Using Alamar Blue assay to measure proliferation inhibition of trastuzumab and its biosimilars

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Abstract

Bioassay is a critical quality attribute for biosimilar development. Therefore, data consistency and reproducibility from bioassay are of great importance. We successfully set up a reliable proliferation assay platform using the Alamar Blue (Resazurin) to enable reproducible evaluation of trastuzumab and its biosimilars. We also found that BT-474 gives higher signal-to-noise ratio and more consistent potency across biosimilars than SKBR-3, suggesting that BT-474 is a better target cell in the proliferation inhibition assay.

Introduction

The human epidermal growth factor receptor 2 (HER2) is an established therapeutic target for HER2 overexpressing cancer cells. The anti-HER2 monoclonal antibody Trastuzumab (Herceptin) substantially extended lives for patients with metastatic and early-stage breast cancers [1] and motivated pharmaceutical companies in discovering biosimilars of this antibody [2].

During biosimilar development, data consistency in bioassays are crucial for demonstration of biosimilarity. Failure to set up reliable bioactivity assays is a hurdle to biosimilar development and evaluation. It is important to identify critical parameters, including choices of type of assays, target cell lines, cell seeding technique, and assay conditions, to investigate how these parameters affect data consistency and reproducibility when analyzing antibody biosimilars.

The proliferation inhibition of trastuzumab biosimilars was usually measured with the tedious Trypan Blue exclusion assay. We report herein Alamar Blue [3] is a efficient and cost-effective cytotoxic method that can be useful for proliferation inhibition assays.

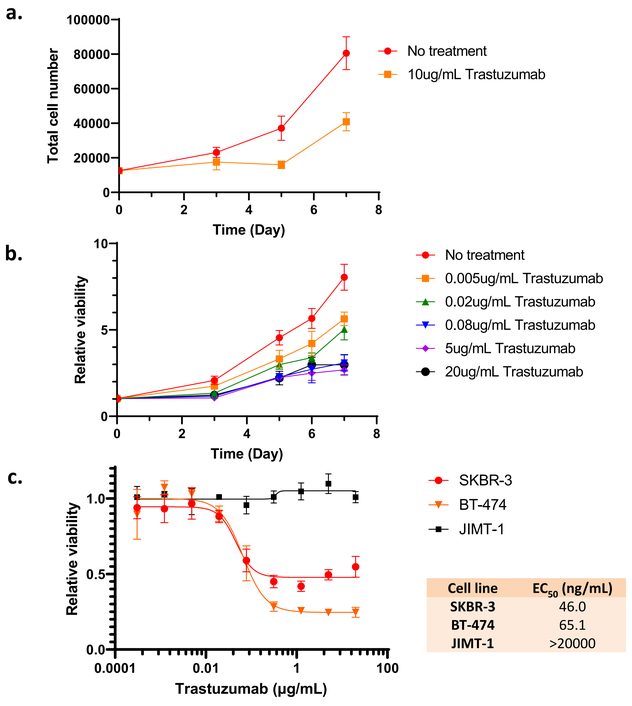
Materials and Methods shorter

Cells were purchased from ATCC and propagated as described previously [4]. Cells were seeded in clear 96-well cell-culturing plates at 5000 cells/well and allowed to recover overnight. Serial dilutions of trastuzumab or its biosimilars were added and incubated for 0, 3, 5 and 7 days with media change and antibody replenishing on these days. Cells were added with 10 µg/mL Alamar Blue (Resazurin) and incubated at 37°C for 3 hours prior to reading the fluorescence (544nm Ex/ 590nm Em). For growth curve detection, Alamar Blue reagent was incubated for exactly 3.0 hours and measurement was performed on Days 0, 3, 5, and 7. Readings relative to Day 0 were used to plot against time.

Results and Discussion

We compared Trypan Blue Exclusion assay (Figure 1a) with Alamar Blue assay (Figure 1b) and found that Alamar Blue assay gives efficient assessment of proliferation inhibition, while allowing high-throughput assay format. Using Alamar Blue assay we were able to measure E_{max} and EC_{50} of anti-proliferation activity of trastuzumab in SKBR-3 and BT-474, and differentiate sensitive (SKBR-3, BT-474) and resistant (JIMT-1) cell lines (Figure 1c).

The potency of trastuzumab and its five biosimilars (denoted as a-e) were measured using Alamar Blue proliferation inhibition assay in two HER2-positive breast cancer cell lines, SKBR-3 (Figure 2a) and BT-474 (Figure 2b). Both of cell lines are commonly used for bioassays of trastuzumab and its biosimilars for lot release and stability testing. Table 1 includes the quantitative results shown in Figure 2, and Figure 3 shows the distribution of E_{max} and EC_{50} of trastuzumab biosimilars. SKBR-3 cells showed lower E_{max} and larger variation. In this case, BT-474 cells likely provide more consistent potency measurement for trastuzumab biosimilars than SKBR-3.



Cell line	EC_{50} (ng/mL)
SKBR-3	46.0
BT-474	65.1
JIMT-1	>20000

Figure 1. Alamar Blue is a reliable assay for proliferation inhibition of trastuzumab. a. Proliferation inhibition of trastuzumab in SKBR-3 measured with Trypan Blue exclusion assay; b. Proliferation inhibition of trastuzumab in SKBR-3 measured with Alamar Blue assay; c. Alamar Blue assay differentiates sensitive (SKBR-3, BT-474) and resistant (JIMT-1) cell lines. Cells were treated with variable concentrations of Trastuzumab for 7 days. All experiments were performed in triplicate with 5% FBS in cell culturing medium.

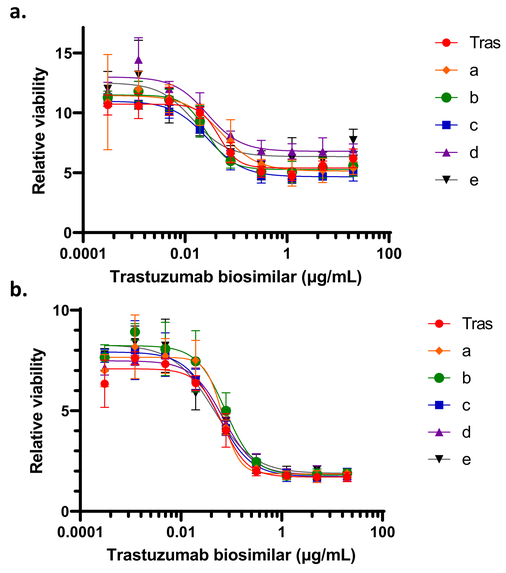


Figure 2. Comparing different cell lines in measuring anti-proliferation of Trastuzumab biosimilars with Alamar Blue cell viability assay. Potency of trastuzumab/reference product and five FDA-approved biosimilars were measured using Alamar Blue proliferation inhibition assay in HER2+ breast cancer cell lines, SKBR-3 (a) and BT-474 (b). Experiments were performed in triplicate with 5% FBS in cell culturing medium, and 7-day treatment.

Table 1. Quantitative analysis of results shown in Figure 2. a. distribution of E_{max} (percent inhibition) and b. EC_{50} (ng/mL) of trastuzumab biosimilars in SKBR-3 and BT-474

	E_{max} (Percent Inhibition)		EC_{50}(ng/mL)	
	SKBR-3	BT-474	SKBR-3	BT-474
Trastuzumab	50.2	71.4	46.0	65.1
Biosimilar a	63.1	76.7	52.3	67.2
Biosimilar b	51.6	76.2	26.5	76.1
Biosimilar c	61.2	79.3	28.2	53.8
Biosimilar d	42.6	73.6	27.4	68.1
Biosimilar e	52.3	73.6	14.3	43.8

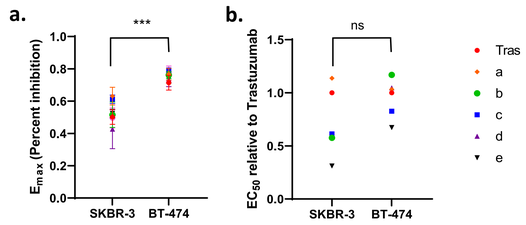


Figure 3. Comparing distribution of E_{max} and EC_{50} of trastuzumab biosimilars in SKBR-3 and BT-474 in Alamar Blue proliferation assay. a. distribution of E_{max}; b. distribution of EC_{50}. ***P<0.0001 by two-way ANOVA.

Conclusion

1. Alamar Blue assay gives efficient assessment of proliferation inhibition, while allowing high-throughput assay format.
2. Trastuzumab and its biosimilars were evaluated in HER2+ cell lines SKBR-3 and BT-474 using Alamar Blue proliferation assay.
3. BT-474 gives higher signal-to-noise ratio and more consistent potency across biosimilars than SKBR-3, suggesting BT-474 as a better target cell in the proliferation inhibition assay.
4. The preliminary data shown in Figures 1-3 and Table 1 are the premise of this study. These experiments will be carefully repeated, and the assays used for the test will be further qualified and optimized using different target cell lines.

References

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