

Trastuzumab therapies in human epidermal growth factor receptor 2 cancer

Jingyun Wu^{1, a, †}, Pianhong Xu^{2, b, †}, Shinong Zeng^{3, †, *}

¹Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

²Bullis School, 10601 Falls Road, Potomac, Maryland, USA

³Department of Biomedical Engineering, University of Southern Science and Technology, Shenzhen, Guangdong Province, China

*Corresponding author: 12012712@mail.sustech.edu.cn, ^ajingyun@live.unc.edu, ^bamberlyxu@gmail.com

†These authors contributed equally.

Abstract: Monoclonal antibodies therapy had shown critical importance in terms of personalized, disease-specific medicine. One example of monoclonal antibody is Trastuzumab, targeting on diseases such as breast cancer. Despite side effects like fever, chills, headache, infection, congestive heart failure, Trastuzumab is proved to be highly effective in experimental studies and clinical practice. Research has shown that through binding with human epidermal growth factor receptor 2, Trastuzumab is able to decrease its signaling pathways and therefore, inhibit cell proliferation and initiate apoptosis. The results of completed clinical trials and ongoing experimental studies, mainly focusing on the combination of trastuzumab and other treatments such as chemotherapy, have shown that compared with monotherapy or traditional conventional treatments such as surgery, radiotherapy, chemotherapy, etc., the combination of trastuzumab has a longer survival period and a lower recurrence rate. Some prime examples are discussed in this review to indicate recent trends of research on this topic. In addition, an in-depth analysis of possible directions of development of this technology is introduced.

Keywords: Monoclonal Antibodies, Trastuzumab, HER-2 Cancer, Therapies.

1. Introduction

Today's medicine has been revolutionized into personalized, disease-specific medicine, and monoclonal antibodies therapy is considered to be a prime example of personalized therapeutics. People's understanding of the concept of targeted therapy like monoclonal antibodies had increased throughout recent years' research on biological pathways involved in cancer pathogenesis.

There are several mechanisms that monoclonal antibodies can act on cancer cells. According to some in vitro studies, monoclonal antibodies can lead to apoptosis in tumor cells through either binding to surface receptors that result in a signaling cascade that triggers a death signal, or blockade of growth factor receptor signaling. For instance, EGFR can be overexpressed so as to cause cancer, and what the monoclonal antibodies can do to prohibit cell proliferation is block ligand binding and receptor dimerization. One typical example of this type of cancer treatment is Trastuzumab, a targeted cancer drug, which is also known as Herceptin, Herzuma, and Ontruzant [1]. Trastuzumab is directed against the overexpressed HER2 in tumor cells. It works through attaching to HER2 on the tumor cell surface and prohibits the cancer cells from growing and dividing by inducing antibody-dependent cell-mediated cytotoxicity [2].

Currently, Trastuzumab is licensed for the treatment of HER2-positive breast cancer, Metastatic HER2-positive breast cancer, and HER2-positive gastric cancer in the United States. The treatment for HER2-positive breast cancer is adjuvant therapy. Patients receiving this therapy usually have completed anthracycline or taxane-based chemotherapy [3]. For Metastatic HER2-positive breast cancer, Trastuzumab can be served as monotherapy or combined with the use of paclitaxel. Recently, FDA approved tucatinib (Tukysa) for use in combination with trastuzumab to treat patients with

HER2-positive breast cancer. For HER2-positive gastric cancer, Trastuzumab is approved for combination use with cisplatin-based chemotherapy [4].

For recent studies, Trastuzumab is used in combination with other monoclonal antibodies or chemotherapy drugs to further investigate new methods of effective treatment of cancer. One ongoing clinical study would be the combination use along with Perioperative Lapatinib, targeting on HER2-Positive Breast Cancer. Besides this, Trastuzumab biosimilar equivalent is also under research for cancer treatment. One example would be HD201 for neoadjuvant treatment in people with HER2-positive early breast cancer. And it demonstrated the equivalence with trastuzumab in terms of efficacy for the total pCR endpoint and safety [5].

The general mechanism for Trastuzumab is that it binds to the extracellular juxtamembrane domain of HER2 to prohibit the HER2 tumor from proliferating or living. The HER2 signaling involves the RAS–MAPK pathway and mTOR pathway. These two pathways promote cell proliferation and inhibit apoptosis [6]. When Trastuzumab binds to the extracellular domain of the HER2 receptor, it decreases the cell signaling through blocking the release of intracellular tyrosine kinase [7]. Furthermore, it is hypothesized that Trastuzumab is recruiting immune effector cells to enhance antibody-dependent cytotoxicity (figure 1). Current studies are attempting to take advantage of this feature to develop HER2-targeted vaccines and combination use of Trastuzumab and activated CD8+ lymphocytes [6].

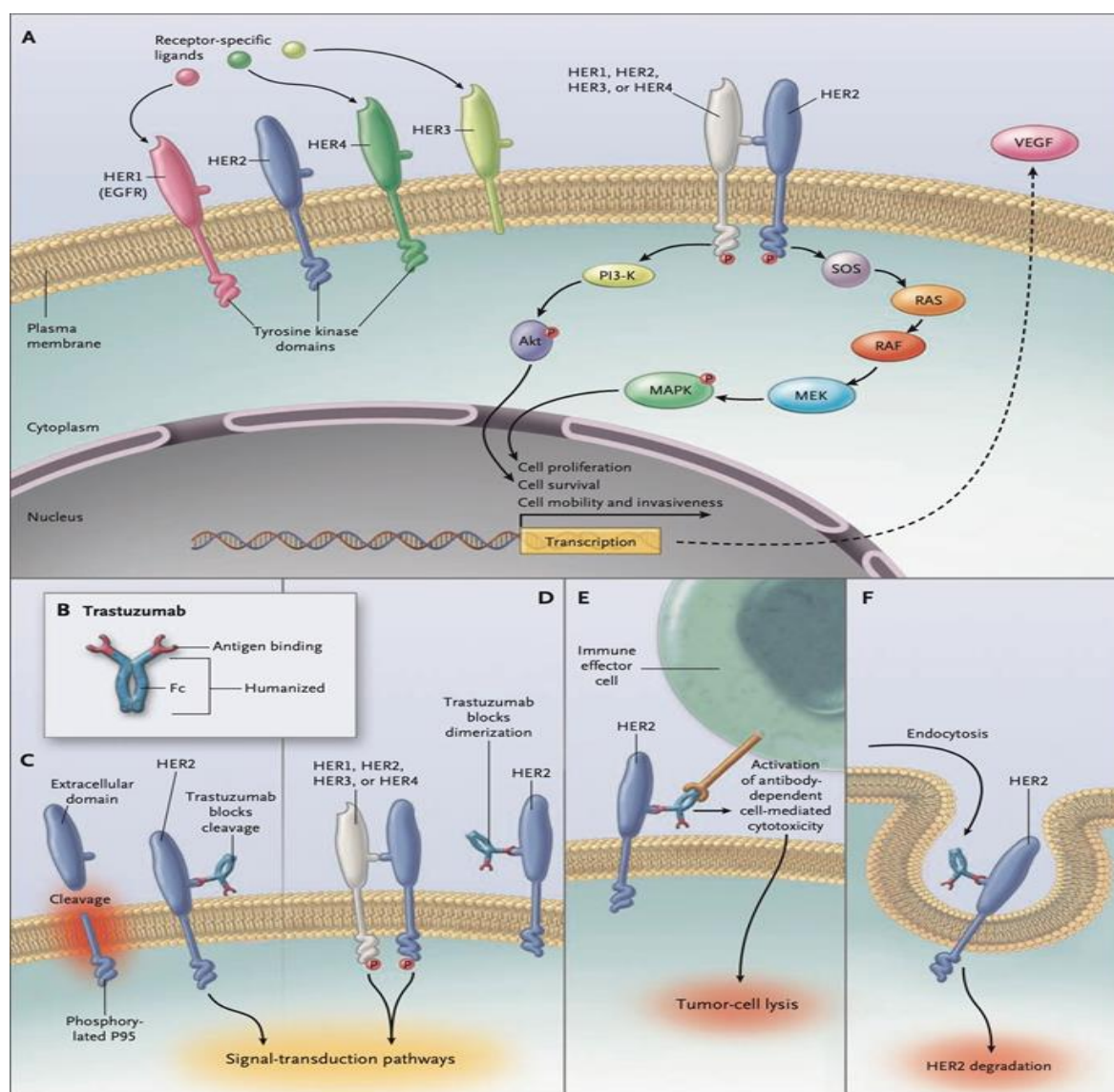


Figure 1. Mechanism of Trastuzumab on HER2 [6].

Trastuzumab is a widely used treatment option for several types of cancers and it has the potential of developing novel combinations with other treatments. Hence, different aspects of this technology are introduced and discussed in this review to explore and propose possible directions in future preclinical studies. This article will introduce the discovery of drugs, the mechanisms of actions of drugs, and the outcome and grade of the phase study when treating HER2 cancer-related Trastuzumab.

2. Summary of previous technology in HER2 cancer treatment

In the past time, HER2 not only can be treated by Trastuzumab alone but also by the combination between Trastuzumab and chemotherapy, which is a great prior. It was also the first humanized monoclonal antibody approved for HER2+ BC, functioning through multiple impressive molecular mechanisms. Moreover, trastuzumab has powerful mechanisms of action to treat HER-2 cancer. One of these pathways is the degradation of the HER2 receptor [8]. The extracellular transmembrane HER2 receptor region has been bound to the trastuzumab, causing it to internalize and be degraded by a ubiquitin ligase called c-CBL. Second, trastuzumab binds to the HER2 receptor, making it cytotoxic. Antibody-dependent cellular cytotoxicity occurs when the IgG1 Fc region of trastuzumab triggers the FcRIII/CD16 found on natural killer cells (ADCC). Lastly, trastuzumab's extracellular HER2 binding reduces proliferative signaling and cellular growth by inhibiting downstream HER2 signaling via the PI3K/AKT and RAS/MAPK pathways. [9].

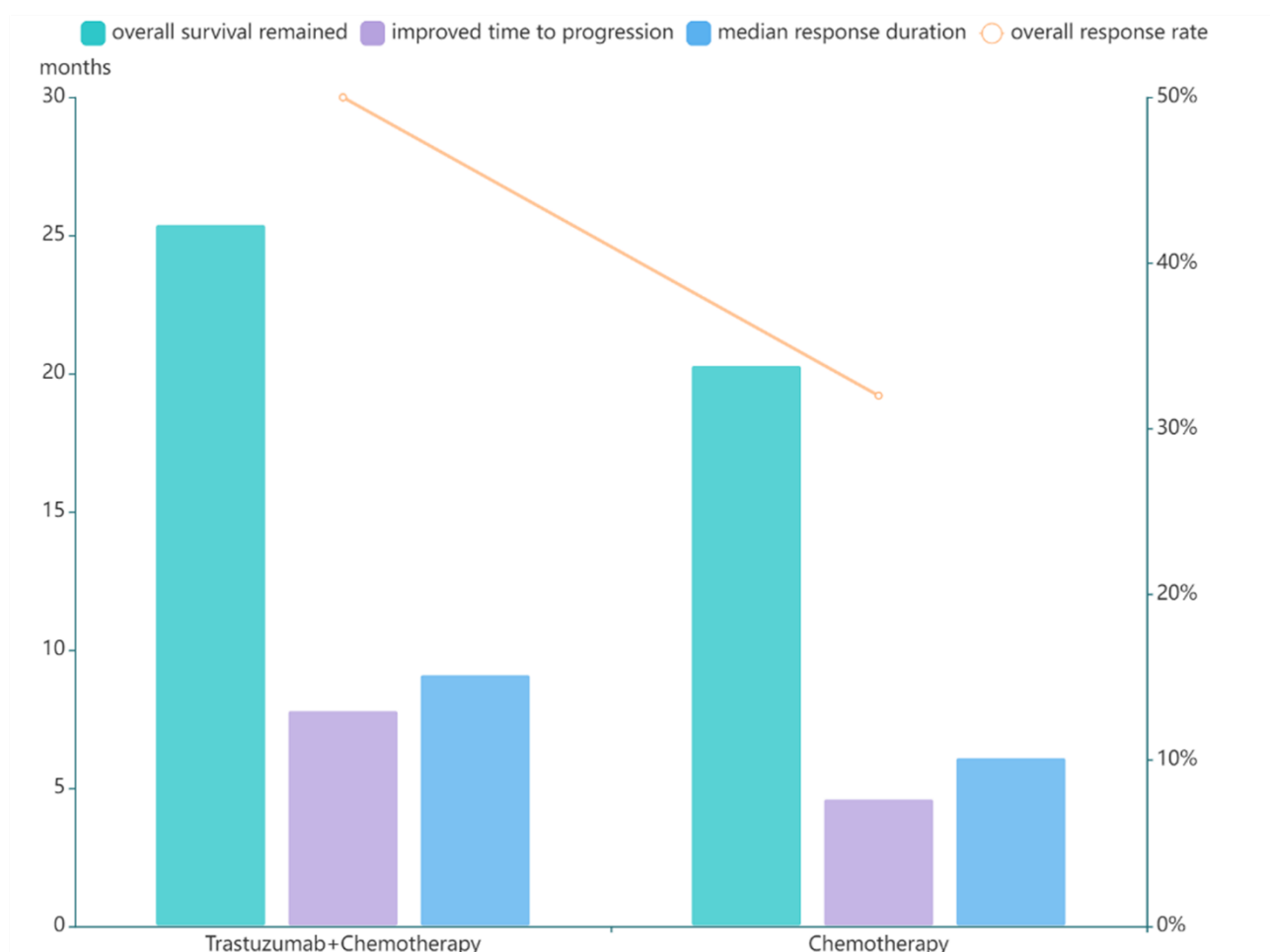


Figure 2. The data between chemotherapy and Trastuzumab + chemotherapy [11].

It showed a few promises as a novel comparatively non-toxic targeted therapy, with reported response rates of more than ten percent. This meant that it became obvious that a considerable amount of women were able to maintain illness stability for long periods (>6 months) [10]. Furthermore,

researchers presented the findings of a multi-center clinical trial of trastuzumab involving more than 450 individuals with breast cancer. The researchers divided the patients into two sectors, and used different approaches with chemotherapy, respectively. Anthracyclines with cyclophosphamide (AC) alone or in combination with trastuzumab were given to patients who had never been exposed to them before. Paclitaxel was given alone or in combination with Trastuzumab to patients who had previously been treated with anthracyclines. After 14 months, the combination between trastuzumab and chemotherapy resulted in a considerably improved time to disease progression (Figure 2, $p=0.0001$), and higher median response duration, which is 9.1 months versus 6.1 months, $p=0.0002$, as well as a significantly higher overall response rate at 50 percent versus 32 percent, $p<0.0001$. Overall survival remained considerably higher during a 29-month follow-up at $p<0.025$, and the result is 25.4 vs. 20.3 months. Every statistic revealed that using trastuzumab in combination with either cyclophosphamide or paclitaxel is more efficient and effective than using the medicine alone. Based on the outcomes of these two pivotal studies, the FDA authorized trastuzumab for the treatment of metastatic breast cancer.

It does, however, have a negative effect, since heart dysfunction was the most serious adverse event reported [10]. In this trial, cardiac dysfunction was seen in 26% of patients who received trastuzumab and AC, 12% of patients who received paclitaxel and trastuzumab, 6% of patients who received AC alone, and 2% of patients who received paclitaxel alone. Trastuzumab's use as adjuvant therapy is still being researched. Because most contemporary adjuvant drugs are based on anthracyclines, their use in this context is hampered by the risk of cardiotoxicity. Therefore, the researchers should improve this technique for the safety of patients. And because the direction is advanced and popular in this field, more innovative and advanced techniques had been made in these two decades.

3. Summary of current technology in HER2 cancer treatment

In the last two decades, a few innovative drugs related to HER2 have been invented, as well as new mechanisms with Trastuzumab to treat HER2 and other diseases. Many new products related to the connection between Trastuzumab and other medicals not only have established or kept doing in the phase study but also have been approved by the FDA. Next, this article will introduce a list of drugs.

Margetuximab is a novel drug to treat HER2 cancer, which is a kind of monoclonal antibody derivative of trastuzumab, that binds to the same epitope of the HER2 receptor with antiproliferative effects and similar affinity as trastuzumab [11]. In preclinical tests, the appropriately designed Fc region was demonstrated to improve ADCC, overcoming trastuzumab resistance, and maintaining efficacy in HER2-low tumors. It has an IgG1 Fc region that is genetically engineered to have up to 6.6x greater affinity for the stimulatory CD16A FcRIIIA found on NK cells and 8.4x less affinity for the inhibitory CD32B FcRIIB found on immune effector cells (NK cells and macrophages) within the innate immune system's ADCC process than trastuzumab.

A phase 1 study included 66 patients with advanced, HER2- overexpressing malignancies (IHC 2+ or 3+) for whom no conventional treatment was available. (NCT01148849) [11]. This trial enlisted the participation of more than 50 people. The MTD was not attained for any regimen, according to the researchers. The bulk of the toxicities were Grade 1 and 2 constitutional symptoms like nausea, pyrexia, and tiredness, which were well-tolerated. In 12 percent and 50 percent of 60 response-evaluable patients, confirmed partial responses and stable disease were observed, respectively; 70% of these patients had previously received HER2-targeted treatment. Tumor reductions were seen in more than half of Response-evaluable cancer patients (78%), including those who had responded for more than 30 weeks. As a result, Margetuximab was well tolerated and showed promise as a single agent. It is still being developed as a single agent and in conjunction with other treatments.

Ado-trastuzumab emtansine (T-DM1) is an antibody-drug conjugate (ADC) of trastuzumab covalently bonded to the chemotherapeutic agent emtansine (DM1), according to certain researchers

[12]. T-DM1 was authorized by the FDA in 2013 for metastatic HER2+ BC, making it the first ADC approved for HER2+ mBC patients. Following the outcomes of the KATHERINE Trial, T-DM1 was recently licensed in the adjuvant setting for patients with the persistent invasive disease after neoadjuvant trastuzumab and chemotherapy. T-DM1 combines trastuzumab's anti-HER2 characteristics with the anti-tubulin properties of high-dose, concentrated DM1 chemotherapy. T-DM1 attaches to HER2 and is internalized into the cancer cell, where it is proteolytically cleaved, releasing and activating DM1 [13].

T-DXd is an innovative HER2 antibody–drug combination that consists of an exatecan derivative called DX-8951 derivative topoisomerase I inhibitor and a monoclonal anti-HER2 antibody. In models of HER2-positive gastric cancer cell lines and xenografts, T-DXd displayed anti-tumor activity [14]. The FDA was the first to approve it for patients with metastatic or unresectable disease. The first and most important problem in developing an ADC is linker stability in plasma, which is vital for reducing systemic toxicity. Trastuzumab's cysteine residues bind to the DXd via an enzymatically cleavable peptide-based linker, which avoids the problem. The researchers performed a DNA topoisomerase I inhibition trial to demonstrate that DXd has ten times the potency of SN-38, used to treat a variety of solid tumors. Therefore, the further efficacy suggests that DXd would like to be an excellent ADC payload selection [15].

T-DXd is anticipated to target the same antigen as trastuzumab while additionally providing a customized cytotoxic payload. T-DXd works by inducing a bystander antitumor impact on tumor cells when a cytotoxic payload is administered. Due to the payload's strong membrane permeability, T-DXd can also diffuse across membranes, infiltrating and damaging surrounding tumor cells. This property is especially useful when targeting malignancies that express the targeted antigen in a heterogeneous manner. Furthermore, preclinical investigations have indicated that DXd is extremely membrane permeable and that T-DXd has a bystander anticancer impact [16]. The dosage and efficacy of T-DXd have been established by results from the phase 1 DS8201 A-J101 study and the phase 2 DESTINY-Gastric01 study (NCT03329690). The goal of the study was to discover the mechanism of treating HER-2 gastric tumors and other HER-2 malignancies (Figure 3). The DESTINY-Gastric01 study met its primary goal of a significantly improved objective response rate for T-DXd versus physician's choice treatment. ($P < 0.001$) (51 percent versus 14 percent, respectively) [17]. Also, as the paper points out, T-DXd had a median duration of confirmed objective response of 11.3 months, which was longer than Chemotherapy plus Trastuzumab. (9.1 months) While it has a few resistances, all patients in both trials experienced a treatment-emergent adverse event, which is known as TEAE. TEAE occurred in one-fifth of participants in the phase 2 trial (Figure 3). The most common grade 3 events in a fifth of patients treated with T-DXd were hematological: white blood cell count decline (16 percent against 21 percent), neutrophil count drop (20 percent against 51 percent), and anemia at 30 percent versus 38 percent. In these two trials, TEAEs caused dose reductions in 16 percent and 32 percent of patients, respectively. Moreover, in the phase 2 study, the two treatment groups had similar TEAEs that resulted in dose reduction, which is 32 percent and 34 percent, respectively [11]. As a result, trastuzumab deruxtecan has a better response than the physician's choice of chemotherapy in this phase 2 study, while it has a few side effects and TEAE, which is that we should concentrate on it.

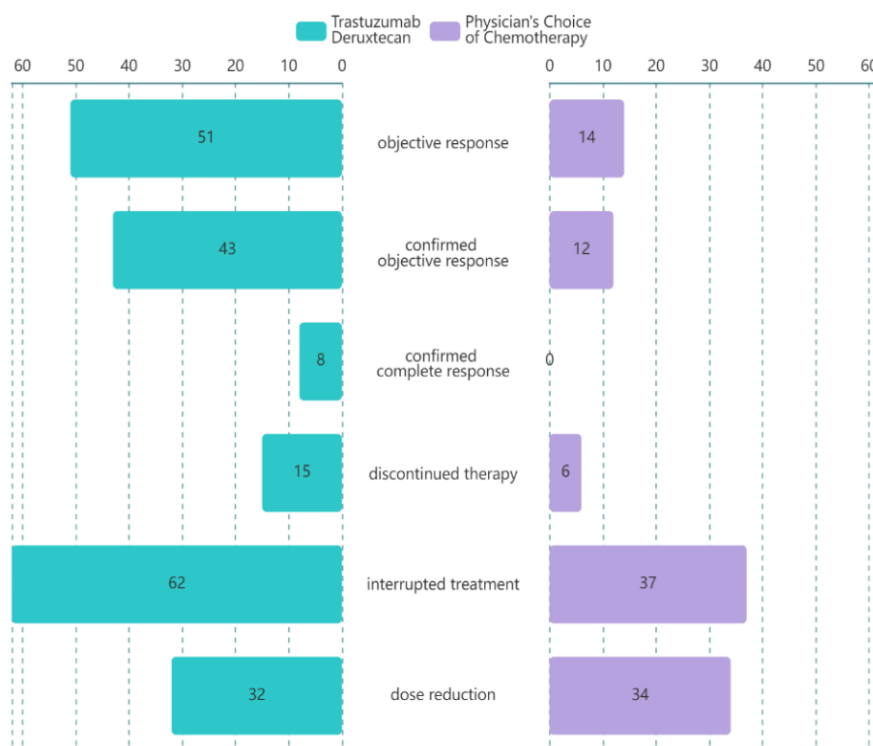


Figure 3. The figure in the phase 2 study compared to the Trastuzumab Deruxtecan with Physician's Choice of Chemotherapy [17].

Therefore, the drug to treat HER2 is so mature that patients can be used safely and risk-freely. And patients can have their own drugs and dosage according to their own diseases. However, this field still has a couple of resistances that we should undertake and overcome like the dose reduction, Interstitial lung disease (ILD) related HER-2 cancer, cardiotoxicity, and so on when treating HER-2 using Trastuzumab. So, in the future, researchers still work on it.

4. Future Direction of Trastuzumab

Ongoing research studies on Trastuzumab generally focus on the improvement of its performance in the clinical setting. For example, many recent studies have concerned themselves with Trastuzumab's role in biomedicine when combined with other antibiotics or treatment methods. One of the most popular combinations of drugs under investigation is the combination of trastuzumab, paclitaxel, pertuzumab and atezolizumab. Randomized trial studies are designed to look into the effect of trastuzumab along with the two other medicines with or without atezolizumab in treating patients with breast cancer that has shown clear signs of spreading to other parts of the patient's body (metastatic). The potential therapeutic effects of other combinations including trastuzumab are also at different stages of testing. For example, ongoing studies continue to identify and categorize how well trastuzumab, vinorelbine tartrate, and avelumab with or without utomilumab work in treating patients with HER2-positive breast cancer [18]. In addition to continued research on pharmaceutical novel combinations of trastuzumab, this drug and its placebo effects are also tested in different cancer stages of patients. Many current research groups devote themselves to test the ability of trastuzumab to decrease chemotherapy in patients who are diagnosed with HER2-Positive Breast Cancer but have no remaining cancer at surgery after limited pre-operative chemotherapy and HER2-Targeted Therapy [19]. Due to the fact that trastuzumab is a relatively mature medicine active in market and widely employed in biomedical institutions, ongoing research usually are identified as clinical trials, which are research studies that involve people. Therefore, the careful design and safety implementation of the research studies of trastuzumab are significant factors to ensure in future studies. On top of these precautions measures, there are many other effects and potential improvements of trastuzumab that can be studied in the near future.

Trastuzumab's use with first-line chemotherapy has been the main therapeutic focus for the past decade. As a monoclonal antibody developed to target the HER2 receptor which is overexpressed by breast, stomach, or esophagus cancer cells, especially commonly expressed in breast cancer cells, Trastuzumab is mostly taken intravenously by patient as a side-treatment along with the most selected standard treatment for cancer, the chemotherapy. However, previous studies have started to investigate the underestimated medical effects of Trastuzumab when used with other types of antibiotics. Since Trastuzumab provides viable therapeutic benefits to cancer patients, its usage with other medicine can be predicted. This is partially because of Trastuzumab's high demand and still growing popularities in the recent years. Since more cancer patients choose this option and oftentimes cancer patients are under multiple medications or treatments at the same time, the effectiveness and health risks of taking Trastuzumab with other types of medicines need to be studied as thoroughly as possible in order to maximize treatment benefits and more importantly, to ensure the safety of the patient. Trastuzumab's effects when applied with other common treatment methods are also rewarding to look into for pharmaceutical research and development purposes. It is possible to create and refine novel medical effects during the investigation of Trastuzumab with other treatments. Until now, many of its combinations with other antibiotics are still under development. For example, Tucatinib has been revealed by recent studies as a highly selective inhibitor of the HER2 tyrosine kinase, but its functions, when combined with Trastuzumab, are still investigational and experimental [20]. Future studies should explore more combination possibilities like this to enrich the treatment options for patients with human HER2-positive metastatic breast cancer as well as to ensure a more secure foundation for the implementation of multiple cancer treatment methods used on patients at the same time in a realistic clinical setting, rather than a controlled laboratory environment.

Not only is the use of Trastuzumab during cancer treatment worthy of further investigations, it is also rewarding to look into its roles after the treatment itself. After the course of the disease treatment for breast cancer and many other types of cancers that are related to the HER2 receptor, the use of Trastuzumab is advised to patient within their treatment period. And most application of Trastuzumab would gradually come to a stop after the disease reaching an end of its deterioration. However, the clinical benefits of continued use of trastuzumab after disease progression are explored and categorized by some previous retrospective and observational studies [21]. These studies primarily look at individual cases of cancer patients whose medication history of Trastuzumab were not particularly confined by their cancer development stages. These studies collect the patients continued health changes and relatively long-term physical status after their cancer progression as they were still under Trastuzumab. They were able to find enough evidence to draw qualitative connections that emphasize the positive effects of taking Trastuzumab after the designated treatment plan. However, more quantitative analyses on the patient response rates and survival outcomes should be done in the near future to understand trastuzumab's effects beyond each specific type of cancer's progression. While assessing the continued effects of Trastuzumab on post-treatment patient, it is also very important to promote the correct use of this monoclonal antibody. It is noticeable that supervised continuation of medication does not equal to unauthorized overuse of drug. The former has the potential to bring patients extra medical benefits; whereas the latter often leads to drug resistance, which is signified by the non-responsiveness of viruses, bacteria, or cancer cells to a certain type of antibody. In order to prevent overuse of Trastuzumab, the safe use of this medication should be studied and recommended to patients.

Due to its wide and common use along with chemotherapy, the side effects of Trastuzumab are observed in many treatments. Such side effects include fever, chills, headache, infection, congestive heart failure, insomnia, cough, and rash. These seemingly common negative effects on patients can be extremely detrimental to cancer patients in some cases. For example, these side effects can pose serious health threats on at-risk populations with other severe or chronic health concerns, which are frequently observed in cancer patients. Furthermore, these side effects can usually greatly increase the overall stress and suffering during the patient's treatment experience, which would in turn, effect

the overall efficacy of curing inevitably. In order to enhance the patient's treatment experience, future studies should focus on reducing these common side effects seen in intravenous trastuzumab.

In summary, the future directions of research on Trastuzumab should primarily focus on discovering novel combinations, assessing the efficacy of the combination, exploring Trastuzumab's effects in different clinical stages, especially those after disease progression, preventing its overuse and reducing existing side effects.

5. Conclusion

Conventional surgical treatment of breast cancer and other diseases has serious physical and mental damage to patients. Moreover, the problem of tumor recurrence after conventional treatment has not been well resolved. The development of monoclonal antibodies such as Trastuzumab has brought light to the solution of HER2-positive tumors. Numerous clinical data show that Trastuzumab has a therapeutic effect on various HER2-positive cancers, which depicts a bright future of patient-specific therapy and clinical trial transformation methods. However, the optimal use of Trastuzumab remains an open question. While experimental studies have shown encouraging results, further investigations should be performed to discover more efficient use of this technology and reduce its side effects.

Reference

- [1] Trastuzumab. Trastuzumab (Herceptin) | Cancer information | Cancer Research UK. (2021, April 16). Retrieved March 9, 2022, from <https://www.cancerresearchuk.org/about-cancer/cancer-in-general/treatment/cancer-drugs/drugs/trastuzumab>
- [2] Iqbal, N., & Iqbal, N. (2014). Human epidermal growth factor receptor 2 (HER2) in cancers: Overexpression and therapeutic implications. *Molecular biology international*. Retrieved March 9, 2022, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4170925/>
- [3] Baselga, J., Perez, E. A., Pienkowski, T., & Bell, R. (2006). Adjuvant trastuzumab: A milestone in the treatment of her-2-positive early breast cancer. *The Oncologist*, 11(S1), 4–12. <https://doi.org/10.1634/theoncologist.11-90001-4>
- [4] Greenblatt, K. (2021, December 4). Trastuzumab. *StatPearls* [Internet]. Retrieved March 9, 2022, from <https://www.ncbi.nlm.nih.gov/books/NBK532246/>
- [5] Nanda, S. (2022, March 7). Trastuzumab biosimilar equivalent to referent drug in neoadjuvant setting. *oncology.medicinematters.com*. Retrieved March 17, 2022, from <https://oncology.medicinematters.com/breast-cancer/biosimilars/troika-trastuzumab-biosimilar-equivalent-neoadjuvant-setting/20197364>
- [6] Hudis, C. A. (2007). Trastuzumab — mechanism of action and use in clinical practice. *New England Journal of Medicine*, 357(1), 39–51.
- [7] Malik, B. (2021, July 5). Understanding how monoclonal antibodies work. *StatPearls* [Internet]. Retrieved March 9, 2022, from <https://www.ncbi.nlm.nih.gov/books/NBK572118/>.
- [8] O'Mahony, D., & Bishop, M. R. (2006). Monoclonal antibody therapy. *Frontiers in bioscience : a journal and virtual library*, 11, 1620–1635.
- [9] Kreutzfeldt, J., Rozeboom, B., Dey, N., & De, P. (2020). The trastuzumab era: current and upcoming targeted HER2+ breast cancer therapies. *American journal of cancer research*, 10(4), 1045–1067.
- [10] Slamon DJ, B Leyland-Jones, S Shak, H Fuchs, VPaton, A Bajamonde, T Fleming, W Eiermann, J Wolter, M Pegram, J Baselga, & L Norton: Use of chemotherapy plus a monoclonal antibody again for metastatic breast cancer that over-express HER2. *N Engl J Med* 344, 783-792 (2001)
- [11] Shitara, K., Baba, E., Fujitani, K., Oki, E., Fujii, S., & Yamaguchi, K. (2021). Discovery and development of trastuzumab deruxtecan and safety management for patients with HER2-positive gastric cancer. *Gastric cancer : official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association*, 24(4), 780–789.
- [12] FDA approves ado-trastuzumab emtansine for early breast cancer. 2019.

- [13] Oostr D and Macrae E. Role of trastuzumab emtansine in the treatment of HER2-positive breast cancer. *Breast Cancer* (Dove Med Press) 2014; 6: 103-113.
- [14] Mishima, S., & Shitara, K. (2021). Trastuzumab deruxtecan for the treatment of HER2-positive gastric cancer. *Expert opinion on biological therapy*, 21(7), 825–830.
- [15] Ogitani Y, Aida T, Hagihara K, Yamaguchi J, Ishii C, Harada N, et al. DS-8201a, a novel HER2-targeting ADC with a novel DNA topoisomerase I inhibitor, demonstrates a promising antitumor efficacy with differentiation from T-DM1. *Clin Cancer Res*. 2016;22(20):5097–108.
- [16] Bang, Y. J., Giaccone, G., Im, S. A., Oh, D. Y., Bauer, T. M., Nordstrom, J. L., Li, H., Chichili, G. R., Moore, P. A., Hong, S., Stewart, S. J., Baughman, J. E., Lechleider, R. J., & Burris, H. A. (2017). First-in-human phase 1 study of margetuximab (MGAH22), an Fc-modified chimeric monoclonal antibody, in patients with HER2-positive advanced solid tumors. *Annals of oncology : official journal of the European Society for Medical Oncology*, 28(4), 855–861.
- [17] Shitara K, Bang YJ, Iwasa S, Sugimoto N, Ryu MH, Sakai D, et al. Trastuzumab deruxtecan in previously treated HER2-positive gastric cancer. *N Engl J Med*. 2020;382(25):2419–30.
- [18] National Cancer Institute (2022). Clinical Trials Using Trastuzumab. NIH. <https://www.cancer.gov>
- [19] National Cancer Institute (2022). Testing the Ability to Decrease Chemotherapy in Patients with HER2-Positive Breast Cancer Who Have No Remaining Cancer at Surgery after Limited Pre-operative Chemotherapy and HER2-Targeted Therapy. NIH. <https://www.cancer.gov>
- [20] Murthy, R. K. Tucatinib, Trastuzumab, and Capecitabine for her2-Positive Metastatic Breast Cancer. *New England Journal of Medicine* 2020, 382 (6), 586–586.
- [21] Pegram, M.; Liao, J. Trastuzumab Treatment in Multiple Lines: Current Data and Future Directions. *Clinical Breast Cancer* 2012, 12 (1), 10–18.