

## Introduction

Conventional two-dimensional (2D) cell cultures are not able to completely describe malignant phenotype that is related to tumorigenicity *in vivo*. In 2D culture conditions cells need to adapt to a flat and rigid surface that can result in altered cell metabolism and functionality (1, 2). Thus, cellular functions and physiological responses present in organs are often lost in 2D cell cultures which are usual standard for testing new chemical entities (NCE). Since 3D structure and environment are crucial for cellular behaviour and tumorigenicity, recently more researchers are adopting 3D cell culture systems to obtain biological insights that are lost in 2D cell culture testing platforms as well as to overcome differences in cell morphology, proliferation and differentiation (3, 4). In the case of breast cancer, 3D cell cultures represent a model for understanding cell proliferation regulation. Furthermore, breast cancer cells grown in 3D can be more resistant to the same doses of chemotherapeutic substances than cells grown in 2D (5, 6).

## Objective

The objective of this study was to compare responsiveness of three breast cancer cell lines (SK-BR-3, T-47D and MDA-MB-231) to known chemotherapeutic substances with different modes of action (doxorubicin, gemcitabine, docetaxel and vinorelbine) in two formats: 2D polystyrene culture plates and 3D Perfecta hanging drop plates.

## Materials and methods

Human breast cancer cell lines SK-BR-3 (HTB-30), MDA-MB-231 (HTB-26) and T-47D (HTB-133) were purchased from ATCC.

For 2D culture, cells were grown in 96 well Cell star polystyrene plates, while for 3D cultures cells were grown in 96 well Perfecta 3D hanging drop plates.

To determine the influence of testing substances on the cell viability, cytotoxicity studies were performed by culturing three cell lines in 2D format for 72 and 96 hours. After which 96 hours were chosen to be a culturing time for 3D format. In both cases  $5 \times 10^3$  cells per well were seeded 4 hours prior the treatment with different starting concentrations for each substance: doxorubicin- $5 \mu\text{M}$ , docetaxel- $50 \text{ nM}$ , vinorelbine- $25 \text{ nM}$  and gemcitabine- $1 \mu\text{M}$ . All substances were further serially diluted 1:3.

Cell viability assay was performed according to the manufacturer's instruction, using Cell titer 96 aqueous solution, MTS kit (Promega).

Statistical analysis: raw and derived data results are given in the form of tables and graphs. Calculation of  $\text{IC}_{50}$  data and curves is made by using Excel tools and GraphPadPrism software. Briefly, individual concentration-effect curves are generated by plotting the logarithm of the tested concentration of tested compounds (X) vs. corresponding percent inhibition values (Y) using least squares (ordinary) fit. Best fit  $\text{IC}_{50}$  values are calculated using Log(inhibitor) vs. normalized response - Variable slope equation.

## References

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## Results

### Dose response studies in 2D format

Cell viability (MTS assay) of SK-BR-3, T-47D and MDA-MB-231 cell lines was determined after 72 and 96 hours treatment with four chemotherapeutic substances.  $\text{IC}_{50}$  values are compiled below (Table 1.).

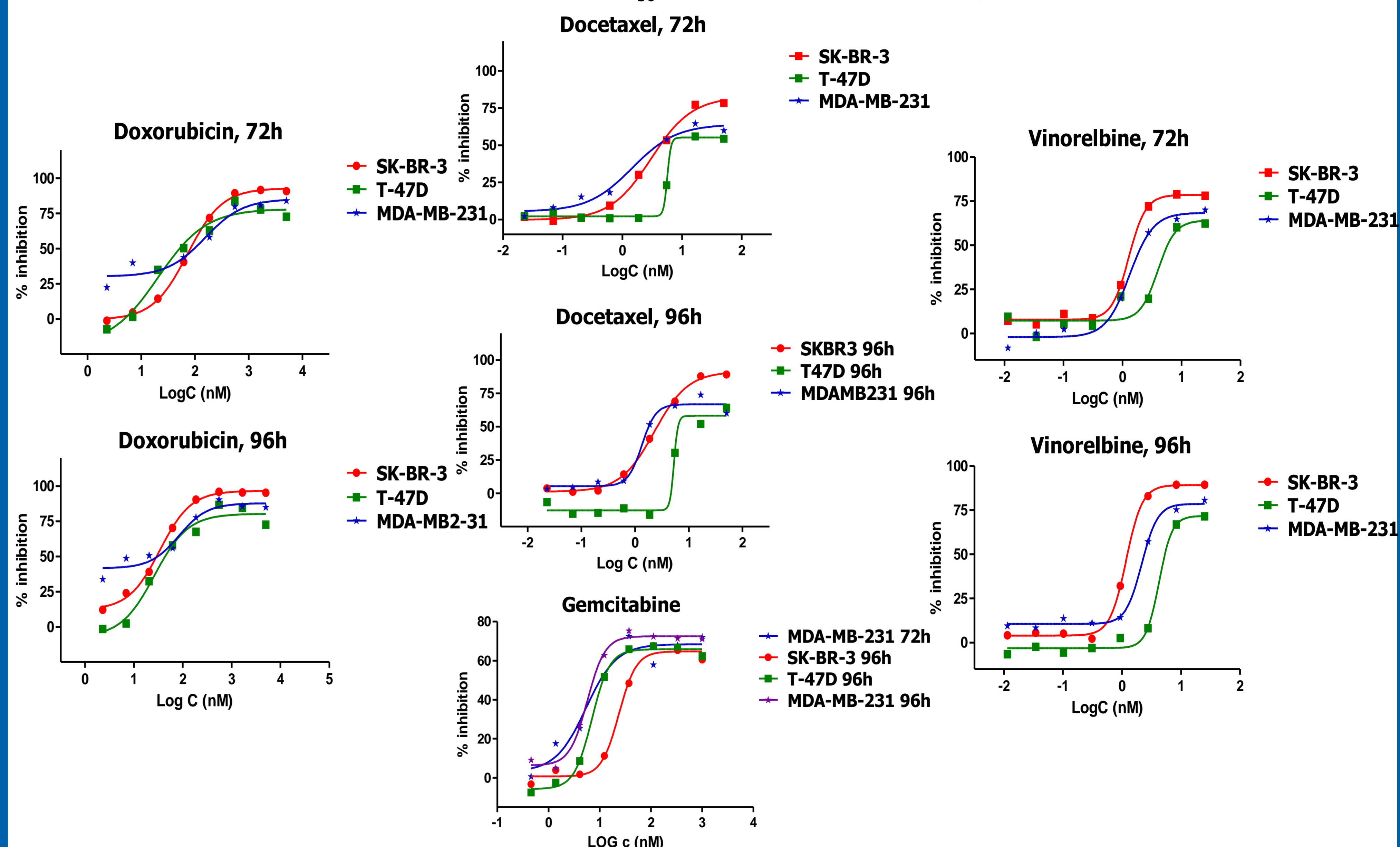


Figure 1. Dose responses curves in 2D format after 72 and 96 hours.

Table 1. Comparison of  $\text{IC}_{50}$  values (in nM) for 2D format from 72 and 96 hours treatment

	72h			96h		
	SK-BR-3	T-47D	MDA-MB-231	SK-BR-3	T-47D	MDA-MB-231
Doxorubicin	73	21	154	34	26	83
Docetaxel	3	6	1	2	5	1
Vinorelbine	1	4	1	1	4	2
Gemcitabine	37	10	5	24	7	6

### Dose response studies in 3D format

Based on the noticed cell viability and reproducibility, 96 hours was chosen to be a culturing time for SK-BR-3, T-47D and MDA-MB-231 cell lines in 3D format.  $\text{IC}_{50}$  values are compiled below (Table 2.).

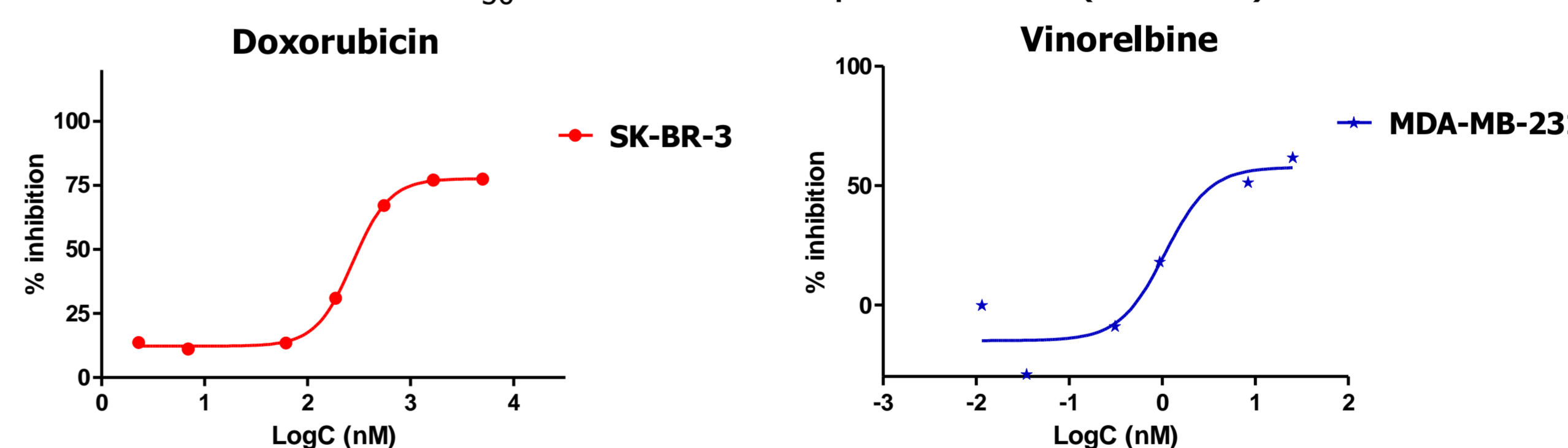


Figure 2. Dose responses curves in 3D format after 96 hours.

Table 2.  $\text{IC}_{50}$  values (in nM) for 3D format from 96 hours treatment

	SK-BR-3	T47-D	MDA-MB-231
Doxorubicin	250	>5000	>5000
Docetaxel	>50	>50	>50
Vinorelbine	>25	>25	1
Gemcitabine	>1000	>1000	>1000

## Conclusions

From our study it is evident that three breast cancer cell lines grown in 3D format are resistant to four chemotherapeutic substances in comparison to cells grown in 2D format. Chosen concentration range for doxorubicin, docetaxel, vinorelbine and gemcitabine are in line with literature (7), needed to span effective dose response curve ( $\text{IC}_{20}$  –  $\text{IC}_{90}$ ) in 2D format. Thus, cells grown in 3D format may be better targets for evaluation of antitumoral activity of different chemotherapeutic substances with known anticancer activity as well as for identification of novel chemical entities with similar action.