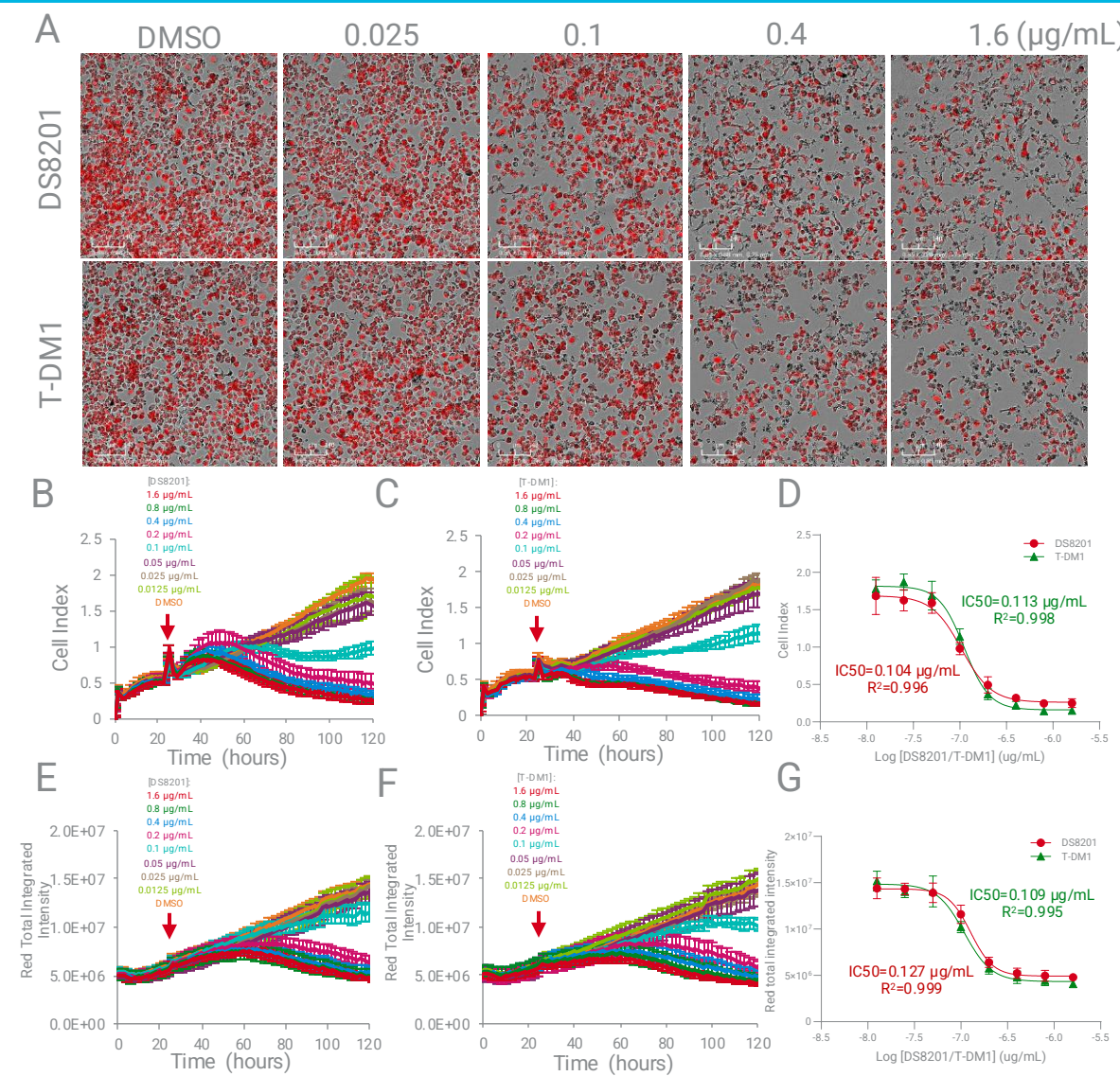
**Conditioned medium transfer assay:** The media from SKBR3 cells treated with DS8201 or T-DM1 for 96 hours were transferred to HER2-positive MCF7-GFP cells seeded 24 hours earlier in the E-Plate VIEW 96 (Agilent, #300601010). Viability of MCF 7-GFP was then monitored and quantified in real-time by imaging and impedance measurements on the RTCA eSight.

## References

1. Staudacher AH, Brown MP. Antibody drug conjugates and bystander killing: is antigen-dependent internalization required? *Br J Cancer*. 2017, 117 (12),1736-1742. DOI:10.1038/bjc.2017.367
2. Szot C, Saha S, Zhang XM, et al. Tumor stroma-targeted antibody-drug conjugate triggers localized anticancer drug release. *J Clin Invest*. 2018, 128 (7), 2927-2943. DOI:10.1172/JCI120481

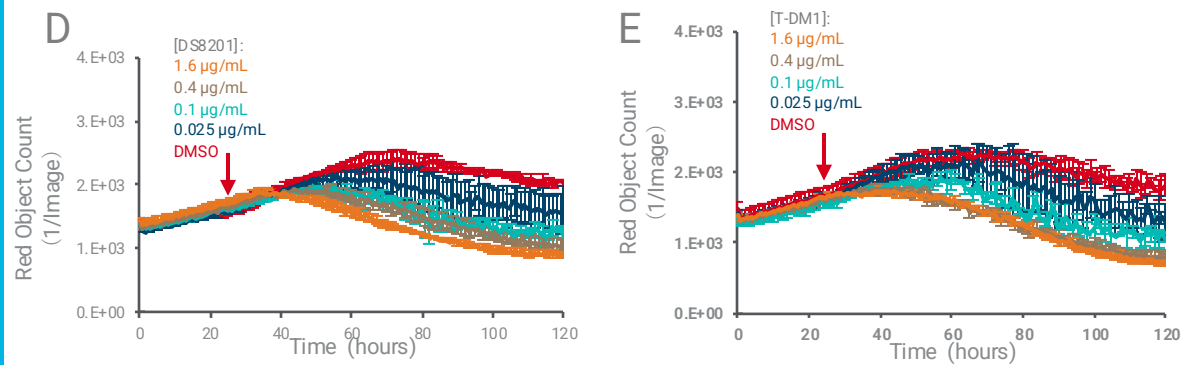
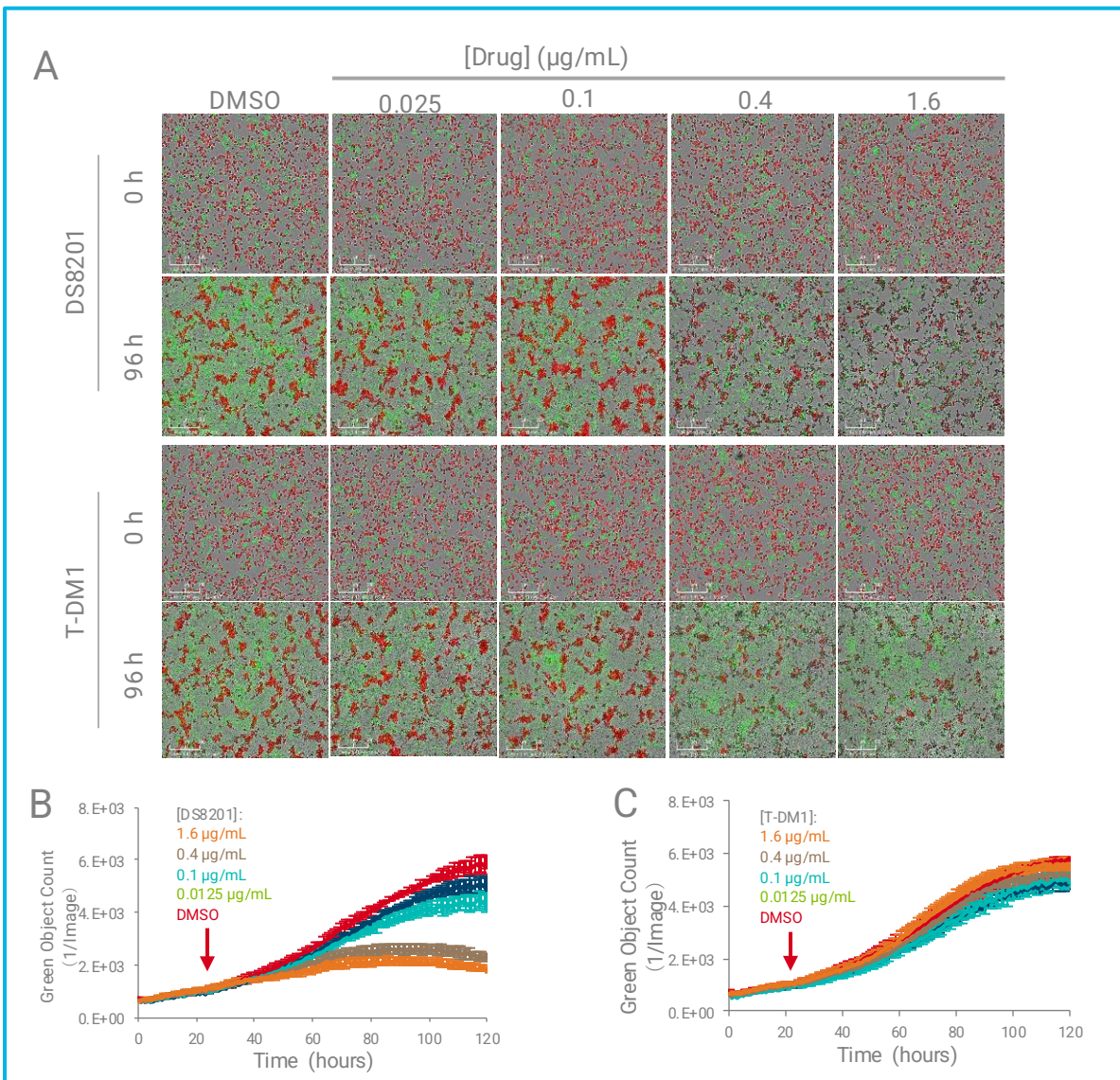
## Results and Discussion

### Assessment of cytotoxicity of ADCs against HER2+ cell line SKBR3 using impedance and imaging



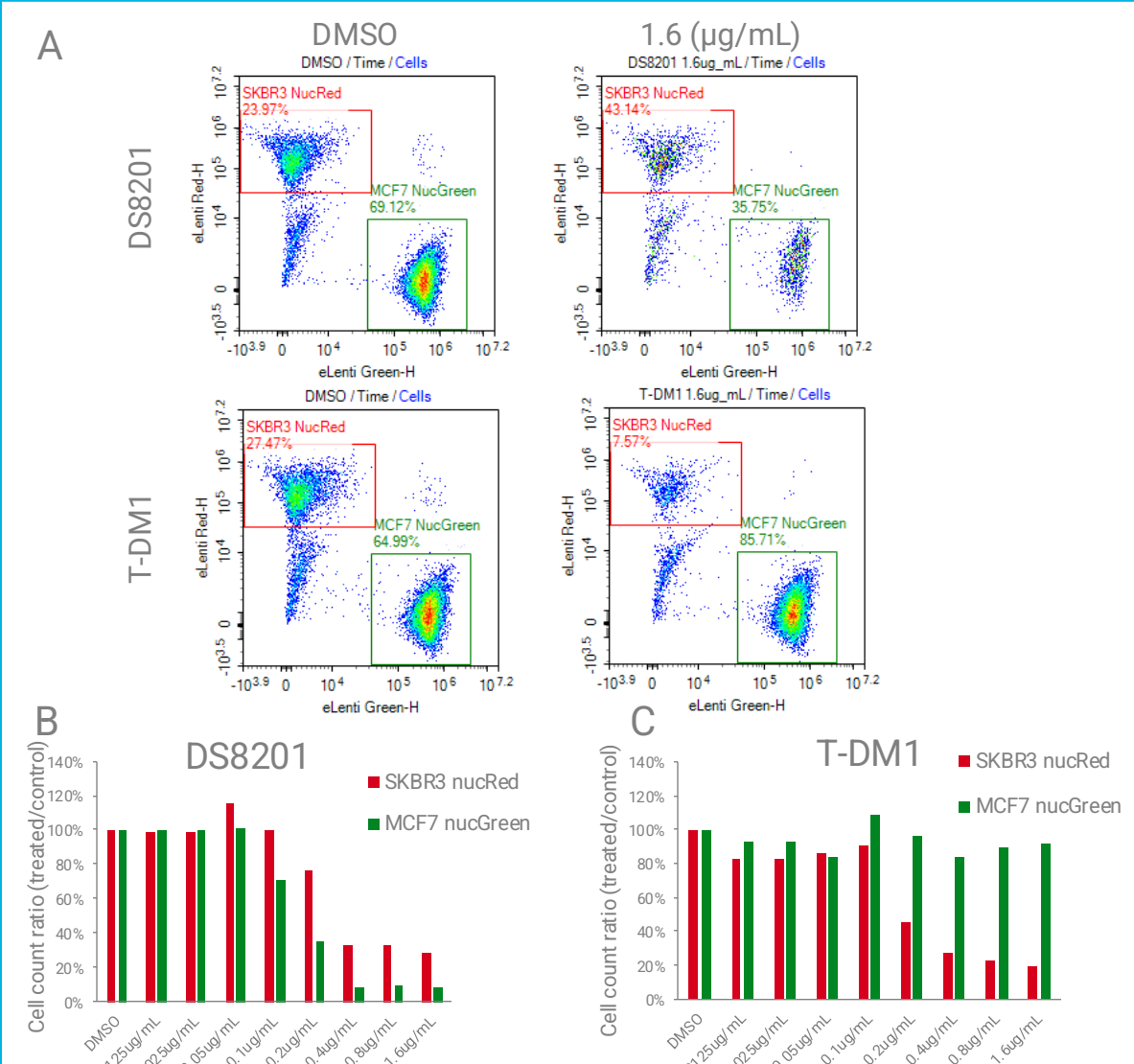
**Figure 1. Measurement of ADC-induced cytotoxicity using the xCELLigence RTCA eSight system.** (A) Representative Images of SKBR3-RFP cells treated with various concentrations of DS8201 and T-DM1. The changes in Cell Index over time after the treatment with DS8201 (B) and T-DM1 (C). (D) Dose-response curves from B and C. Red total integrated intensity varying over time for DS8201 (E) and T-DM1 (F). (G) Dose-response curves of red total integrated intensity at 96 hours postdrug. Impedance data is comparable with the imaging data.

### Evaluation of bystander killing effect of two ADCs on HER2-positive and HER2-negative cells using coculture



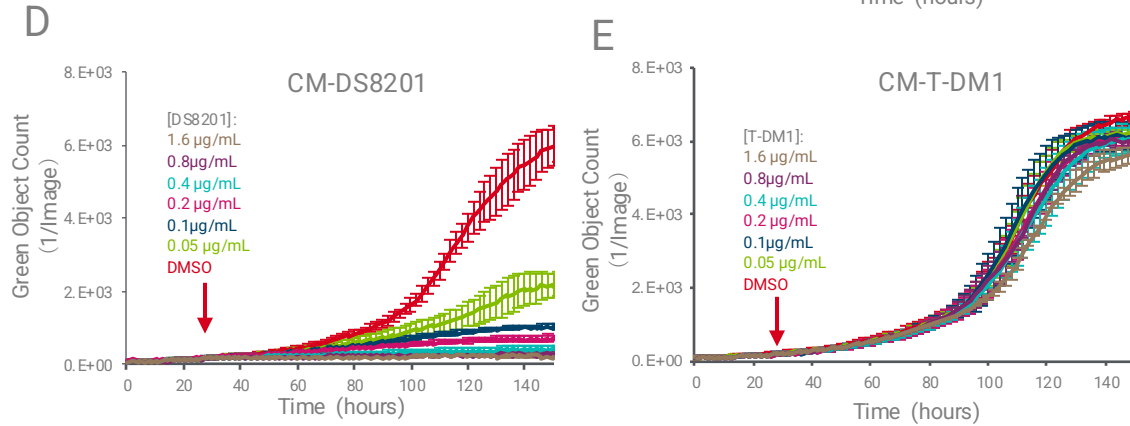
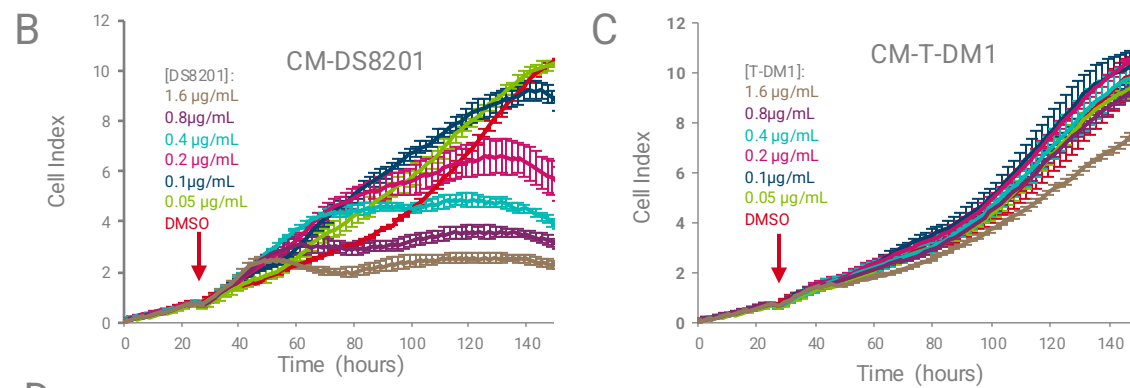
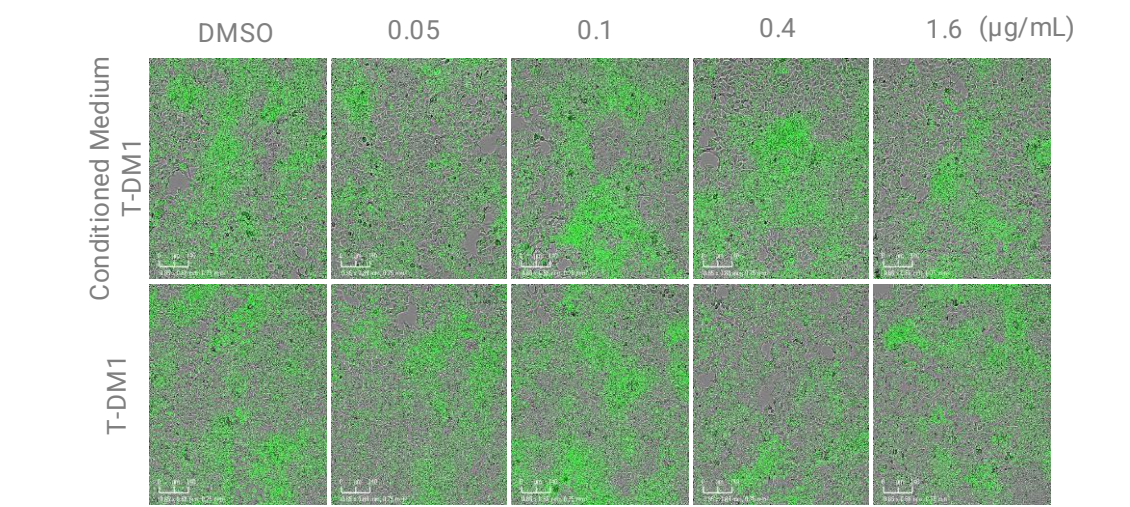
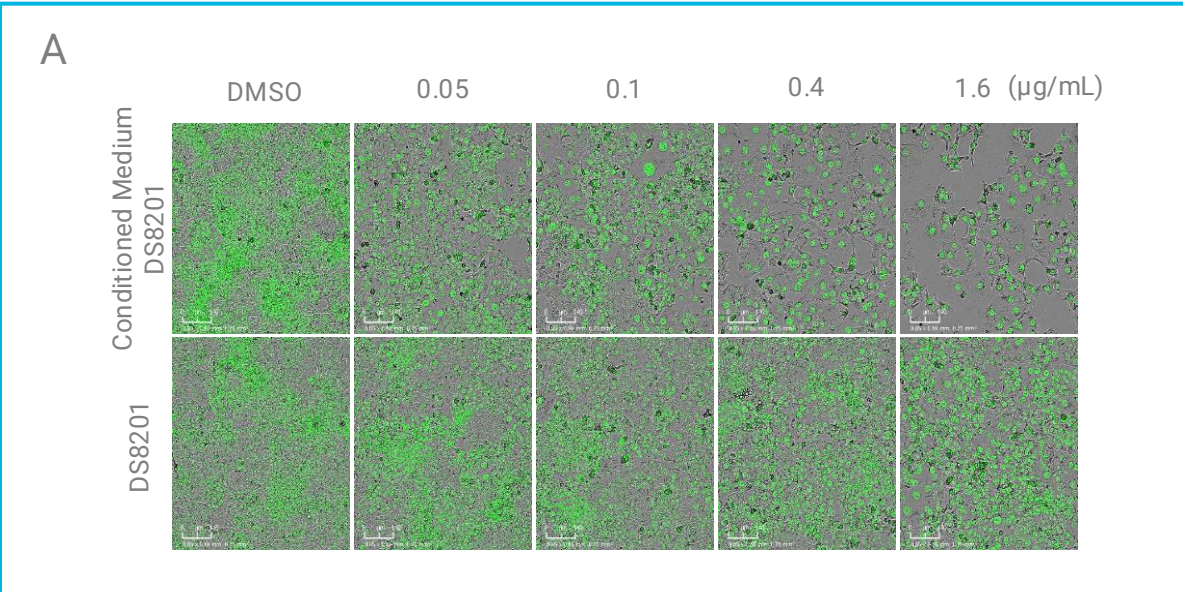
**Figure 2. Bystander killing effect of ADCs in the coculture of HER2-positive and HER2-negative cells.** (A) Images show MCF7-GFP (3K cells/well) cocultured with SKBR3-RFP (6K cells/well) at 0 and 96 hours after treatment with DS8201 and T-DM1 at various concentrations. MCF7-GFP cell counts through green channel over time when cocultured with SKBR3-RFP and treated with DS8201 (B) and T-DM1 (C). SKBR3-RFP cell counts over time when cocultured with MCF7-GFP and treated with DS8201 (D) and T-DM1 (E).

### Evaluation of bystander killing effect by flow cytometry in the coculture assay



**Figure 3. Evaluation of bystander killing effect using flow cytometry in a coculture assay.** (A) Proportion of viable SKBR3 and MCF7 cells at 96 hours after treatment with DS8201 and T-DM1 at various concentrations. Cell count ratio from panel A after adding DS8201 (B) or T-DM1 (C) to the cells.

### Evaluation of bystander killing effect of ADCs on HER2-negative MCF7-GFP cells using conditioned media



**Figure 4. Evaluation of the bystander killing effect of ADCs on MCF7 cells using conditioned medium.** MCF7-GFP cells were seeded at 3,000 cells/well in an E-Plate 96 for 24 hours before replacing their culture medium with conditioned media collected from SKOV3 cells treated with DS8201 or T-DM1 gradients for 96 hours. (A) Brightfield and green fluorescent channel images obtained from the conditioned media (CM) treated MCF7 and MCF7 directly exposed to DS8201 or T-DM1. In cells treated with ADC-conditioned media (B-E), (B) and (C) are time courses of Cell Index, and (D) and (E) are cell counts through the green channel.

## Conclusions

• In the coculture assay, results revealed that DS8201 treatment led to the death of HER2-negative MCF7 cells in the presence of SKBR3 cells, indicative of a bystander effect. In contrast, T-DM1 did not affect MCF7 viability under similar conditions. Both DS8201 and T-DM1, however, induced cytotoxicity in HER2-positive SKBR3 cells.

• In the conditioned medium transfer assay, medium from SKBR3 cells treated with DS8201 significantly reduced MCF7 cell viability, demonstrating a bystander effect. Conversely, conditioned media from T-DM1-treated SKBR3 cells did not impact MCF7 viability. Neither DS8201 nor T-DM1 exhibited direct cytotoxic effects on MCF7 cells.

• Using the Agilent xCELLigence RTCA eSight to assess the bystander effect of ADCs can optimize the efficacy, safety, and pharmacokinetics of therapeutic ADCs during biopharmaceutical development.