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Magnetic Resonance Imaging in Drug Development

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Abstract: Using a popular diagnostic method such as magnetic resonance imaging (MRI) for imaging cell cultures can contribute to scientific research. With an optimally selected testing protocol, it will be possible to image cell lines using MRI at the cellular level and then implement pharmaceutical substances into ba-days and test their effectiveness in cell cultures. Preliminary studies on pharmaceutical substances show that the application of MRI for *in vitro* experimental studies yields satisfactory differences in signal intensity, so it turns out to be important to implement *in vitro* studies and then try to transfer them to *in vivo* studies. Trastuzumab is an IgG1 monoclonal antibody that binds selectively to human epidermal growth factor receptor 2. It is used to treat breast cancer in patients whose tumors overexpress the Her-2 receptor. The implementation of cell culture research is helping to understand cell biology. Pharmaceutical research using 3D cell culture provides biologically relevant models for determining drug activity in tumor tissue, which can contribute to assessing response to therapies and improving drug resistance. Attempts to use MR relaxation times measured in cancer cell cultures help assess the biological changes of the applied drug therapy. Relating the results of relaxation times to the results of abundance and viability will allow assessment of the effect of Trastuzumab on viability in cell cultures without interference.

Keywords: tracking; drug; cell culture; MRI; relaxation time.

1. Introduction

In recent years, breast cancer has been increasing, one of the three most common cancers worldwide [1]. It is the most common type of cancer among women, affecting more than two million women annually. HER receptors play a major role in the pathogenesis of breast cancer. The HER receptor family includes receptors: HER1, HER2, HER3, and HER4. Too many copies of the ERBB2 gene encoding the HER2 protein affect its overexpression in the tumor cell [2]. Overexpression of the HER2 receptor is found in about 20-25% of breast cancer cases, which affects the course and treatment of breast cancer [3]. Immunotherapies used to treat this type of cancer are usually based on monoclonal antibodies [4]. One example is Trastuzumab class IgG1, which exhibits therapeutic activity against the HER2 receptor-positive domain. Trastuzumab is a biologic anticancer agent approved by the American Food and Drug Administration (FDA) in 1998 [5]. It is a large protein molecule with a molecular weight of 145,531 Da [6]. It acts against the extracellular domain of the HER2 receptor, binding to the membrane-bound subdomain IV of the extracellular portion of the HER2 protein. Binding to the receptor inhibits the transmission of division signals to the cell's nucleus,

slowing tumor growth. When HER2 is overexpressed, cancer cells proliferate at a higher rate due to higher growth factors. In addition, they show greater drug resistance [7]. The main indications for the implementation of treatment with Trastuzumab are the treatment of breast cancer with tumor overexpression of HER2, palliative treatment, early-stage cancer, metastatic breast cancer, and gastric cancer with metastasis. As for the mode of delivery, the drug Trastuzumab is mainly administered intravenously by infusion (the solution administered is 21 mg/mL), but recent reports also consider subcutaneous delivery. In therapies, it is used alone or in combination with chemotherapy. We have several developed treatment regimens with varying duration of therapy. It is a certain predetermined time, for example, 6 months, until the maximum clinical response, until the occurrence of progression, or even after its occurrence, for example, in the case of dissemination to the central nervous system (due to the fact that the trastuzumab molecule does not penetrate the central nervous system - hence it does not act in this area). The monoclonal antibody Trastuzumab is a mirror image of the protein receptor, which can attach to the receptor and thus block the growth factor, slowing tumor development. It stops the process during the G1 phase of the cell cycle, resulting in decreased proliferation. Normal cells have two copies of the HER2 gene with a small number of receptors on the surface, while in the case of cancerous lesions, we have additional copies of the HER2- gene, and thus more receptors on the cell surface. In the case of overexpression, there is a chance of a positive response to therapy with Trastuzumab. Compared to the chemotherapeutics used, the antibodies show lower cytotoxicity. Herceptin therapy began in 1998 [8]. In 1998 the monoclonal antibody Trastuzumab and capecitabine were approved for the treatment of metastatic breast cancer, and in 1999 the same antibody was used by Wong [9] in the first published studies. And in 1999, Goldenberg showed in preclinical studies both *in vitro* and *in vivo* that combination therapy with Paclitaxel or carboplatin significantly improves the growth inhibition of HER2 tumor cells, with the best results in the combination of Herceptin with the drug Paclitaxel. In addition, in 1999, Schaller et al. in a study described therapy for metastatic breast cancer using humanized antibodies against the HER2 receptor protein. The study also showed that weekly combination therapy with Paclitaxel and Trastuzumab showed a therapeutic response in 83% of treated HER2-positive patients. In contrast, subsequent *in vivo* studies have observed a high response rate of up to 80% in combination therapy of Trastuzumab with vinorelbine [10]. The effects of combination chemotherapy are associated with a 20% reduction in mortality. It should be noted, however, that there are reports that discontinuing anti-HER2 therapy results in tumor growth inhibition, which may be related to the re-expression of HER2 due to mitogenic signaling [11]. Thus, it can be said that preliminary results suggest that Trastuzumab, in combination with chemotherapy, leads to better clinical outcomes [12]. Adding Trastuzumab therapy to other systemic therapies leads to a striking increase in response and, thus, survival [13]. Combining Trastuzumab with Pertuzumab provides a more comprehensive HER2 receptor blockade. The Cetin group has studied and reported the efficacy of Lapatinib and Capecitabine in combination in metastatic breast cancer [14]. The drug compounds exhibit cytotoxicity at a lower level than standard chemotherapeutics. The low efficacy of drug therapies in treating various types of cancer is due to the unknown mechanism of action of the drugs used in the therapies. Targeted drug delivery to the cancer cells turns out to be crucial. In therapies, it is used alone or in combination with chemotherapy. Trastuzumab administered as (neo)adjuvant therapy in radically treated patients with cancer with HER2+ receptor overexpression improves overall survival. A recent study compared Trastuzumab therapy with endocrine therapy to Trastuzumab therapy in

combination with chemotherapy. For patients with hormone receptor-positive and HER2-positive metastatic breast cancer, Trastuzumab, in combination with endocrine therapy, was not inferior to Trastuzumab plus chemotherapy [15]. The monoclonal antibody Trastuzumab is a mirror image of a protein receptor that can attach to the receptor and thereby block growth factors slowing tumor growth. It stops the process during the cell cycle's G1 phase, resulting in reduced proliferation. The duration of Trastuzumab therapy is selected individually, and the optimal time to achieve a complete response is unknown. Studies over the years have shown that the average treatment time was 4.1 years, and 54% of patients experienced a complete response (CR) within 9 months of treatment. The maximum treatment time to achieve a CR was 27 months, and treatment lasting 2 or more years showed better survival [16]. Trastuzumab biosimilar drugs are increasingly being used in therapies, improving patient access to breast cancer treatment by providing safe and effective therapy compared to reference trastuzumab [17,18]. The registered biosimilar drug is Trastuzumab-pkrb (Herzuma). Trastuzumab drug delivery is performed intravenously and subcutaneously, resulting in comparable therapy outcomes. On the other hand, Biosimilar drugs are administered only intravenously and are often used in patients in whom other chemotherapeutics are administered concurrently [19]. Used therapies with Trastuzumab carry the risk of side effects. The most common adverse effect of Trastuzumab is cardiotoxicity, which is a limiting factor in the safe use of the drug. Often, such side effects result in premature discontinuation of the applied therapy. Trastuzumab treatment-induced cardiotoxicity (TIC) is not dose-dependent and does not occur in all patients [20]. Recent studies have evaluated N-terminal brain natriuretic peptide (NT-proBNP), creatine kinase-MB (CK-MB), myoglobin, and selected biochemical and clinical factors as predictors of trastuzumab treatment-induced cardiotoxicity. These have been found not to be predictors of cardiotoxicity, and there is a continuing need to identify biomarkers of TIC [21]. Left ventricular dysfunction (LVD) and heart failure (HF) are relatively common and severe manifestations of cardiotoxicity in cancer therapy [22]. The exact mechanism of trastuzumab cardiotoxicity is unknown, and there is no specific drug to prevent it in clinical practice [23]. Therefore, cardiac monitoring is important during Trastuzumab therapy. Studies show that in patients with metastatic breast cancer, Trastuzumab-induced cardiotoxicity was more common during the first 4 years of treatment [24]. The study shows control of cardiac function during trastuzumab therapy by performing an ultrasound echocardiogram (UCG) before starting Trastuzumab and 6 months after starting Trastuzumab in the adjuvant group and having a UCG before Trastuzumab for the metastatic group. The results show that in an adjuvant group (14,501 patients), 34.7% of patients received adequate UCG control, but many patients still did not receive optimal UCG control [25]. The use of cell cultures in research enables drug testing to select biopharmaceutical compounds and improve their efficacy. 2D cell cultures are routinely used in biotechnology, biopharmacy, and toxicology research. Among other things, they make it possible to assess the effects of drugs on their viability [26]. Studies using cell cultures to predict drugs' toxic and therapeutic properties are common. Cell cultures show varying degrees of similarity to cells present in a living organism, especially in these conditions that reproduce 3D cultures. Research on pharmaceuticals using 3D cell cultures provides biologically relevant models for determining drug activity in tumor tissue, which can contribute to assessing the response to applied therapies and improving drug resistance. Recent research has used radioactively labeled Trastuzumab (TRZ) loaded with solid lipid nanoparticles (SLN). Studies of the mechanism of apoptosis of TRZ-SLN with the MCF-7 breast cancer cell line were conducted. *In vitro* studies showed that TRZ-SLN was biocompatible and effectively

induced apoptosis in MCF-7 cells, while *in vivo* studies in rats yielded a profile of prolonged release in the blood circulation compared to free drug solution by evaluating pharmacokinetic parameters. 99mTc-labeled radioisotope-loaded SLN preparations loaded with TRZ are potential theranostic agents based on their characterization profiles, *in vitro* cellular uptake and apoptosis induction capacity, and *in vivo* pharmacokinetic profiles [27]. Of note is Trastuzumab resistance, which occurs in about 20% of patients receiving Trastuzumab in adjuvant relapse. In addition, about half of patients with HER2-positive metastatic breast cancer develop resistance to Trastuzumab within 1 year. The study presents an evaluation of the relationship between angiogenesis and trastuzumab resistance using a trastuzumab-resistant cell line (SKBR3-TR). Culture supernatant from SKBR3-TR cells significantly increased endothelial cell sprouting, and αB-crystallin increased the ability of the cells to activate mTOR in endothelial cells, thereby promoting angiogenesis [28]. Interactions between estrogen receptors and ER) and erbB tyrosine kinase (RTK) receptors are also important, as they influence HER2+ progression. We evaluated the gene expression dynamics in HER2+ breast cancer patients treated with the neoadjuvant PFHPert. It was found that modulating erbB2 receptors by inhibiting CDK4 / 6 triggers sustained aging in breast cancer of estrogen receptors and ErbB2 [29]. Implementing 3D cell culture studies and evaluating the drug's efficacy with Trastuzumab emtansine (Kadcyla®, T-DM1) on five breast cancer cell lines (BT-474, SK-BR-3, MDA-MB-361, MDA-MB-175, and MCF-7) is presented. The study confirmed a difference in T-DM1 drug activity in 3D spheroids or aggregates compared to 2D cultures. This may be related to tumor heterogeneity and less efficient internalization of T-DM1, which are not present in 2D cell cultures [30]. We also have a report on determining the effect of simultaneous irradiation and T-DM1 on HER2+ breast cancer cell lines. A study was performed on five breast cancer cell lines with different levels of HER2 expression to evaluate the therapeutic effect of T-DM1 in combination with radiation. The results showed significant mortality due to the intracellular effects of DM1 on the cell cycle with significant blocking of the G2 / M phase. HER2 expression shows radioresistance, but T-DM1 is not a radiation-sensitive agent under experimental conditions that allow cell survival [31]. The study determined cellular responses to the drug trastuzumab, Paclitaxel, carboplatin, and a combination of the three drugs using the XTT cytotoxicity assay. The analysis showed that the levels of matrix metalloproteinase MMP and the proteins TIMP-1, TIMP-2 were more highly expressed in HER2 BCSCs than in primary culture, and HER2 BCSCs showed greater drug resistance than HER2 BCSCs [32].

The magnetic resonance imaging method allows us to obtain information on the density distribution of atomic nuclei, for example, ^{1}H, but also ^{13}C, ^{14}N, ^{17}O, ^{23}Na, ^{39}K, ^{43}Ca [33]. The main information we get with nuclear magnetic resonance imaging is the density distribution of atomic nuclei. Because the nucleus of the hydrogen atom has the highest gyromagnetic coefficient among atomic nuclei, and because of the high concentration of hydrogen in the human body, an image of the density distribution of this particular element is obtained using MRI. Thus, in the study, we get a very good image of soft tissues (e.g., the brain), distinguishing this method from X-ray computed tomography, which is best for imaging bones. In addition, after the RF pulse is turned off, the magnetization returns to thermal equilibrium. There are two relaxation times: T_1 (longitudinal or spin network) and T_2 (transverse or spin/spin). By measuring the magnetization relaxation times - T_1 and T_2, it is possible to obtain information about certain physical and chemical properties of tissues [34]. There is a need to develop a non-invasive method of monitoring the effects of drugs and accurately analyzing the effects of pharmaceutical substances on the human body. In magnetic resonance, with the change of TE

and TR parameters, the signal intensity changes; selecting these parameters accordingly based on the data obtained, we are able to plot relaxation curves and determine T_1 and T_2 times. One of the methods for determining T_1 and T_2 relaxation times is the SR method.

We can describe the signal intensity by the relation:

$$IS \approx PD\left(1 - e^{\frac{-TR}{T_1}}\right)e^{\frac{-TE}{T_2}}$$

In the above equation, PD stands for proton density-dependent parameter, TE- echo time, the time between the excitation pulse and the measurement, and TR- repetition time. By measuring the signal intensity based on the designated areas of interest (ROI), we plot a graph of the signal intensity, sequentially determining the location for which the IS takes values of 63% at T_1, while at T_2, it loses 63% (i.e., it takes a value of 37% of the IS).

For T_1, this is represented by the relationship:

$$\lim_{TE\to 0}\left(PD\left(1 - e^{\frac{-TR}{T_1}}\right)e^{\frac{-TE}{T_2}}\right) = PD\left(1 - e^{\frac{-TR}{T_1}}\right)$$

$$\left(1 - e^{\frac{-TR}{T_1}}\right)_{TR=T_1} \approx 0,63212 \approx 63\%$$

For T_2, this is represented by the relationship:

$$\lim_{TR\to \infty}\left(PD\left(1 - e^{\frac{-TR}{T_1}}\right)e^{\frac{-TE}{T_2}}\right) = PD\, e^{\frac{-TE}{T_2}}$$

$$\left(PD\, e^{\frac{-TE}{T_2}}\right)_{TE=T_2} \approx 0,36788 \approx 37\%$$

Although the primary nucleus that meets the resonance condition is hydrogen in ^{1}H MRI imaging in clinical instruments, it is also possible to image other elements such as fluorine ^{19}F, carbon isotope ^{13}C, phosphorus ^{31}P, ^{15}N, oxygen ^{17}O, sodium ^{23}Na. Since hydrogen is the most abundant in the human body, it is the most commonly used element. Due to its similar resonant frequency, the fluorine nucleus is increasingly used in scientific work [35,36]. In particular, in the study of various cancer types in diagnostic and spectroscopic studies involving ^{19}F-labeled anticancer drugs. Cell culture studies offer a controlled and systematic way to study cellular and molecular properties associated with the disease. ^{19}F MRI allows imaging without a tissue background [37], which is extremely important for bad studies of pharmaceutical substances. Among other things, they enable monitoring of drug delivery [38], assessment of metabolism, tracking of labeled cells [39-41], and the ability to visualize novel fluorinated drug conjugates [42]. The pharmaceutical market includes a group of drugs that contain fluorine nuclei in their formulation. In 1957, 5-fluorouracil (5-FU), the first fluorinated drug, was introduced to the pharmacy market [43], which has been used, for example, in trials in patients with colorectal cancer metastasizing to the liver [44]. In 1977, per-fluorocarbon compounds (PFCs) were introduced and first analyzed with ^{19}F MRI. PFCs form an emulsion and can carry drugs [45].

2. Materials and Methods

HTB-125 and CRL-2314 cell lines for further culture and research and culture media are from American Type Culture Collection (ATCC®) (ATCC). PO BOX 1549 MANASSAS, VA 20108 was purchased through LGC standards (Łomianki, Poland). Sodium bicarbonate was sourced from Honey-well Fluka, Collagen bovine type Lyophilized Fibrous Powder from

Tendon was sourced from Advanced BioMatrix (USA), while Penicillin - Streptomycin - Neomycin Solution Stabilized, Fetal Bovine Serum (FBS), Epidermal growth factor (EGF) were sourced from Sigma Aldrich, and were used to prepare complete growth media for cells under sterile conditions in Al-pina's laminar chamber. Trastuzumab (150mg) in the form of powder for infusion solution came from "Roche Polska Sp. z o.o." (Warsaw, Poland) company with limited liability.

As recommended, the HTB-125 line cells were cultured in ATCC Hy-bri-Care Medium, catalog no. 46-X (lgs standards), supplied as a powder, dissolved in 1l of water for infusion, then added 1.5 g/l of sodium bicarbonate. In the next step, the solution was filtered through 0.22 µm syringe filters under a laminar chamber into sterile bottles and stored at a temperature of 4ºC. To obtain a complete growth medium, supplement the basal medium with 30 ng/ml of mouse EGF (growth factor) and fetal bovine serum (FBS) to a final concentration of 10%. All the components of the medium were heated to a temperature of 37oC. Then the complete growth medium was prepared as follows: to 27 mL of basal medium (ATCC-46-X Hybri-Care Medium) were added 3 mL of Fetal Bovine Serum (FBS) and 300 µL of Penicillin antibiotic and 39 ng/mL of EGF(Epidermal Growth Factor from mouse) in our case in a portion of 0.45 µL. The basal medium for the CRL-2314 cell line is RPMI-1640 medium formulated by ATCC, ATCC 30-2001, which is in liquid form. In order to uz-skin a complete growth medium, fetal bovine serum (ATCC 30-2020) should be added to the basal medium to a final concentration of 10%, and the antibiotic, in our case Penicillin. All the components of the medium were heated to a temperature of 37oC. Then the complete growth medium was prepared as follows: 3 mL of Fetal Bovine Serum (FBS) and 300 µL of the antibiotic Penicillin were added to 27 mL of RPMI-1640 medium. Both HTB-125 and CRL-2314 cell lines were incubated in an atmosphere of 5% CO_2 and 37ºC. The incubator was from Biogenet, while the carbon dioxide supplied to the incubator was from Air Liquide. For cell counting, 10µl was taken from the suspension of cells after trypsinization (suspended in 1 ml of culture medium) and adequately diluted in PBS. The number of cells was automatically counted with Merck's Muse Cell Analyzer count and viability kit. Prepared ependorphs with coiled cells, i.e., CRL-2314 and HTB-125 lines, and the same lines with the addition of 10 uL of Trastuzumab solution (1 mg/1 ml of injection water) to determine T_1 and T_2 relaxation times were subjected to magnetic resonance imaging. The experiment aimed to see if Trastuzumab therapy would cause changes in relaxation times (Fig. 1).

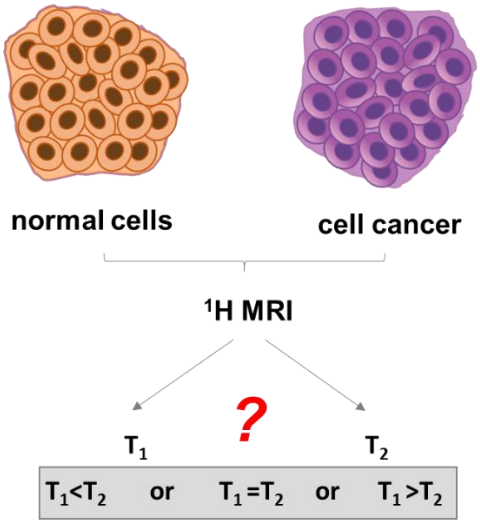

Figure 1. Measurement of T_1 and T_2 relaxation times - are there any noticeable differences?

Using the magnetic resonance imaging method, we assessed the possibilities of imaging with a 1.5 Tesla apparatus of cell cultures based on assessing the longitudinal (T_1) and transverse (T_2) relaxation times. Prepared Ependorfs with centrifuged cells, i.e., line CRL-2314 and HTB-125, and with the same lines with the addition of 10 µL of Trastuzumab solution (1 mg/1 ml of water for injection) to determine T_1 and T_2 relaxation times were examined by magnetic resonance imaging. T1 and T2 relaxation times were measured with a 1.5 T Optima MR360 magnetic resonance apparatus from General Electric Healthcare (Milwaukee, Wisconsin, USA). The camera was supported in software version SV23. Prepared Ependorfs were scanned using Fast Spin-Echo (FSE) sequences with axial projection using a single-channel coil. The scan matrix was 320 x 224, the field of view (FOV) 6 cmx6 cm, and a section thickness of 1 mm, spacing 0.5 mm, and NEX=2. For the T_1 time, the measurements were made in 12 steps with a repetition time (TR) in the range of 78-15,000 ms (78, 100, 200, 500, 700, 1000, 1500, 2000, 3000, 5000, 10000, 15000 ms) with constant echo time (TE) of 3 ms. Example images for determining the relaxation time are shown in Figure 2. Based on the obtained DICOM images, the analysis was performed, and ROI measurements were made for 12 images for each of the samples, based on which the relaxation time T_1 was determined. However, in the case of T_2 relaxation time, a series of 12 steps were performed with the same scanning parameters, except for the repetition time, which was constant and amounted to 10000 ms, echo time in the range from 11.8-300 ms (11.8, 20, 42, 68, 85, 102, 130, 160, 200, 230, 260, 300 ms) and NEX=3. Increasing the NEX value increases the SNR and the scan time. After the test, the prepared samples were incubated for 24 hours under the conditions of 5% CO_2 and 37°C. After this time, the entire test was repeated in exactly the same setting and with the same scanning parameters.

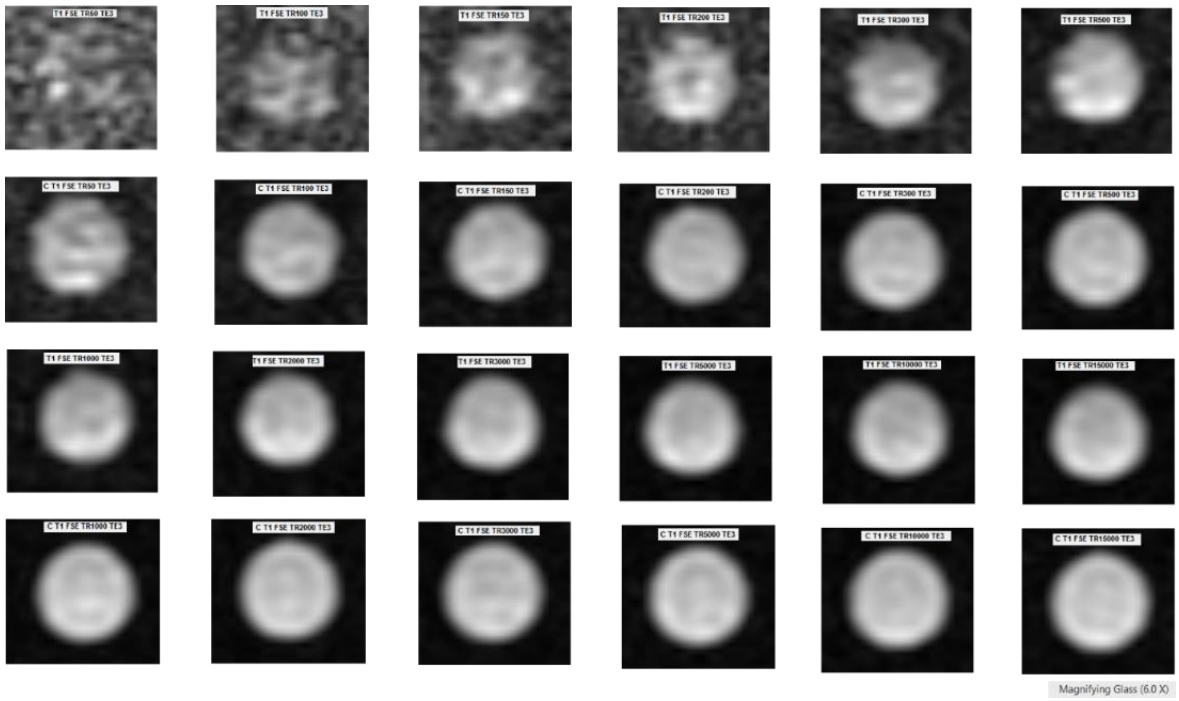

Figure 2. Exemplary pictures for determining the relaxation time.

The results of the viability and the number of cells were checked after repeated MRI examination; the result is presented in the table below. Results of T_1 and T_2 relaxation times for the HTB-125 and CRL-2314 cell line and medium and cells after treatment with Trastuzumab are shown below (Tab. 1).

Table 1. Results of T_1 and T_2 relaxation times for the HTB-125 and CRL-2314 cell lines.

Test sample						
HTB-125	**HTB-125 + Trastuzumab**	**Medium**	**CRL-2314**	**CRL-2314 + Trastuzumab**	**Medium**	**Solution with Trastuzumab**
T_1 relaxation time (ms)						
2914,96	2885,11	3025,39	2892,57	2509,04	3056,73	3334,31
T_2 relaxation time (ms)						
143,26	160,30	150,70	154,10	154,01	160,50	147,70
Results after 24h						
T_1 relaxation time (ms)						
2495,61	2691,10	2774,68	1801,66	1759,88	2761,24	3020,91
T_2 relaxation time (ms)						
152,95	153,29	164,36	148,80	143,35	169,72	201,05

Table 2 shows the cell counts of the cell lines in question.

Table 2. Cell counts of the cell lines for the HTB-125 and CRL-2314 cell lines.

Cell line			
CRL-2314		**CRL-2314 + Trastuzumab**	
Live	Total	Live	Total
54%	$3,58*10^6$	59,1%	$3,72*10^6$
Results after 30h			
44,05%	$4,13*10^6$	51,6%	$3,46*10^6$
HTB-125		HTB-125 + Trastuzumab	
Results after 30h			
Live	Total	Live	Total
91,25%	$2,7*10^6$	91,75%	$3,46*10^6$
89,80%	$2,12*10^6$	87,4%	$1,73*10^6$

4. Discussion

Trastuzumab is an IgG1 monoclonal antibody that binds selectively to the human epidermal growth factor receptor 2. It is used to treat breast cancer in patients whose tumors overexpress the Her-2 receptor. The implementation of cell culture studies is helping to understand cell biology. Research on developing tissue engineering, biomedical engineering, drug delivery systems, and new drugs is being conducted using cell lines. Developing an imaging method to monitor the effectiveness of applied therapies is crucial. Evaluating the state of cells before and after the application of therapies in a way that is non-invasive to cell lines allows the study scheme to be transferred to *in vivo*. Measuring relaxation times *in vitro*, will sequentially allow the procedure to be transferred to clinical trials. MR relaxation times measured in cancer cell cultures help to assess the biological changes of the applied drug therapy. Measuring T1 and T2 times makes it possible to assess cells' state and check drug therapy's effectiveness. Based on the studies performed, we noticed differences in relaxation times between untreated cells and cells treated with Trastuzumab. Unfortunately, in the case of the 1H method, we cannot imaging without background from hydrogen nuclei, so research is currently being conducted on fluorine-containing drugs. Currently, the quality of medical images obtained allows imaging of cell cultures using a 1.5 Tesla field. Studies on pharmaceuticals show that the clinical MR scanner used for *in vitro* experimental studies yields satisfactory differences in signal intensity, allowing them to be transferred to *in vivo* studies. Preclinical imaging, particularly molecular imaging, can be used to assess biological processes at the cellular and molecular levels. It is a valuable tool that can improve cancer detection at a very early stage of development or at a pre-symptomatic stage. In addition, it can be used to evaluate the efficacy of new therapies, such as personalized, targeted, and combination therapies [46]. Assessment of metabolism, proliferation, degree of hypoxia, angiogenesis,

apoptosis, and gene and receptor expression is extremely important in preclinical studies [47]. Evaluating therapeutic responses or lack of response to treatment at an early stage makes it possible to initiate a new treatment regimen and ultimately improve the efficacy of therapy [48]. Also, analysis of HER2 receptor expression is important for monitoring the response and efficacy of Trastuzumab treatment [49]. Recent studies present magnetic polystyrene nanoparticles conjugated with Herceptin-Agsbox used as a theranostic agent in breast cancer [50]. In addition, Fe_3O_4/P(NIPAM-AA-MAPEG) nanogels (MPLs) based on in situ loading of doxorubicin (DOX) were presented. Acrylic propionic acid (AA) molecules were conjugated with Herceptin antibody [51]. Quantitative longitudinal changes in tumor cell heterogeneity and vascularity in response to Trastuzumab treatment in HER2+ breast cancer were made, and the ability to predict response to treatment was confirmed [53]. Studies show that Trastuzumab *in vivo* is heterogeneously distributed, with the tumor microenvironment playing a key role [54]. The use of 3D culture and density classification is possible using T1 and T2 MRI. Cell culture MRI is a tool uniquely suited for this task. Our results demonstrated the potential of using the MR scanner to detect Trastuzumab in tumor cells. Measurements of T1 and T2 relaxation times can be used to assess drug efficacy *in vitro*. Our results show that it is possible to image cell cultures in the 1.5 Tesla field, and the implementation of non-invasive MRI imaging studies using Trastuzumab may affect the development of drug delivery systems and improve their effectiveness by finding the optimal drug modification.

5. Conclusions

Magnetic Resonance Imaging *in vitro* is a useful tool for analytical studies of cell cultures. Due to changes in water content, we can provide the viability information base on changes in relaxation times T1 and T2. The useful structure of cell culture for MRI is 3D culture. The measurements of relaxation times before and after drug treatment are useful for studying drug efficacy.

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Conflict of interests

The authors declare no conflict of interest.

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