In vitro comparison of Kadcyla® and Enhertu® in breast cancer with varying HER2 expression: proliferation, internalization, bystander effects and toxicity

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Introduction

HER2-positive breast cancer is a subtype characterized by the overexpression of the human epidermal growth factor receptor 2 (HER2), which promotes aggressive tumor growth. Antibody-drug conjugates (ADCs) such as trastuzumab emtansine (Kadcyla®, T-DM1) and trastuzumab deruxtecan (Enhertu®, T-DXd) have shown efficacy in targeting HER2-positive tumors, but their effectiveness across varying levels of HER2 expression remains a topic of ongoing investigation.

This poster contains comparative data for Kadcyla® and Enhertu® and provides examples of the many possible assays in the development of ADCs.

Method

Surface Plasmon Resonance (SPR): HER2 binding to trastuzumab, Kadcyla®, and Enhertu® was investigated. All data was collected on a Biacore 8K+ (Cytiva). Antibodies and ADCs were captured using an anti-Fc IgG immobilized on a Series S CM4 chip (Cytiva). HER2 binding was measured in single-cycle kinetics mode in HBS-P+ and sensorgrams were fit using 1:1 Langmuir model (black line).

Proliferation: Real-time cell analysis was performed by measuring cell impedance using xCELLigence technology. Tumor cells were plated and test articles were added after 24 hours for 7 days.

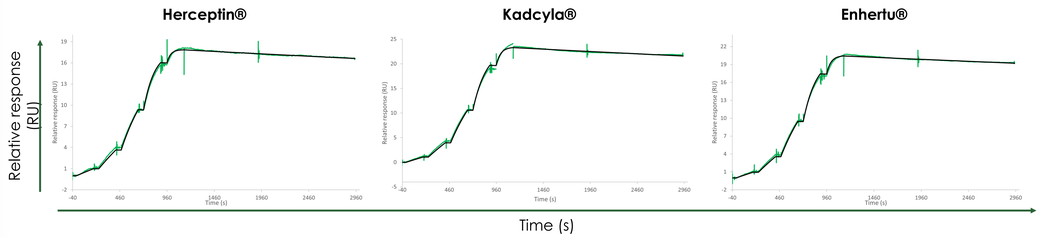
Internalization: The antibody trastuzumab was labeled with Zenon™ pHrodo™ iFL Green (Fab fragment, ThermoFisher) and added to the tumor cells. Uptake was analyzed by flow cytometry at different time points.

Bystander Assay: Her2-expressing tumor cells were co-cultured with Her2-negative and luciferase-expressing tumor cells and treated 24h later. Her2-negative tumor cell viability was determined by measuring luciferase activity.

In Vivo Hollow Fiber Model: Tumor cells grow inside Hollow Fibers, which consist of a semipermeable membrane that holds the cells in place and allows the influx of nutrients and drugs. A set of three fibers, each loaded with a tumor cell line, was implanted subcutaneously and intraperitoneally in mice for a two-week treatment.

Potency Assay: SK-BR-3 cells were plated in 96-well plates and a dilution series of reference Kadcyla® and a Kadcyla® test sample was added. Fluorescence was measured using a Tecan Infinite M200 with excitation at 530 nm and emission at 590 nm. .

Results



Binding Model	Target	Analyte	Chi² (RU²)	k_a (1/Ms)	k_d (1/s)	K_D (M)	R_{max} (RU)
1:1 Kinetic	Herceptin®	HER2	3.39E-02	1.08E+06	3.38E-05	3.14E-11	20.5
1:1 Kinetic	Kadcyla®	HER2	5.92E-02	1.05E+06	4.21E-05	4.01E-11	23.4
1:1 Kinetic	Enhertu®	HER2	3.39E-02	1.08E+06	3.38E-05	3.14E-11	20.5

Fig. 1: Surface Plasmon Resonance (SPR))
HER2 binding to Herzeptin® (Trastuzumab), Kadcyla®, and Enhertu® is depicted. Trastuzumab and ADCs were captured using an anti-Fc IgG immobilized on a Series S CM4 chip and data were collected on a Biacore 8K+ (Cytiva).

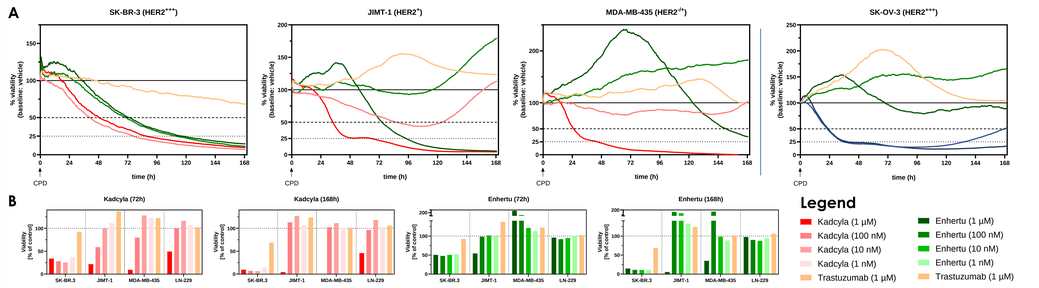


Fig. 2: Real-time cell analysis by measuring cell impedance with xCELLigence technology.
Breast cancer cells with different levels of HER2 expression (high / medium / low), an ovarian cancer cell line with high HER2 expression and a glioblastoma cell line without HER2 expression were plated on 96-well plates and treated with the indicated concentrations of Kadcyla® (trastuzumab emtansin), Enhertu® (trastuzumab deruxtecan) and Herceptin® (trastuzumab): (A) Time course of viability (measured twice per hour) compared to the untreated control up to day 7 (168h) for the two highest concentrations; (B) the graphical representation at day 3 (72h) and day 7 (168h) of all ADC concentrations tested.

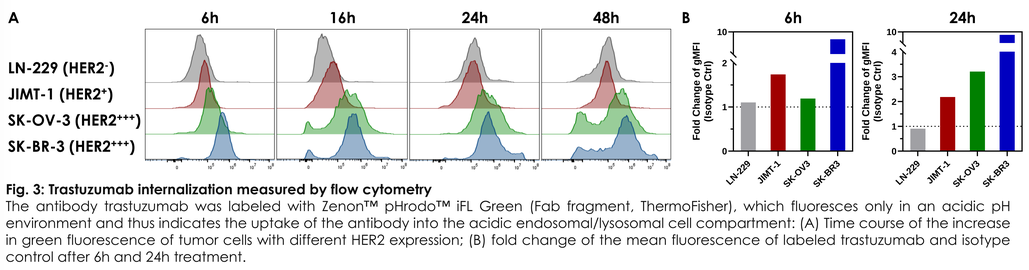


Fig. 3: Trastuzumab internalization measured by flow cytometry
The antibody trastuzumab was labeled with Zenon™ pHrodo™ iFL Green (Fab fragment, ThermoFisher), which fluoresces only in an acidic pH environment and thus indicates the uptake of the antibody into the acidic endosomal/lysosomal cell compartment: (A) Time course of the increase in green fluorescence of tumor cells with different HER2 expression; (B) fold change of the mean fluorescence of labeled trastuzumab and isotype control after 6h and 24h treatment.

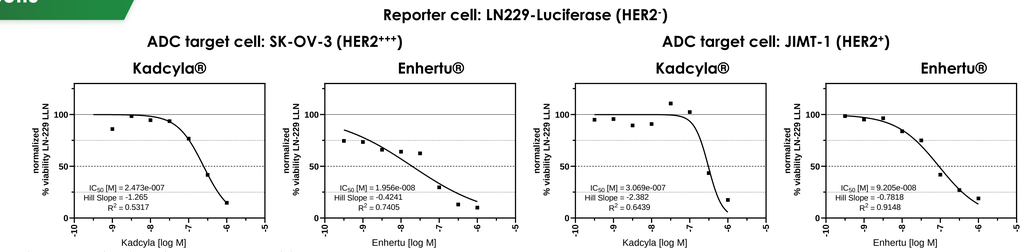


Fig. 4: Investigation of bystander cell killing.
Tumor cells with varying levels of HER2 expression (ADC target cells) were combined in a 3:1 ratio with luciferase-positive glioblastoma cells without HER2 expression (reporter cells) and treated with a semi-logarithmic concentration series of Kadcyla® and Enhertu®. After 10 days of incubation, luciferase activity was determined as a measure of live glioblastoma cells.

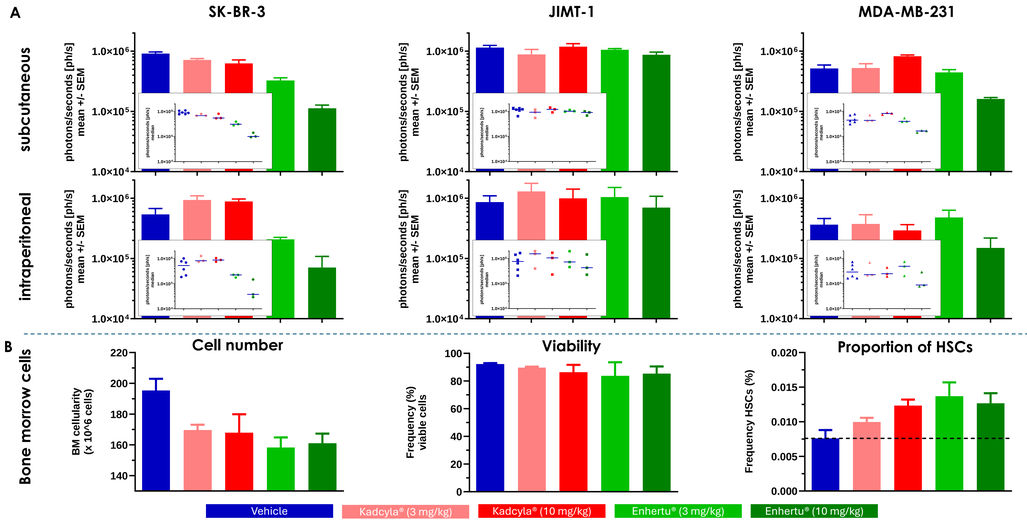
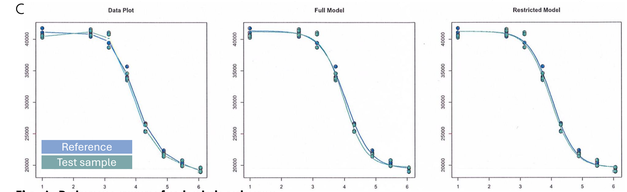


Fig. 5: In vivo efficacy in the Hollow Fiber Model and bone morrow toxicity analysis.
(A) On day -1, SK-BR-3, JIMT-1, and MDA-MB-231 tumor cells were loaded into Hollow Fibers. The next day, three Hollow Fibers loaded with each of the different cell lines were implanted in two different compartments: subcutaneous and intraperitoneal. Starting on day 1, mice were treated once with vehicle, Kadcyla®, and Enhertu® as indicated by the colors. On day 15, the study was terminated, and the Hollow Fibers were harvested. Cell number was determined in each fiber using the CellTiter Glo® assay. (B) In addition to fiber removal, bone marrow cells were isolated and analyzed by flow cytometry. Hematopoietic stem cells (HSCs) were gated negative for lineage markers (CD3e, Ter-119, B220, Nk1.1, C



Relartive Potency	
Potency ratio	1.03401
95% Confidence Interval	0.99624 – 1.07321
Relative Confidence Interval	96.35% - 103.79%

Fig. 6: Potency assay for batch release.
SK-BR-3 cells were plated in 96-well plates and a dilution series of reference Kadcyla® and a Kadcyla® test sample was added. Fluorescence was measured

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Kadcyla® and Enhertu® are both based on the monoclonal antibody Trastuzumab, but differ in linker (non-cleavable MCC vs. cleavable tetrapeptide-based) and payload (tubulin polymerisation inhibitor vs. topoisomerase I inhibitor), resulting in different outcomes:

- **Kadcyla® eliminates target cells faster than Enhertu®**

Conclusion

- **Herceptin® (Trastuzumab), Kadcyla® and Enhertu® show similar binding capacities to HER2**
- **Enhertu® does not inhibit SK-OV-3 (HER2+++) cells**
- **Enhertu® shows increased bystander killing**
- **SK-BR-3 and SK-OV-3 cells show different internalization of Trastuzumab**
- **Enhertu® inhibits tumor growth in the Hollow Fiber Model in vivo**
- **Kadcyla® and Enhertu® cause bone marrow cell toxicity**
- **Setting up a potency assay for ADC batch release is possible.**