Effect of Kadcyla® and Enhertu® on Breast Cancer: Proliferation, Internalization, Bystander and In Vivo Hollow Fiber Assay

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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 study aims to compare the effects of Kadcyla and Enhertu on cell proliferation, ADC internalization, and bystander cytotoxicity across cancer cell lines with different HER2 expression levels: SK-BR-3/SKOV-3 (high HER2 expression), JIMT-1 (moderate HER2 expression), MDA-MB-435 (low HER2 expression) and unrelated glioblastoma LN-229 cells (no expression).

The xCELLigence system, which works via label-free impedance measurements on living cells, was employed to monitor real-time cell proliferation and viability. Internalization of Trastuzumab was quantified using flow cytometry. The degree of internalization was determined by measuring the fluorescence intensity of the cell populations. To evaluate the potential bystander killing effect, a co-culture system was established. High and medium HER2-expressing ADC target cells SKOV-3 and JIMT-1 cells were co-cultured with luciferase-labelled non-HER2 expressing LN-229 reporter cells. After treatment with Kadcyla or Enhertu, luciferase assays were conducted to assess whether the cytotoxic payload released by the ADCs in HER2-positive cells could affect the viability of neighboring HER2-negative cells. Finally, Kadcyla was tested in comparison to Trastuzumab in the In Vivo Hollow Fiber assay.

Results

Proliferation

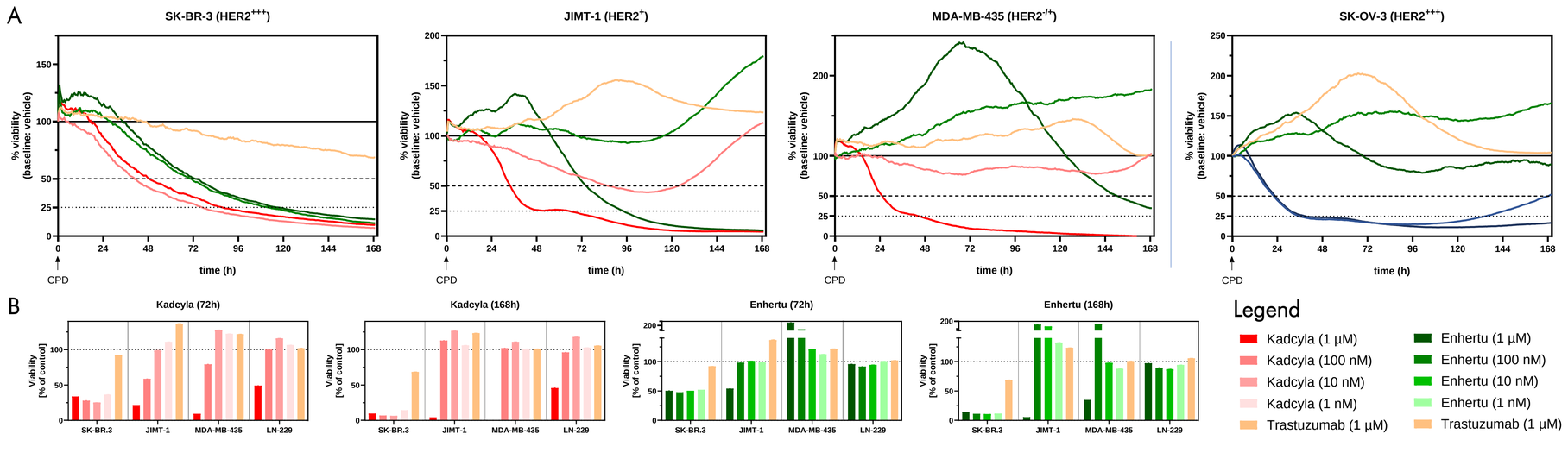

Fig. 1: Real-time cell analysis through cellular impedance measurement with xCELLigence technology.
Breast tumor cells with different HER2 expression (high / medium / low) and an ovarian cancer cell line with high HER2 expression and a glioblastoma cell line without HER2 expression as comparison were plated on 96 well plates and treated with the indicated concentrations of Kadcyla® (Trastuzumab-Emtansin), Enhertu® (Trastuzuma-Deruxtecan) and Trastuzumab: (A) time course of viability (measured twice per hour) compared to the untreated control until 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.

Internalization

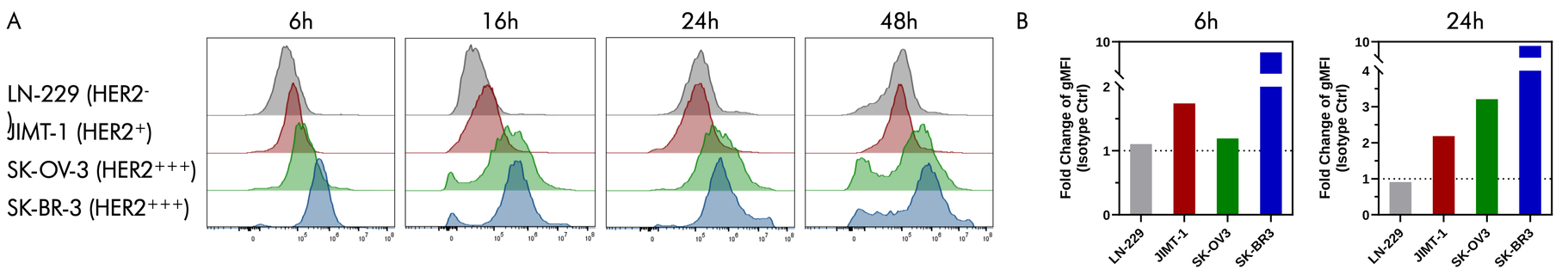

Fig. 2: Internalization of trastuzumab measured by flow cytometry.
The antibody trastuzumab was labeled with Zenon™ pHrodo™ iFL Green (Fab fragment, ThermoFisher) which fluoresces only in an acidic pH environment and thus indicates uptake of the antibody into the acidic endosome/lysosome cell compartment: (A) time course of the increase in green fluorescence of the tumor cells with different HER2 expression; (B) fold change of the mean fluorescence of the labeled trastuzumab and the isotype control after 6h and 24h treatment.

Bystander Assay

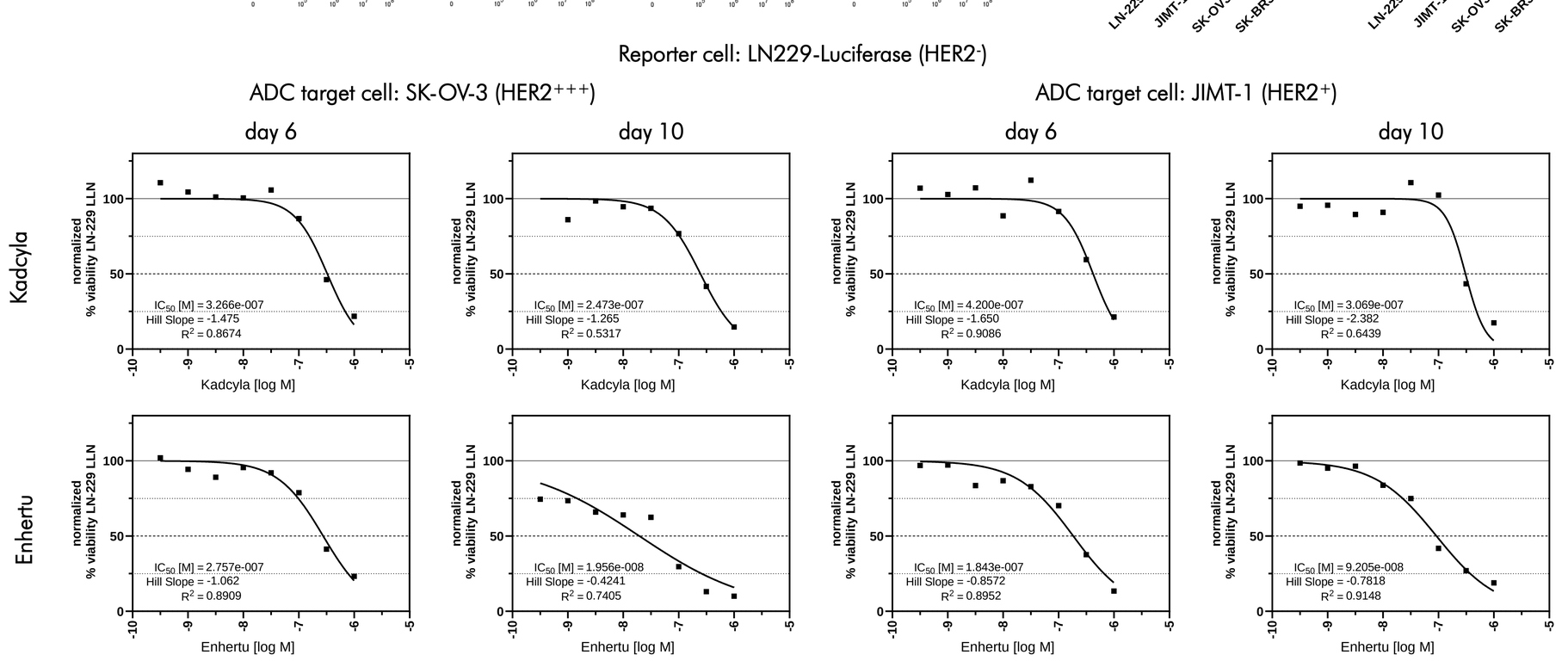

Fig. 3: Investigation of bystander killing.
Tumor cells with different HER expression (ADC target cells) were combined in a ratio of 3:1 with luciferase-positive glioblastoma cells without HER2 expression (reporter cell) and treated with a semi-logarithmic concentration series of Kadcyla® and Enhertu®. After 6 or 10 days of incubation, the luciferase activity was determined as a measure of the living glioblastoma cells.

In Vivo Hollow Fiber Assay

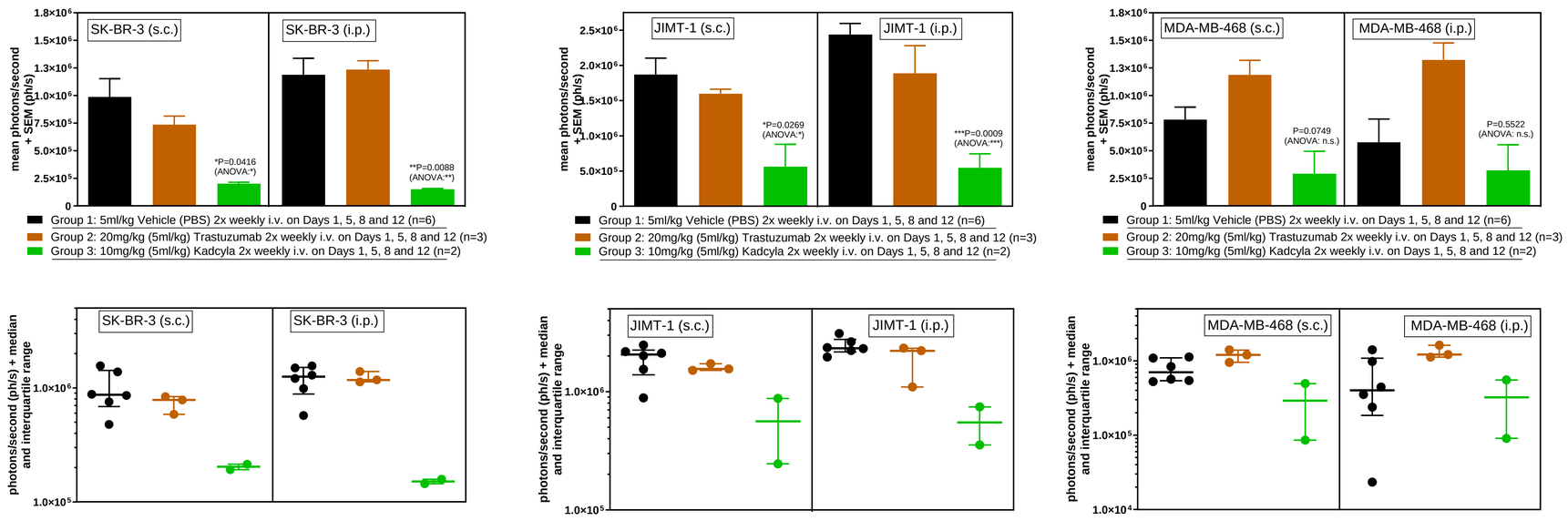

Fig. 4: In vivo efficacy.
On day -1, the SK-Br-3, JIMT-1 and MDA-MB-468 tumor cells were loaded into Hollow Fibers. The next day, three hollow fibers loaded with each of the different cell lines were implanted into two different compartments: subcutaneous and intraperitoneal. From Day 1, the mice were treated with vehicle, Trastuzumab or Kadcyla as indicated below each figure. On Day 15, the study was terminated and the fibers were removed. The cell count and viability in each fiber was determined by CellTiter Glo® assay.

Conclusion

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

- **Kadcyla® eliminates the target cells faster than Enhertu®**
- **Enhertu® shows increased bystander killing even at low concentrations**
- **Enhertu® does not inhibit the highly Her2-expressing cell line SK-OV-3**
- **SK-BR-3 and SK-OV-3 cells show different internalization of Trastuzumab**
- **Kadcyla® inhibits tumor growth in vivo depending on the Her2 expression level**

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