FULL ARTICLE

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Synergistic effect of oxytetracycline as a combination treatment with Carboplatin on MCF-7 breast cancer cell line

Efeito sinérgico da oxitetraciclina em um tratamento combinado com Carboplatina na linhagem de células cancerígenas pulmonares MCF-7

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ABSTRACT

In breast cancer treatment, chemotherapy resistance is a major problem where many receptive tumors rebound and develop resistance. When provided in combination, cancer drugs are most successful, thus reducing the risk of developing resistant cancer cells. However, the evaluation of combination therapies has increased rapidly in recent years. Consequently, by repurposing old treatments, the discovery of additional medicines that may interact synergistically with chemotherapy is considered a current medical aim through discovering a new cancer medication or therapeutic strategy. The purpose of this research is to increase the anti-cancer activity of carboplatin (CP) by increasing the apoptotic effect of breast cancer cells (MCF-7) during in vitro experiments in combination with oxytetracycline. Our results showed a high synergistic effect between oxytetracycline and carboplatin, MCF-7 representative cell treated with carboplatin with/without different concentrations of oxytetracycline (5% and 10% of IC50). Oxytetracycline, which potentiated the action of carboplatin and/or had notable activity was reported as a single agent. This research demonstrated the synergistic relationship between oxytetracycline and carboplatin in viability assays. Surprisingly, our findings suggest that inhibiting treatment strategies can extend carboplatin's therapeutic window, potentially allowing for cancer therapy.

Keywords: Carboplatin. Oxytetracycline. Breast cancer cells (MCF-7). In vitro assay. Chemotherapy.

RESUMO

No tratamento do câncer de pulmão a resistência à quimioterapia é o maior problema no qual muitos tumores receptivos apresentam um rebote e desenvolvem a resistência. Quando oferecidas em combinações, as drogas anticancerígenas apresentam maior taxa de sucesso, reduzindo assim o risco de desenvolvimento de células cancerígenas resistentes. Contudo, a avaliação das terapias de combinação tem crescido muito rapidamente. Consequentemente, por reaproveitamento de tratamentos antigos, a descoberta de novos medicamentos adicionais que podem interagir sinergicamente com a quimioterapia que é considerada como auxílio médico na corrente busca à descoberta de novas medicações anticancerígenas ou estratégias terapêuticas. O propósito da presente pesquisa é aumentar a atividade anticancerígena da Carboplatina (CP) pelo incremento do efeito apoptótico de células de câncer pulmonar (MCF-7) em experimentos *in vitro* pela combinação com oxitetraciclina. Os resultados obtidos confirmaram elevado efeito sinérgico entre oxitetraciclina e Carboplatina em células MCF-7 representativas tratadas com Carboplatina, com e sem diferentes concentrações de oxitetraciclina (5% e 10% de IC50). A oxitetracilina que potencializou a ação da Carboplatina e/ou teve uma notável atividade relatada como um agente isolado. A pesquisa demonstrou a relação sinérgica entre oxitetraxiclina e Carboplatina nos ensaios de viabilidade. Surpreendentemente, os resultados obtidos sugeriram que as estratégias de tratamento inibidor podem aplicar um janela terapêutica da Carboplatina com potencial para a terapia do câncer.

Palavras-chave: Carboplatina. Oxitetracilina. Células de câncer pulmonar (MCF-7). Ensaio in vitro. Quimioterapia.

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Introduction

Drug resistance remains the most significant barrier to cancer patients achieving cures. Most chemotherapy drugs target rapidly dividing cancer cells by interfering with the synthesis of DNA. Although potent, many of these compounds are nonspecific, causing damage to normal tissue, and leading to a high rate of mortality among cancer patients. Chemotherapy treatment responses are also influenced by the increase in drug resistance (Alfarouk et al., 2015; Jin et al., 2020). As a consequence, drug combinations became a new model for cancer care, resulting in the development of highly complicated regimens (Vasan et al., 2019). Furthermore, there are a variety of approaches to dose intensity, including shorter chemotherapy administration intervals (Gray et al., 2019; Narayan et al., 2020). Recently, progress in the design of repurposing compounds, combined with increased knowledge of tumor biology, has resulted in improved therapeutic cancer therapy (Rudrapal et al., 2020; Yadav & Talwar, 2019). Also, drug repositioning takes fewer funds and efforts than finding alternative indications for an existing drug or a failed drug. Chemoresistance is described as the insensitivity of cancer cells to therapy, which is considered a major cause of anti-breast cancer chemotherapy failure (Ji et al., 2019). Despite several attempts to improve the sensitivity of current chemotherapeutic drugs and resolve drug resistance in breast cancer, the results remain unsatisfactory. Breast cancer treatment needs optimum clinical effectiveness. Patients could get the most benefit from their treatment when significantly reducing recurrence, resistance, and adverse effects, as well as maintaining a healthy quality of life, attributable to a carefully selected combination of treatment modalities (Fisusi & Akala, 2019).

Material and Methods

Growth medium of MCF-7 breast cancer cells line. Mammary adenocarcinoma cell lines (MCF-7) were grown in DMEM/F12 media supported with antibiotics (50 IU/mL penicillin and 50 g/mL streptomycin) enriched with 10% newborn calf serum (Life Technologies, Grand Island, NY, USA). The cells were held in a humidified inside environment at 37°C with 5% CO2.

MTT assay for the viability of cells. When the cells reached semi-confluent of 75% flask, they were detached by 10% of trypsin and then resuspended in completed media. After that they were plated onto a plate of 96-wells. After seeding, the cells were incubated for 24 h and the samples of plated cells were handled in duplicate through media containing 1 to 100 M carboplatin with/without a fixed dose of oxytetracycline (5% and 10% of IC50), cells were exposed to drugs for 72 h. Sargent and Taylor's MTT cell viability assay was used (Ulukaya et al., 2004). MTT reagent was added to each well at a final concentration of 0.5 mM, and the cells were incubated in an incubator set at 37°C with 5% CO2 for 3 h. Then the cells dissolved in 200 L of DMSO after the MTT solution was collected. The cell cytotoxicity of stain was measured by using a microplate reader set to 570 nM for each experiment used in this study. The data were standardized to the number of viable cells in each well, which represented the number of untreated control wells.

Viability and cell survival Testing (live/dead® cytotoxicity/viability kit): - Calcein AM and ethidium homodimer1 reagents are the main components of the assay kit (Thermo Fisher Scientific). Calcein AM detects intracellular esterase activity and cell membrane integrity, indicating the presence of live cells. Enzymatically transforming calcein AM to green fluorescent calcein is considered the main achievement, while the red fluorescent dye ethidium homodimer-1 binds to the nucleic acids of membrane-damaged cells, creating a bright red fluorescence that indicates the presence of dead cells. Both reagents were combined with PBS at the following ratios to make a live/dead staining solution: Samples were stained with 1:200 calcein AM and 1:50 ethidium homodimer-1 in PBS, washed 2-3 times in PBS and incubated with the live/dead staining solution for 30 min at room temperature while shielded from light. The dye solution was then washed away, and the samples were washed 2-3 times with PBS. Green and red fluorescence were used as indicators of live/dead cells in confocal microscopy to evaluate cell viability. Consequently, the percentage of cells was calculated by counting the live and dead cells in each sample's specific region. The cell counting technique used Image J software(Younus, 2019).

Trypan blue assay: Seeding of cells in 12-well plates and incubated overnight before being exposed to the drugs for 72 h as directed. The supernatant medium was removed from the cells and trypsinization was used to separate the cells to determine viability. After cells detachment, they were mixed with their supernatants for each sample separately. Then the suspension was centrifuged at 150 g for 3 min. As consequence, the supernatant was removed and the pellet was resuspended with 0.5 mL of a new medium. Finally, by mixing 0.4 percent (v/v) dye trypan blue (Sigma-Aldrich) with an equal amount of cell suspension, the viable and non-viable cells were counted under light microscopy by using a Neubauer hemocytometer (Jawad, 2020). The viability percentage was calculated using the following metrics:

$$Viability\% = number\ of\ viable\ cells\ x\ 100\ / \left(number\ of\ dead\ cells + number\ of\ live\ cells\right) \quad (1)$$

Results

The activity of oxytetracycline evaluated on the breast cancer cell line: - In cell growth assays of breast cancer cells, the anticancer activity and potency of oxytetracycline were evaluated, and the IC_{50} was 64.4 µM, which was calculated as a single agent for growth inhibition reported three times (n=3., S.D) (Figure 1).

Confirmation of carbpoplatin's antiproliferative activity when combined with fixed oxytetracycline doses.

To determine carboplatin's activity when combined with oxytetracycline. MCF-7 cells were exposed for 72 h to indicate the concentrations of carboplatin alone and in combination with a fixed concentration of oxytetracycline (Figure 2), then the cells were stained with MTT. The oxytetracycline concentrations used in cell growth assays were those that had a 5% and 10% effect on their own (referred to as f.a. (fraction affected) = 5% (13.94 µM) and 10% (15.62 µM).

Proliferation and viability of cells. After 72 h of cell culture, the prevalence of viable versus dead cells was determined by live/dead staining samples of seeded cells. The sample was repeated at each concentration. Green and red fluorescence under confocal microscopy were used as indicators of live/dead cells to measure cell viability. In each sample's specific area under confocal, the percentage of viable and dead cells was calculated.

The results showed a combination effect of carboplatin with oxytetracycline as a synergistic activity in cell lines assessed at the concentration fraction of oxytetracycline evaluated (Figures 3, 4). These data indicate that oxytetracycline could be an effective choice for combining with carboplatin, which led to further studies focusing on this combination.

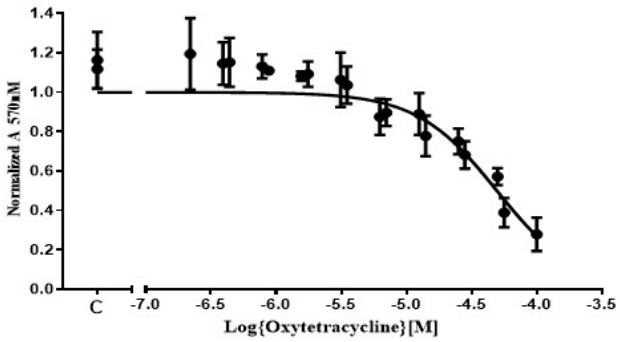

Figure 1 – The figure shows the effects of oxytetracycline on MCF-7 cell lines calculated by graph pad prism (version 9) after 72 h of exposure to a range of oxytetracycline concentrations. the number of surviving cells was determined by using the MTT assay and the IC_{50} 64.4 µM. The results of the cytotoxicity experiment are repeated three times. Control sampling is indicated by the letter "C" on the x-axis.

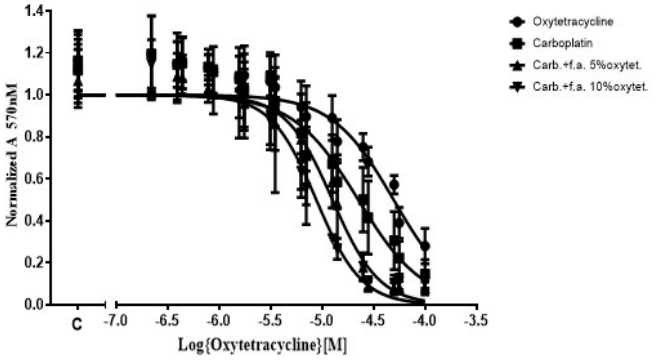

Figure 2 – The figure shows measuring the cytotoxic effect of breast cancer cell lines (MCF-7). The combination between oxytetracycline and carboplatin was counted by graph pad prism (version 9), after exposing the cells to a variety of carboplatin concentrations for 72 h. The MTT reagent was used to estimate the number of surviving cells. create dose-response curves by using curve fitting. The results are repeated three times (mean SD, n 3), and expressed as a portion of the top of the curve in each experiment. Control sampling is indicated by the letter "C" on the x-axis and the percentage of cells affected by oxytetracycline is marked by the numbers 10% and 20%.

Cell viability assay (trypan blue) after treatment strategy of combination drugs

Trypan blue staining (Figure 5) was used to support the previous findings and that the drug combinations triggered the cell death effect of the drug combinations on cell viability. After the cells displayed synergy during cell growth assays, these experiments were carried out. Synergy was observed in both of the oxytetracycline concentrations tested. The results revealed more cell death than would be

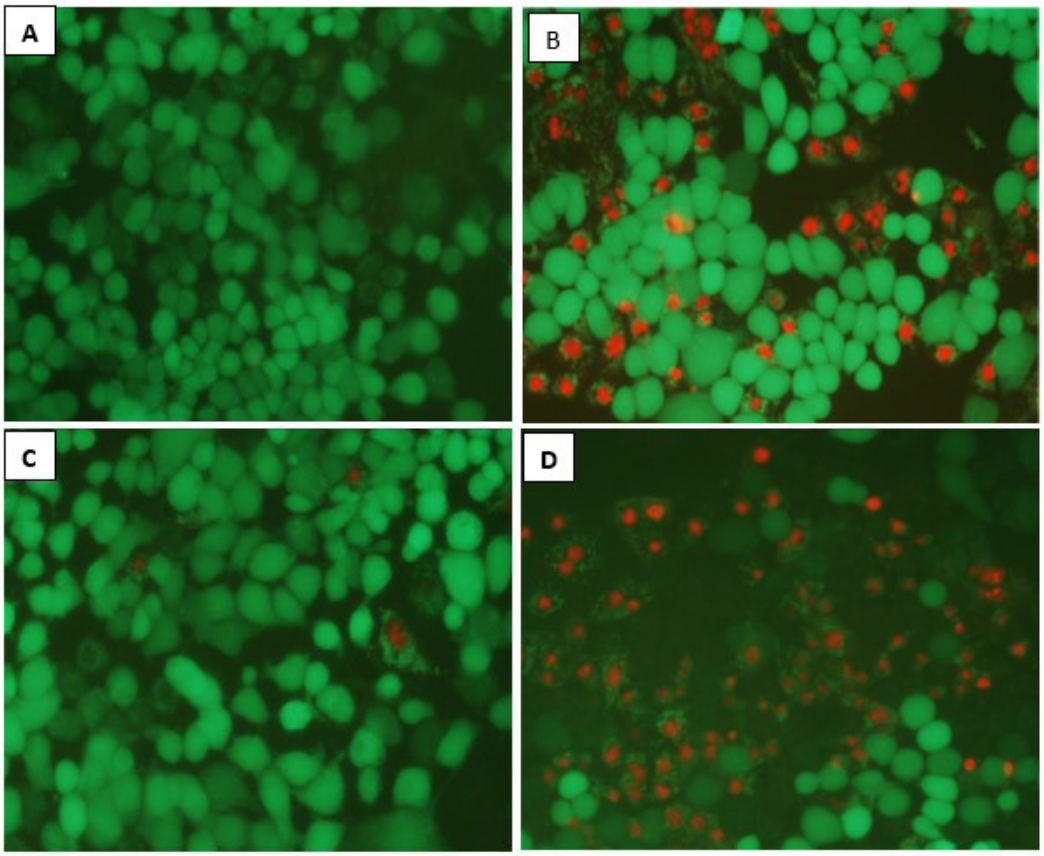

Figure 3 – The figure shows the proliferation and viability of MCF-7 cells counted (dead cells to live cells) after 72 h of treatment. The untreated cells (A) were used as a control or treated with the IC_{50} of carboplatin (36.41 μM) for 24 h (B), 5% f.a. oxytetracycline only (C) or 5% f.a. oxytetracycline only with carboplatin (D). Live cells are green and dead is red. The results are representative of three experiments. The images were taken by confocal microscope with a 100 μm scale.

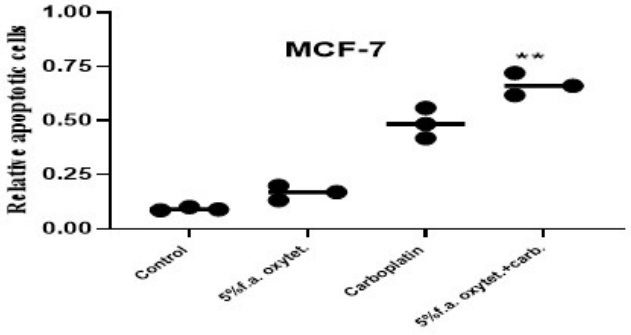

Figure 4 – The figure measures dead cells after 72 h of treatment of MCF-7 cells. Cells were treated with carboplatin at IC_{50} (36.41 μM) with/without 5% (13.94 μM) oxytetracycline IC_{50}. Untreated cells were used as a control for the drugs' potential effects on cell number concerning the number of survival cells. The results were repeated three times (n=3, mean + SD). The results in cells treated with drug combination were significantly different from carboplatin alone (paired t-test; *, P< 0.05; **, P< 0.01).

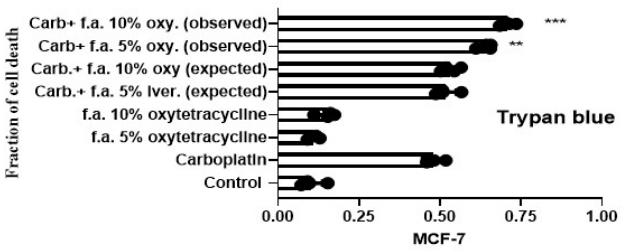

Figure 5 – The figure shows the combined effect of oxytetracycline with carboplatin on breast cancer MCF-7 cell lines. The cells were exposed to carboplatin with a dose of IC_{50} (36.41 μM) with/without affected concentration of 5% (13.94 μM) and 10% (15.62 μM) from oxytetracycline IC_{50}. After repeating the experiment three times, the number of dead cells was determined by staining with trypan blue. The percentage (%) of viable cells to dead cells is counted under light microscopy. Samples exposed to the combination showed significantly more dead cells than would be anticipated if the drugs acted additively by using the Bliss independence criterion ((paired t-test; *, P< 0.05; **, P< 0.01; *** P< 0.001).

expected if the drugs were combined additively. The Bliss independence criterion was used to estimate the effect if the drugs were combined additively.

Discussion

Cancer is one of the main causes of death worldwide (Zhang et al., 2020). Resistance is now one of the most

difficult problems of cancer treatment. Resistance is found in all kinds of cancer and all treatments, such as molecularly targeted therapy, immunotherapy, and chemotherapy. As a result, cancer treatment uses multidrug regimens to increase efficacy and reduce the high drug doses needed (Wang et al., 2019). Besides that, cancer is a diverse form of the disease with a variety of pathogenesis and molecular pathways (Montemagno & Pagès, 2020). Therefore, targeting several pathways to inhibit tumor growth and enhance survival is advantageous. The creation of new drugs, increasing technological advances and expanded understanding of the human neoplastic disease are creating opportunities to reduce the death rate from cancer (Hopkins, 2009; Nowak-Sliwinska et al., 2019; Zhang et al., 2020). Patients will have better access to medications because of drug repurposing, which will again lower costs during the lengthy and complicated treatment process. In contrast, repurposing is time-consuming and inefficient due to the thousands of existing drugs and diseases, as well as the heterogeneity of patients and diseases, especially cancers (Bertolini et al., 2015; Nowak-Sliwinska et al., 2019). This study found that combining carboplatin with oxytetracycline can increase the cytotoxic activity of the drug carboplatin, currently used to treat breast cancer. Although researchers studied oxytetracycline as a potential additive to carboplatin, oxytetracycline, like carboplatin, inhibited the growth of breast cancer cell lines as a single agent, but it was less effective than carboplatin.

Using different assays in the MCF-7 cell line, oxytetracycline improved synergistically with carboplatin in inducing apoptosis. Oxytetracycline potentiated carboplatin's activity in inducing cell death, as measured by estimating live/dead cell viability and counting cell viability under the light microscope by cell staining with trypan blue. Furthermore, all treated cells showed significant morphological microscopic changes. The cooperation between carboplatin and oxytetracycline observed by using different assays lend and supports this observation.

The studies presented here did not address the mechanistic rationale for the cooperation between carboplatin and oxytetracyclines. Carboplatin causes cell death by inhibiting replication and transcription (Sousa et al., 2014). On the other hand, oxytetracycline has not shown a significant impact on cancer replication or transcription. The mechanism underlying the cooperation between these two drugs is unlikely to be this one. However, it provides a strong indicator of synergism for clinical application. Interestingly, tetracyclines interfere with several pathways which contribute to cancer cell growth including a) inhibition of protein synthesis of mitochondria; b. inhibition Matrix of metalloproteinase; c) function as an angiogenesis inhibitor; d) eradicate the stem cell of cancer; e) tumor downregulation of DNA-PK, and reduce sensitization (Koltai, 2015). Consequently, oxytetracycline has already been investigated as an anti-cancer agent by other researchers, which could help in understanding the basis for carboplatin-oxytetracycline cooperation. Oxytetracycline had a similar effect on rat liver regeneration as tetracycline, indicating that the mitochondrial translation process was involved in the production of functionally active cytochrome C oxidase (Koltai, 2015). Oxytetracycline, on the other hand, has a negligible effect on hemopoietic cell proliferation, except for T lymphoid cells, whose division is inhibited (Van Den Bogert & Kroon, 1982). Our findings indicate the need for further research into the oxytetracycline and carboplatin combination.

Conflict of Interest

None.

Ethics Statement

This research is done by lab and we did not apply in domestic, lab animals or we did not apply for clinical treatment in human therefore we do not issues Ethics for labrotory assays according to our university roles.

References

Alfarouk KO, Stock CM, Taylor S, Walsh M, Muddathir AK, Verduzco D, Bashir AHH, Mohammed OY, Elhassan GO, Harguindey S, Reshkin SJ, Ibrahim ME, Rauch C. Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. Cancer Cell Int. 2015;15:71. http://dx.doi.org/10.1186/s12935-015-0221-1. PMID: 26180516.

Bertolini F, Sukhatme VP, Bouche G. Drug repurposing in oncology: patient and health systems opportunities. Nat Rev Clin Oncol. 2015;12(12):732-42. http://dx.doi.org/10.1038/nrclinonc.2015.169. PMid:26483297.

Fisusi FA, Akala EO. Drug combinations in breast cancer therapy. Pharm Nanotechnol. 2019;7(1):3-23. http://dx.doi.org/10.2174/2211738507666190122111224. PMid:30666921.

Gray R, Bradley R, Braybrooke J, Liu Z, Peto R, Davies L, Dodwell D, McGale P, Pan H, Taylor C, Barlow W, Bliss

J, Bruzzi P, Cameron D, Fountzilas G, Loibl S, Mackey J, Martin M, Del Mastro L, Möbus V, Nekljudova V, De Placido S, Swain S, Untch M, Pritchard KI, Bergh J, Norton L, Boddington C, Burrett J, Clarke M, Davies C, Duane F, Evans V, Gettins L, Godwin J, Hills R, James S, Liu H, MacKinnon E, Mannu G, McHugh T, Morris P, Read S, Wang Y, Wang Z, Fasching P, Harbeck N, Piedbois P, Gnant M, Steger G, Di Leo A, Dolci S, Francis P, Larsimont D, Nogaret JM, Philippson C, Piccart M, Linn S, Peer P, Tjan-Heijnen V, Vliek S, Mackey J, Slamon D, Bartlett J, Bramwell VH, Chen B, Chia S, Gelmon K, Goss P, Levine M, Parulekar W, Pater J, Rakovitch E, Shepherd L, Tu D, Whelan T, Berry D, Broadwater G, Cirrincione C, Muss H, Weiss R, Shan Y, Shao YF, Wang X, Xu B, Zhao D-B, Bartelink H, Bijker N, Bogaerts J, Cardoso F, Cufer T, Julien J-P, Poortmans P, Rutgers E, van de Velde C, Carrasco E, Segui MA, Blohmer JU, Costa S, Gerber B, Jackisch C, von Minckwitz G, Giuliano M, De Laurentiis M, Bamia C, Koliou G-A, Mavroudis D, A'Hern R, Ellis P, Kilburn L, Morden J, Yarnold J, Sadoon M, Tulusan AH, Anderson S, Bass G, Costantino J, Dignam J, Fisher B, Geyer C, Mamounas EP, Paik S, Redmond C, Wickerham DL, Venturini M, Bighin C, Pastorino S, Pronzato P, Sertoli MR, Foukakis T, Albain K, Arriagada R, Bergsten Nordström E, Boccardo F, Brain E, Carey L, Coates A, Coleman R, Correa C, Cuzick J, Davidson N, Dowsett M, Ewertz M, Forbes J, Gelber R, Goldhirsch A, Goodwin P, Hayes D, Hill C, Ingle J, Jagsi R, Janni W, Mukai H, Ohashi Y, Pierce L, Raina V, Ravdin P, Rea D, Regan M, Robertson J, Sparano J, Tutt A, Viale G, Wilcken N, Wolmark N, Wood W, Zambetti M. Increasing the dose intensity of chemotherapy by more frequent administration or sequential scheduling: a patient-level meta-analysis of 37 298 women with early breast cancer in 26 randomised trials. Lancet. 2019;393(10179):1440-52. http://dx.doi.org/10.1016/S0140-6736(18)33137-4. PMid:30739743.

Hopkins, AL. Predicting promiscuity: a fossil record for exoplanets, News & Views. 2009; 462(112): 167-168.

Jawad MJ. Translational studies to support the use of pitavastatin as a cancer therapeutic [thesis]. Reino Unido: Keele University; 2020.

Ji X, Lu Y, Tian H, Meng X, Wei M, Cho WC. Chemoresistance mechanisms of breast cancer and their countermeasures. Biomed Pharmacother. 2019;114:108800. http://dx.doi.org/10.1016/j.biopha.2019.108800. PMid:30921705.

Jin KT, Lu ZB, Chen JY, Liu YY, Lan HR, Dong HY, Yang F, Zhao YY, Chen XY. Recent trends in nanocarrier-based targeted chemotherapy: selective delivery of anticancer drugs for effective lung, colon, cervical, and breast cancer treatment. J Nanomater. 2020;2020:1-14. http://dx.doi.org/10.1155/2020/9184284.

Koltai T. Tetracyclines against cancer: a review In: RESEARCH Gate. Berlin: 2015. 29 p. http://dx.doi.org/10.13140/RG.2.1.4097.1600.

Montemagno C, Pagès G. Metastatic heterogeneity of breast cancer: companion and theranostic approach in nuclear medicine. Cancers (Basel). 2020;12(4):821. http://dx.doi.org/10.3390/cancers12040821. PMid:32235331.

Narayan RS, Molenaar P, Teng J, Cornelissen FMG, Roelofs I, Menezes R, Dik R, Lagerweij T, Broersma Y, Petersen N, Marin Soto JA, Brands E, van Kuiken P, Lecca MC, Lenos KJ, In 't Veld SGJG, van Wieringen W, Lang FF, Sulman E, Verhaak R, Baumert BG, Stalpers LJA, Vermeulen L, Watts C, Bailey D, Slotman BJ, Versteeg R, Noske D, Sminia P, Tannous BA, Wurdinger T, Koster J, Westerman BA. A cancer drug atlas enables synergistic targeting of independent drug vulnerabilities. Nat Commun. 2020;11:2935. http://dx.doi.org/10.1038/s41467-020-16735-2. PMid:32523045.

Nowak-Sliwinska P, Scapozza L, Ruiz i Altaba A. Drug repurposing in oncology: compounds, pathways, phenotypes and computational approaches for colorectal cancer. Biochim Biophys Acta Rev Cancer. 2019;1871(2):434-54.

Rudrapal M, Khairnar JS, Jadhav AG. Drug repurposing (DR): an emerging approach in drug discovery. In: Badria FA, editor. Drug repurposing: hypothesis, molecular aspects and therapeutic applications. London: IntechOpen; 2020.

Sousa GFD, Wlodarczyk SR, Monteiro G. Carboplatin: molecular mechanisms of action associated with chemoresistance. Braz J Pharm Sci. 2014;50(4):693-701. http://dx.doi.org/10.1590/S1984-82502014000400004.

Ulukaya E, Colakogullari M, Wood EJ. Interference by anti-cancer chemotherapeutic agents in the MTT-tumor chemosensitivity assay. Chemotherapy. 2004;50(1):43-50. http://dx.doi.org/10.1159/000077285. PMid:15084806.

Van den Bogert C, Kroon AM. Effects of oxytetracycline on in vivo proliferation and differentiation of erythroid and lymphoid cells in the rat. Clin Exp Immunol. 1982;50(2):327-35. PMid:7151329.

Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. Nature. 2019;575(7782):299-309. http://dx.doi.org/10.1038/s41586-019-1730-1. PMid:31723286.

Wang X, Zhang H, Chen X. Drug resistance and combating drug resistance in cancer. Cancer Drug Resist. 2019;2(2):141-60. PMid:34322663.

Yadav V, Talwar P. Repositioning of fluoroquinolones from antibiotic to anti-cancer agents: an underestimated truth. Biomed Pharmacother. 2019;111:934-46. http://dx.doi.org/10.1016/j.biopha.2018.12.119. PMid:30841473.

Younus ZM. Design and fabrication of hydrogel scaffolds for osteochondral tissue regeneration [thesis]. Reino Unido: Keele University; 2019.

Zhang Z, Zhou L, Xie N, Nice EC, Zhang T, Cui Y, Huang C. Overcoming cancer therapeutic bottleneck by drug repurposing. Signal Transduct Target Ther. 2020;5(1):113. http://dx.doi.org/10.1038/s41392-020-00213-8. PMid:32616710.

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