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ORIGINAL ARTICLE

Expression and Characterization of Trastuzumab in Baby Hamster Kidney Cell Line to Check its Anti-Cancer Activity in HCC 1954

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ABSTRACT

OBJECTIVE: To investigate Trastuzumab's production and anti-cancer effects on hepatocellular carcinoma (HCC1954) cells.
METHODOLOGY: This study was conducted in IMBB/CRiMM, The University of Lahore, Pakistan. A humanized IgG1 monoclonal antibody (Trastuzumab) with a high affinity for the extracellular domain of HER2 was produced. Antibody includes humanized sequences. Its anti-proliferative effect was quantified using the MTT assay on the Hepatocellular carcinoma (HCC1954) & BHK. SDS-PAGE confirmed HER2 protein expression. Statistical Analysis was accomplished using Social Package for Statistical Sciences (SPSS) version 17.0 (SPSS, Chicago, IL, USA).
RESULTS: The expressed monoclonal antibody (Trastuzumab) exhibited anti-cancer activity, specifically targeting and inhibiting cell proliferation in the HCC1954 breast cancer cell line. The MTT quantified the antibody's anti-proliferative effects, & SDS-PAGE confirmed HER2 protein expression. The quality & expression levels of Trastuzumab were optimized, paving the way for its local manufacturing and use as a therapeutic option in Pakistan.
CONCLUSION: Trastuzumab effectively targets HER2, disrupting cancer-promoting pathways, which may provide therapeutic benefits. The study supports the feasibility of local production in Pakistan, which could improve treatment access and affordability for patients.

KEYWORDS: HER 2, Trastuzumab, Breast cancer, Baby Hamster Kidney (BHK), HCC, MTT Assay

INTRODUCTION

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells in the body. It occurs when genetic mutations disrupt normal cellular processes, leading to unregulated cell division and resistance to apoptosis. Environmental factors, lifestyle choices, or genetic predispositions can cause these mutations. Cancer can affect nearly any tissue or organ and manifests in various forms, such as carcinomas, sarcomas, and hematologic cancers. Despite advancements in early detection, treatment, and prevention, cancer remains one of the leading causes of death worldwide. Understanding the molecular mechanisms driving cancer is crucial for developing effective therapies[1]. Trastuzumab is a monoclonal antibody primarily used to treat HER2-positive breast and gastric cancers. It targets the HER2/neu receptor, inhibiting cancer cell growth and activating the immune system to destroy the cells. Commonly known by the brand name Herceptin, Trastuzumab is administered via intravenous infusion. It improves survival rates with chemotherapy, especially in early and advanced stages of HER2-positive cancers[2].

Increasingly modern genomic microarray investigations have likewise validated the nearness of a few particular characteristic types of malignant growth: luminal A, luminal B, and basal[3]. Consequent to this underlying examination, the claudin-low subtype was additionally perceived as another unmistakable sub-atomic type. In approximately 70% of cases, the atomic breast malignant growth types correspond with the outflow of HER2, Estrogen receptor (ER), and Progesterone receptor (PR)[4]. Currently, research is concentrating on the clinical utility of atomic profiling that may soon supplant conventional immunohistochemical staining, as beginning proof recommends that sub-atomic profiling might be progressively precise in foreseeing anticipation. Trastuzumab is expected to do a key job for remedial treatment for breast tumors and is viewed as a consideration for patients with HER2-positive breast cancer (BC)[5]. Trastuzumab has been embroiled in cardiovascular unfavorable impacts, constrained helpful reaction against cerebrum metastases and treatment obstruction. Other up-to-date problems incorporate ailment steps, including Trastuzumab, suitable therapy conspires, planning of chemotherapy routine (accompanying or successive organization), ideal treatment span, and perfect organization course[6]. Ongoing updates of worldwide agreement rules for treating BC incorporating HER2-positive BC have addressed these issues[7].

Trastuzumab (Herceptin) is a humanized IgG1 kappa light chain mAb in which corresponding determining districts (CDR) of a HER2-explicit mouse mAb were joined to human immune response structure regions through hereditary designing. In any case, a noteworthy number of patients with HER2-overexpressing breast malignancy will be, at first or in the long run, resistant to HER2-based treatment with trastuzumab[8]. Understanding the resistance mechanisms from Trastuzumab is essential for advancing new restorative methodologies along these lines. Between 25% and 30% of breast malignant growths overexpress HER2-neu, a receptor of the HER family related to poorer visualization and protection from treatment. Overexpression is characterized as 3+ invulnerable histo-chemical recoloring (IHC 3+) or positive in situ hybridization (FISH positive). The accessibility of an acculturated monoclonal immunizer (Trastuzumab) coordinated with an outside bit of this receptor has significantly changed the treatment and anticipation of these patients. As a solitary specialist, Trastuzumab can instigate a 30% reaction rate in HER2-overexpressing tumors, and the expansion of Trastuzumab to chemotherapy as contrasted and chemotherapy alone is related with a noteworthy enhancement in target reaction rate, length of reaction and general survival (middle survival, 25.1 versus 20.3 months; P=0.01). The most well-studied mechanisms of

common resistance to Trastuzumab are: (1) interference with the binding of Trastuzumab to HER2; (2) increased activation of downstream signaling pathways of HER2; (3) activation of alternative pathways; and (4) lack of activation of the mechanisms for the immune-mediated killing of tumor cells. Most resistance mechanisms to Trastuzumab have been identified in the preclinical model system and have not yet been established in clinical samples. There is an urgent goal for this field, which is to define which of the mechanisms above are clinically relevant. Clinical resistance is likely multifactorial, just like all other anticancer drugs. The drug trastuzumab is used in the treatment of HER2-positive breast cancer. Primary breast cancers overexpress HER2 in approximately 25% to 30% of cases. In both in vitro assays and animal studies, Trastuzumab has been demonstrated to inhibit the proliferation of human tumor cells that overexpress HER2. The drug acts as an antibody-dependent cellular cytotoxicity mediator by causing selective cell death due to the binding of the antibody to the HER2 overexpressing cells[9]. HER2-positive breast cancer is characterized by aggressive disease progression and an unfavorable prognosis. Although Trastuzumab has demonstrated significant efficacy in targeting HER2-driven pathways, its limited availability in many regions poses a major challenge, particularly in resource-constrained settings. This study seeks to develop and optimize Trastuzumab locally for therapeutic application, aiming to improve the accessibility and affordability of this critical treatment for patients in Pakistan.

METHODOLOGY

Antibody Preparation

This study was conducted in IMBB/CRiMM, The University of Lahore, Pakistan, from 2021 to 2022. The plasmid (catalogue number 61884, p VITRO Trastuzumab IgG2) was obtained from the Add gene for this study. Upon receipt, the plasmid was plated on an LB agar plate to develop colonies. A single colony was inoculated in 10 mL LB broth and incubated overnight at 37ºC in a shaking incubator. Stocks with 80% glycerol were prepared and stored at -20ºC. To isolate the plasmid, 25 mL of LB broth was inoculated with the plasmid culture and incubated overnight. The following day, growth indicated resistance gene presence in the bacteria. The culture was centrifuged in 1.5 mL portions at 6000 rpm, with 6 mL of bacterial pellet obtained. After drying the pellet, solutions were added sequentially to facilitate plasmid extraction, followed by centrifugation and ethanol washing, after which the DNA pellet was eluted in TE buffer and stored at -20ºC.

Culturing of cell lines

BHK21 cells were cultured in a T75 flask, washed with PBS, and treated with trypsin before incubating at 37ºC with 5% CO_2. The DMEM medium was prepared with 500 mL distilled water, sodium bicarbonate, DMEM powder, fetal bovine serum, and antibiotics, sterilized with a 0.22-micron filter, and stored at 2-8ºC. For splitting, the BHK21 cells were washed, trypsinized, centrifuged, and resuspended in 7 mL DMEM. Cell counting was performed using a hemocytometer, with cell density calculated based on average counts across squares.

Transfection

Cells at 70-90% confluence were seeded in a 6-well plate for transfection. Lipofectamine 2000 transfection reagent was mixed with DMEM, and DNA was prepared in a separate tube; both were incubated before combining and added to the cells. After 48 hours, images were taken in a cell imaging station. RPMI 1640 media preparation followed similar steps with sterile techniques. Gel electrophoresis was performed with a 1% agarose gel in TE buffer and ethidium bromide for DNA visualization under UV. SDS-PAGE was conducted to analyze protein samples, with resolving and stacking gels prepared sequentially. After running, the gel was stained and de-stained to reveal protein bands.

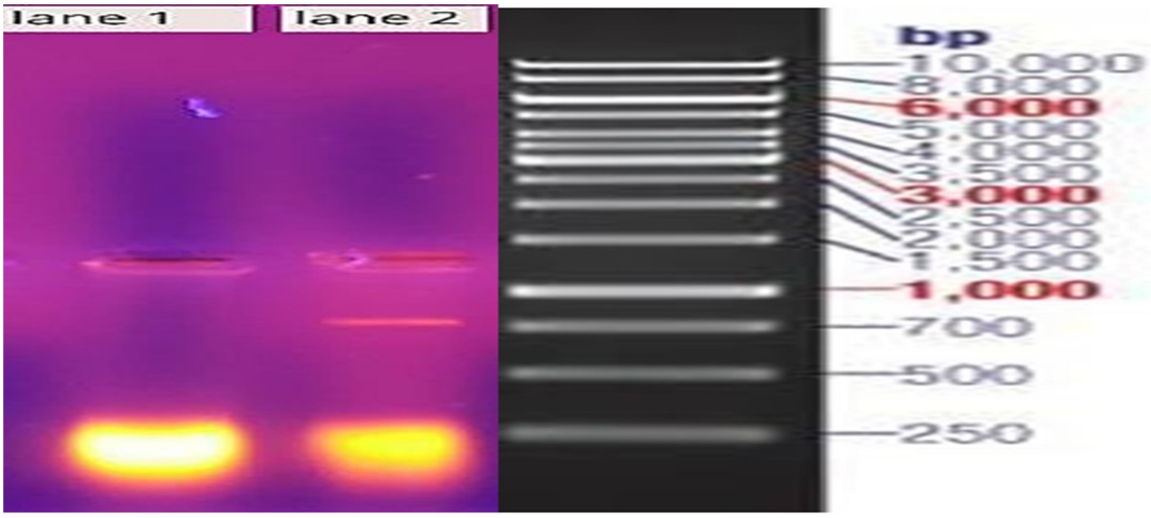
MTT Assay

The MTT assay was used to assess cell viability. Suspended cells were counted and diluted, with HCC1954 cells incubated overnight. Different drug dilutions were applied, and MTT reagent was added after 48 hours. The cells reduced MTT to formazan, indicating glycolytic activity. After incubation, DMSO dissolved the formazan, and absorbance was measured with an ELISA plate reader. Statistical Analysis was accomplished using the Social Package for Statistical Sciences (SPSS) version 17.0 (SPSS, Chicago, IL, USA).

RESULTS

The successful plasmid DNA isolation was confirmed through gel electrophoresis following the manual miniprep plasmid isolation procedure. A 1% agarose gel was prepared and run at 80–100 V under optimal conditions. The resulting gel revealed a distinct band corresponding to the expected size of the pVITRO plasmid carrying the target HER2 protein. This clear and sharp band confirmed the plasmid DNA's presence and validated the isolation process's success. The gel displayed the expected band size corresponding to HER2, as shown in **Figure I:**

Figure I: Shows a DNA electrophoresis result



Lanes 1 and 2 represent experimental DNA samples, while the rightmost lane is a DNA ladder with fragment sizes labeled in base pairs (bp) for molecular weight reference. The experimental bands correspond to approximately 6,000 bp and 3,000 bp, indicating successful DNA fragments of expected sizes.

Morphological Analysis of transfection

The figure shows a morphological analysis of transfected cells to evaluate their efficiency and cellular response to transfection. Microscopic view of BHK21 cells in a T75 culture flask post-transfection with trastuzumab plasmid DNA isolated via the manual plasmid isolation method.

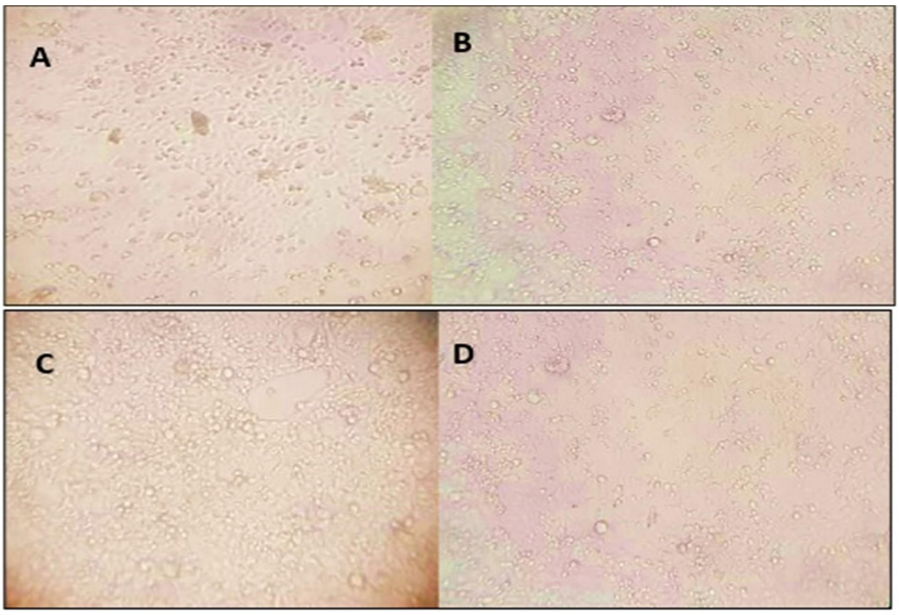
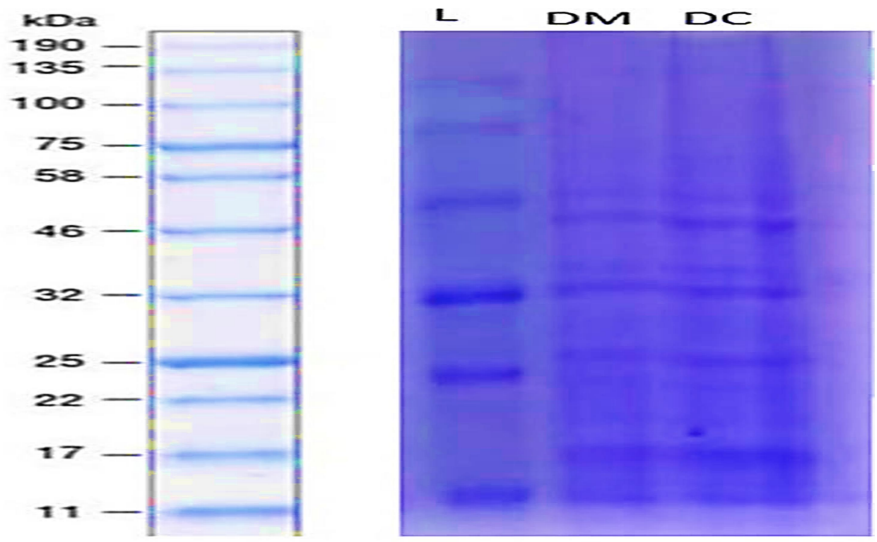


Figure II: A showing before transfection in T75 cultural flask. **B** shows 70% confluent cells before transfection. **C** illustrates the microscopic view of BHK21 cells post-transfection with trastuzumab plasmid isolated by the manual method, while **D** shows the cells transfected with plasmid isolated using a kit method.

SDS-PAGE Analysis

Protein expression was assessed using SDS-PAGE analysis on a 15% gel. Lane 1 represents the control sample (no transfection), Lane 2 displays protein expression from plasmid DNA isolated manually, and Lane 3 shows protein expression from plasmid DNA isolated using a column-based method.

Figure III: Figure shows transfection with trastuzumab plasmid DNA. Protein expression analysis was performed using SDS-PAGE on a 15% gel. Lane 1: Control sample (no transfection); Lane 2: Protein expression from manually isolated plasmid DNA; Lane 3: Protein expression from column-purified plasmid DNA.



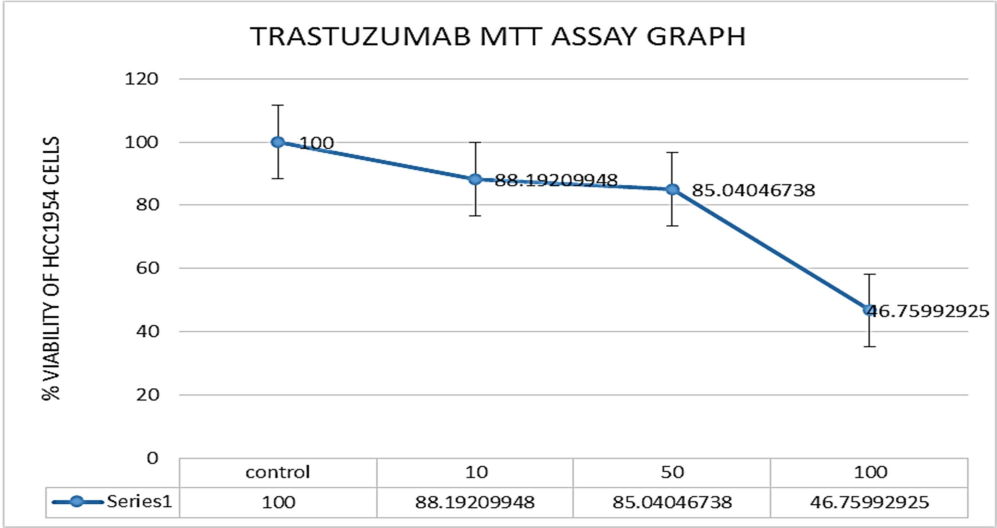
MTT Assay

The MTT assay was conducted to evaluate the viability of HCC1954 cells after treatment with varying concentrations of Trastuzumab. Data was used to generate a graph demonstrating the relationship between drug concentration and the percentage viability of HCC1954 cells. The control (untreated) sample showed no drug effect, and as trastuzumab concentration increased, the cancer cell viability decreased accordingly. The results, depicted in multiple graphs, reveal 50% viability at a concentration of 10 µM/mL without transfection **Graph I**. When DNA isolated by the miniprep method was applied, 79% viability was observed at 10 µM/mL and 90% at 50 µM/mL. DNA isolated by the column method produced 88% viability at 10 µM/mL, 85% at 50 µM/mL, and a significant reduction to 46% at 100 µM/Ml Figure 4 and **Table I**.

Table I: MTT assay presents the measured parameters' mean and standard deviation values across different treatment groups

	Control	10µg/ml	50µg/ml	100µg/ml
Mean	98.67	88.96	83.92	44.81
Std. Deviation	1.528	0.8589	3.431	1.496

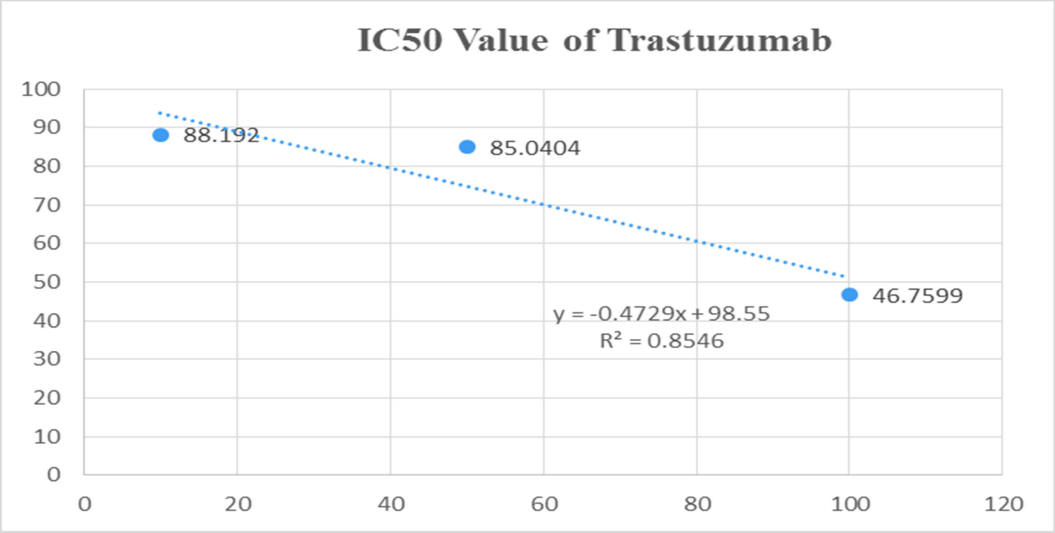
Figure IV: This graph illustrates the different drug concentrations and viability percentages of HCC1954 cells. 85% at 50 µM/mL, and a significant reduction to 46% viability at 100 µM/Ml



IC50 evaluation

The IC50 value, representing the drug concentration required to achieve 50% cancer cell killing, was calculated for Trastuzumab. Using MTT assay data, the IC50 was determined to be approximately 102.66 µM, as shown in Figure 5, where the regression equation Y=0.4729X+98.55Y = 0.4729X + 98.55Y=0.4729X+98.55 was used to derive this value. The IC50 value underscores Trastuzumab's effectiveness at a concentration of 102.66 µM for achieving 50% cell viability.

Figure V: Expresses the IC 50 value of Trastuzumab. IC 50 value of Trastuzumab showing the value of 102.66 uM showing 50% of cell viability at the concentration of 102.66µM



DISCUSSION

Trastuzumab humanized monoclonal antibody, a therapeutic drug for the treatment of breast cancer against human epidermal growth factor receptor (HER2). U.S Food and Drug Administration approved around 70 monoclonal antibodies for the various phases of clinical and experimental development of the cancer[10]. In last few decades, adapted treatment has played a cumulative role in administration of breast cancer patients. The adverse prognostic factor, nearly 25% of hostile breast cancers have HER2 receptor overexpression[11]. Trastuzumab is an approved drug by the Food and Drug Administration (FDA) for patients with progressive breast cancers which express human epidermal growth factor receptor positive, and Trastuzumab is presently therapeutic choice and most prevalent cure for the breast cancer subtype.

As a single agent in metastatic breast cancer first-line treatment, it was found to be less than 40 %, and the median response time was between 9 and 12 months[12]. We confirmed expression of the drug by isolating the plasmid that was sourced from Add gene and then getting the appropriate band by performing gel electrophoresis. In this study we have focused on the anti-proliferative effect of Trastuzumab in breast cancer so we first cultured bhk21 cells in T75 cultural flask at optimum conditions[13]. When the cells become 70- 90% confluent, we performed transfection in order to introduce our plasmid DNA to the desired cells. The selected cells were expanded and antibody was purified from the media after transfection, after getting the transfection results, we performed MTT Assay to check the anti-cancer activity and % viability of this drug. For MTT Assay of this drug we required a breast cancer cell line[14-16]. So, we cultured HCC 1954 cells in RPMI media in T75 cultural flask. For finding out the anti-cancer activity and % viability of HCC1954 cells after treating them with trastuzumab MTT assay was performed. IC50 value of the drug was calculated by using MTT Assay data[17]. After that the purification and expression of the Trastuzumab was confirmed by performing SDS (poly acrylamide gel electrophoresis)[18], and it confirmed that the expression of Trastuzumab against the target protein receptor her2.

In this study we have focused on the optimization of the expression of Trastuzumab by using different breast cancer cell lines and the results came out extraordinary and the quality and action of Trastuzumab on breast cancer cell line was confirmed[19]. Through this research, we have developed an optimized protocol for the production and expression of Trastuzumab, paving the way for its application in therapeutic studies and potential patient testing. The protocol ensures high-quality production of Trastuzumab, making it suitable for large-scale manufacturing and clinical use. This is particularly significant for countries like Pakistan, where the local production of Trastuzumab can improve accessibility and reduce costs, offering a more affordable treatment option for HER2-positive breast cancer patients[20]. The anti-cancer efficacy of Trastuzumab was confirmed through in vitro studies on breast cancer cell lines, demonstrating its potential to inhibit the growth and progression of HER2-overexpressing cancer cells. These findings not only validate the therapeutic action of Trastuzumab but also highlight its potential to significantly impact cancer treatment outcomes in resource-constrained settings[21,22].

CONCLUSION

In this study, we optimized the purification protocol for Trastuzumab using BHK21 and HCC1954 cell lines. Trastuzumab, a humanized monoclonal antibody, effectively induces cell cycle arrest and apoptosis and inhibits angiogenesis, particularly in HER2-positive breast cancer. Plasmid DNA encoding trastuzumab was isolated, and its integrity was confirmed via electrophoresis. The purified antibody showed significant anti-proliferative effects with an IC50 of 102.66 µM in an MTT assay on HCC1954 cells. SDS-PAGE further confirmed protein expression. These results demonstrate Trastuzumab's potential as an effective anti-HER2 therapeutic agent for breast cancer.

Ethical permission: University of Lahore, Pakistan, ERC letter No. IMBB/BBBC/22/182.
Conflict of Interest: No conflicts of interest, as stated by authors.
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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically.

AUTHOR CONTRIBUTION

Azhar MM: Literature search, review, study design
Maqbool T: Designing of questionnaire, data collection
Altaf A: Statistical data analysis
Akbar A: Literature search
Mustafa I: Data interpretation
Qureshi ZH: Drafting

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