The Synergistic Antitumor Activity of Colchicine and Chloroquine

Albader Mohammed Moafa , Fares Ghaleb Alotaibi , Noufa Ibrahim Mater Hakami , Abdulrahman Mabrouk Al-Rashidi , Ali Mohammed Alsalem , Adel Mohammed Juraybi , Fayez Abdullah Alosaim , RASHED Mohammed ALRUWES , Ibrahim Ali Alhaqwe , Fares Mohammed Saleh Alqahtani , Ahmad Nasser Alqahtani , Mubarak Dowihi Fatem Almutiri

Abstract:

The investigation of the synergistic antitumor activity between chloroquine and colchicine has been conducted in the context of treating breast cancer. Breast cancer is the most prevalent malignancy among women and a global health concern. Developing resistance to drugs presents an obstacle to the efficacy of chemotherapy. The objective of this research endeavor was to investigate the potential of colchicine and chloroquine in combination as a supplementary or alternative therapy to traditional chemotherapy. The study's objective was to evaluate the cytotoxic, anti-proliferative, and apoptosis-inducing effects on breast cancer cell lines. The results of the study indicated that colchicine and chloroquine, when combined, exerted a noteworthy synergistic impact on breast cancer cells. Enhanced cytotoxicity, inhibition of proliferation, induction of morphological changes, and apoptosis were all outcomes of the combination therapy. The synergistic effect between chloroquine and colchicine was confirmed by isobologram analysis, which revealed a high degree of synergy. The results of this study underscore the potential efficacy of combining colchicine and chloroquine as a therapeutic strategy for breast cancer. Combination therapy provides the benefit of increased effectiveness with the potential to decrease the dosage of traditional treatments, mitigate adverse effects, and surmount resistance to chemotherapy. Additional research and clinical trials are necessary to validate and apply these results in the field.

Keywords: Synergistic effect, colchicine, chloroquine, combination therapy, breast cancer.

1. Introduction

Cancer is a major public health issue around the world, and it is the second leading cause of death. Cancer is a category of diseases characterized by the uncontrollable growth and spread of abnormal cells. If the spread is not stopped, it can lead to death (1). Cancer therapy tries to destroy cancer cells while causing the least amount of harm to normal cells (2). Localized and / or systemic therapies, as well as supportive therapies, are used in cancer treatment to reduce side effects (3).

Europe accounts for 23.4% of all cancer cases and 20.3% of all cancer deaths worldwide. America has 13.3% of the global population and is responsible for 21.0% of global incidence and 14.4% of global mortality. Cancer mortality rates in Asia and Africa (57.3% and 7.3%, respectively). Breast cancer is a global health problem and the most common cancer affecting women around the world and the second leading cause of cancer related death after lung cancer. Breast cancer in women (6.6 percent), Breast cancer is the most prevalent cancer diagnosed in women (24.2 percent). Breast cancer is the most frequent female tumor in Iraq, with the most new cases in 2018 (4).

In general, breast cancer affects more women than any other type of cancer, and it is especially prevalent in Iraq, where 23% of all female cancer cases worldwide were caused by breast cancer (5). It is the most frequent type of malignancy in the Iraqi population in general, accounting for almost one-third of all recorded female malignancies and nearly one-quarter of all female cancer deaths (6).

2. Breast Cancer

Breast cancer has become one of the biggest hazards to Iraqi female health during the previous two decades, with a clear increase in incidence rates (7). Cell lines are an important experimental tool in cancer research because they provide an infinite supply of a relatively homogeneous cell population capable of self-replication that can be widely distributed to facilitate comparative studies (8).

The development of drug resistance in tumors counteracts the therapeutic effects of chemotherapeutic drugs,

resulting in more aggressive tumor recurrence and worse prognoses for cancer patients (9). Colchicine is a well-known and potent microtubule targeting agent; however, colchicine's therapeutic value against cancer is limited by its toxicity to normal cells. However, there is no evidence that it has cytotoxic potential against lung cancer cells at clinically acceptable or lower concentrations that are minimally toxic to non-cancerous cells (10).

Autophagy is an effective intracellular catabolic process that uses lysosomal degradation to degrade aberrant cellular protein aggregates and damaged organelles. However, it is required for cellular homeostasis and renovation (11). The autophagy process begins with the formation of the phagophore and concludes with the death of the autophagosome. However, cell biologists have been interested in the cellular and molecular mechanisms of this pathway since the late 1950s (Y) (12). Under some conditions, autophagy is regarded a cytoprotective process in cancer therapy, and it is implicated in the development of therapy resistance as a clinical barrier to successful cancer treatment, leading to a poor prognosis in cancer patients (13).

3. Chloroquine

As an antimalarial medication, chloroquine (CQ), a 4-aminoquinoline, has been utilized for decades. It is frequently advised to administer CQ in conjunction with primaquine in order to mitigate the risk of Plasmodium vivax recurrence (14). At present, CQ is recognized as a protonated, weakly basic medication that induces an increase in pH and accumulates within the food vacuole of parasites. This accumulation hinders the malaria parasite's ability to degrade the hemoglobin of the host red blood cell and halts its growth (15). The precise mechanism necessitates additional inquiry. CQ, akin to other antiparasitic medications, has demonstrated promise in the therapeutic management of cancer and various other ailments (16). CQ, functioning as lysosomotropic compounds, induces a pH elevation within the lysosome from 4.5 to 6. This pH variation is detrimental to the functionality of lysosomal enzymes. The term for this process of action is lysosomotropic effect (17, 18). Hence, CQ has the capability to modulate a multitude of biological processes through its interactions with transcriptional factors, enzymes, and receptors; these interactions dictate the therapeutic effects in cancer (19).

Autophagy inhibition will produce anticancer effects. As

an FDA-approved inhibitor of autophagy. Currently, this specific function has been the subject of extensive research. By preventing the degradation of autophagic proteins like light chain 3B-II (LC3B-II), CQ obstructs the last stage of the autophagy process (20,21). CQ thus inhibits the recycling and production of vital nutrients and metabolites, resulting in the destruction and injury of tumor cells. Moreover, by promoting cell apoptosis and cell cycle arrest, inhibition of the final stage of autophagy enhances the cytotoxic effects on cancer cells. By regulating immunity, tumor cells can be prevented from escaping. A number of studies have demonstrated that the application of ultra-low concentrations of chemotherapeutics to tumor cells results in an upregulation of gene expression associated with tumor antigens, immunity, and inflammation (22-26). This upregulation enhances the cell's capability to utilize CTLs for the purpose of inducing immunogenic death of tumor cells.

The initial documentation of the impact of modest concentrations of CQ on the immunogenicity of tumor cells was presented in a recent study (27). The aforementioned investigation observed that the concurrent application of CQ and 5-fluorouracil to HCT-116 colon cancer cells resulted in a substantial upregulation of dendritic cell maturation and an increase in the expression of surface markers, such as CD80 and CD86, as measured by their cell lysates. In addition, dendritic cells stimulated the apoptosis of tumor cells and increased the production of CTLs. Following this, they observed an upregulation of tumor-associated carcinoembryonic antigen family gene expression in the cancer cells that had been treated. However, the precise mechanism underlying this effect remains unknown; it could potentially be associated with CQ's inhibitory effect on autophagy. By altering the pH of the lysosome, CQ has also been observed to have a direct effect on the differentiation and function of numerous immune cells; this effect is described in detail in the aforementioned review (28).

Regulating a diverse array of critical signaling molecules, CQ also serves multiple purposes in malignancies. Platinum medications are widely acknowledged as the cornerstones of epithelial ovarian cancer treatment (29). On the contrary, cancer cells that have developed resistance to anticancer drugs are capable of enduring DNA damage by bypassing cell cycle checkpoints or utilizing DNA repair pathways (30,31). CQ, on the other hand, can reverse the drug resistance of cancer cells by

upregulating the expression of p21WAF1/CIP1. The relationship between this function and autophagy inhibition remains obscure (32). Numerous additional functions of CQ are reliant on its autophagy inhibitory effect. CQ, on the other hand, inhibits the expression of STAT3, which regulates CXC chemokine receptor 4 (CXCR4); thus, the stemness of esophageal squamous cell carcinoma cells is diminished. The aforementioned process operates autonomously from autophagy, as evidenced by the unchanged expression of crucial molecules ATG7 and BECN1 in the autophagy pathway (33); this indicates that CQ may have additional, as yet unidentified effects that are not reliant on autophagy inhibition. Furthermore, CQ has the ability to modulate the activity of signaling molecules, such as NF-κB and p53, thereby demonstrating its anticancer properties (34).

CQ, an autophagy inhibitor that has obtained approval from the FDA, has garnered significant interest and has been the subject of comprehensive research regarding its potential clinical applications. Numerous clinical investigations utilizing CQ singly or in combination for the treatment of cancer have been undertaken thus far (35, 36). The majority of results suggest that the concurrent use of CQ and other medications was well tolerated, and the maximal tolerated dose was higher than when CQ was administered alone. Nevertheless, these trials failed to identify any noteworthy disparities between the treatment and control groups, nor did they detect any substantial enhancement in overall survival. This may also be attributable to the tiny sample sizes utilized in the phase I clinical trials; therefore, a larger sample size should be utilized to further investigate and assess the efficacy of CQ.

4. The Pharmacological Effects of Bioactive Compounds

Over the last two decades, there has been a rise in interest in the pharmacological effects of bioactive compounds on cancer treatment and prevention. It has been shown to possess numerous anti-cancer activities in various cancer cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells (37,38).

The half maximum inhibitory concentration (IC50) of a pharmacological inhibitor is a measure of its ability to inhibit AMJ13. The IC50 value is a quantitative measure used to determine how much of a given inhibitory medication is present.

(Colchicines, Choloroquin, and Co-treatment) is required to prevent the spread of breast cancer. This is because the effect of chemotherapy on cells differs according to type, and this is related to changes in cancer cells after being treated with drugs. The effect of (Colchicines, Choloroquin, and Co-treatment) on breast cancer cell lines was shown to be very clear.

Other studies have employed various strategies, one by halving the provided dose of a chemotherapeutic drug (rituximab or doxorubicin) to lessen chemotherapy toxicity (39). Tamoxifen, an anti-estrogen medication, is currently used widely in the prevention and treatment of estrogen receptor positive breast cancer (40). However, many patients acquire tamoxifen resistance and suffer from severe adverse effects (41).

The results implied that synergism inhibitor had more effect in inhibition of proliferation, anticancer growth action, and caused increase in cytotoxicity and lead to induced morphological changes and apoptosis. The in vitro results of this study revealed that increasing the concentrations each of COL and CLQ in AMJ13 increases cytotoxicity and improves anti proliferation against AMJ13 (39). COL and CLQ were discovered to have a significant effect on breast cancer cells. The combination of COL and CLQ had the strongest effect on cancer cell lines. CI values revealed high synergism between COL and CLQ (CI < 1) in AMJ13 cell lines. They have also considered cytotoxicity due to the absence of any death rate greater than 50%. AO/PI assay results demonstrated that combination therapy was the best inducer of apoptosis, which agreed with our previous studies. tumor development and cancer prevention Our findings support the study's goal (41).

Isobologram analysis, or the Chou-Talalay equation (Combination index), demonstrating the synergistic effect of COL and CLQ in six different doses used of AMJ13. Isobologram analyses in AMJ13 showed synergistic effect between drugs. Combination medicines provide significant benefits in terms of improved efficacy, decreased cancer toxicity, and reduced drug resistance development. As a result, these benefits have become a standard for the treatment of various diseases and are a potential option in cases of unmet medical need (Foucquier and Gued, 2015). There are numerous methods for determining the synergy of two or more chemotherapy regimens combined to treat a variety of diseases and tumors; these methods were used by the CompuSyn program (42,43).

The inhibitory rates were seen in the cytotoxicity activity of the combination (COL and CLQ) on cell lines. These findings

revealed a synergistic effect of six combination concentrations employed on AMJ13 cell lines. The current study's findings reflected great important of combination therapy in the treatment of breast cancer, and it was discovered that the high rates obtained from the combination of COL and CLQ in the cell lines tumors AMJ13 may also be effective in treating other types of cancer (38).

Morphological alterations and apoptosis observed following treatment with Colchicines and Chloroquin are dependent on the combination index determined for COL and CLQ for 72 hours. Apoptosis was visible as red cells in AO/PI stained cell by fluorescent microscopy and treated cells, while healthy cells were green. Apoptosis is a natural process of programmed cell death that can be triggered by a range of physical and chemical causes and is precisely managed by the organism. Although there are three major signaling channels in apoptosis (mitochondrion, death receptor, and endoplasmic reticulum signaling pathways), apoptotic signaling is frequently integrated and amplified at the mitochondrial level (44-52).

5. Conclusion

The current investigation discovered an alternate or supportive treatment for chemotherapy or other conventional treatments by employing the inhibitor (COL) or (CLQ) or their combination. In addition, anti-tumor effectiveness and cancer cell growth inhibition were obtained through apoptosis induction. This can be an alternate treatment as a combination therapy that can be used to reduce the dose of chemotherapy or other conventional treatments while keeping the same or greater anti-proliferative activity and overcoming chemotherapy resistance or other treatments. The objective of combination therapy is to assault tumor cells through many mechanisms of action in order to prevent cancer cells from acquiring resistance to therapy (Kumar et al., 2014). Furthermore, one of the goals of this study was to lessen the harmful side effects of chemotherapy or other conventional treatments in breast cancer cell lines by using combination therapy (virotherapy and phytotherapy). When using chemotherapy with combination therapy, this can be done by lowering the provided dose while keeping the same or stronger anti-tumor activity

6. Suggestions for Improvement

- Determine the specific mechanisms by which the anticancer effects of chloroquine are exerted. Gaining insight into the

manner in which chloroquine interacts with cellular processes, including autophagy and lysosomal function, can yield significant knowledge regarding its efficacy and the possibilities it holds for combination therapies.

- Perform meticulously planned clinical trials to assess the effectiveness and safety of chloroquine, both as a standalone treatment and as an adjuvant for breast cancer. Ruled-down, large-scale clinical trials have the capacity to furnish substantial empirical support regarding the therapeutic advantages, ideal dosage, duration of treatment, and possible adverse effects.
- Investigate the potential of chloroquine to counteract drug resistance in cells of breast cancer. To determine whether chloroquine can sensitize resistant cells to conventional chemotherapy drugs, examine its effect on drug-resistant mechanisms, including DNA repair pathways and cell cycle checkpoints.
- The potential synergistic effects of chloroquine in combination with other anticancer agents should be investigated. Examine the efficacy of combination therapies comprising chloroquine in conjunction with immunotherapies, targeted therapies, or conventional chemotherapy medications in order to ascertain whether such treatments offer synergistic or additive advantages.

- Conduct additional research to explore the immunomodulatory properties of chloroquine in relation to breast cancer. Examine its effects on the function of immune cells, the microenvironment of the tumor, and the induction of immunogenic cell death. Further investigation into the capacity of chloroquine to augment the immune response against cancer cells may be facilitated by this study.

- Research on Populations and Disparities: Undertake population-based investigations to ascertain the prevalence and risk factors associated with breast cancer across various geographical areas, including Iraq. Determine the socioeconomic, environmental, and genetic determinants of the elevated incidence of breast cancer in specific populations. Developing targeted prevention strategies and expanding healthcare access for at-risk populations can be aided by this research.

By devoting their future investigations to these specific domains, scientists have the potential to advance the field of breast cancer research and potentially discover novel approaches for the disease's management and treatment. Subsequently, the results may facilitate the development of individualized and more efficacious therapeutic strategies.

References

1. Chou, T.C., 2010. Drug Combination Studies and Their Synergy Quantification Using the Chou-TalalayMethodSynergy Quantification Method. Cancer research, 70(2), pp.440-446.
2. Coleman, M.P., Quaresma, M., Berrino, F., Lutz, J.M., De Angelis, R., Capocaccia, R., Baili, P., Rachet, B., Gatta, G., Hakulinen, T. and Micheli, A., 2008. Cancer survival in five continents: a worldwide population-based study (CONCORD). The lancet oncology, 9(8), pp.730-756.
3. Foucquier, J. and Guedj, M., 2015. Analysis of drug combinations: current methodological landscape. Pharmacology research & perspectives, 3(3), p.e00149.
4. Fournier, P. and Schirrmacher, V., 2013. Oncolytic Newcastle disease virus as cutting edge between tumor and host. Biology, 2(3), pp.936-975.
5. Freshney, R.I., 2015. Culture of animal cells: a manual of basic technique and specialized applications. John Wiley & Sons.
6. Guo, X., Zhang, X., Wang, T., Xian, S. and Lu, Y., 2016. 3-Bromopyruvate and sodium citrate induce apoptosis in human gastric cancer cell line MGC-803 by inhibiting glycolysis and promoting mitochondria-regulated apoptosis pathway. Biochemical and biophysical research communications, 475(1), pp.37-43.
7. Katiyar, R.S., Singhvi, N.R., Kushwaha, R.V., Ramji, L. and Suryanarayana, N., 2009. VA-mycorrhizal association in arjuna and jamun trees in forest of Bhandara region, Maharashtra, India. International Journal of Plant Sciences (Muzaffarnagar), 4(1), pp.229-232.
8. Lazarus, D.B., Kotrc, B., Wulf, G. and Schmidt, D.N., 2009. Radiolarians decreased silicification as an evolutionary response to reduced Cenozoic ocean silica availability. Proceedings of the National Academy of Sciences, 106(23), pp.9333-9338.
9. Liu, L., Yan, L., Liao, N., Wu, W.Q. and Shi, J.L., 2020. A review of ULK1-mediated autophagy in drug resistance of cancer. Cancers, 12(2), p.352.
10. Al-Hashimi, M.M.Y. and Wang, X.J., 2014. Breast cancer in Iraq,

incidence trends from 2000-2009. Asian Pacific Journal of cancer prevention, 15(1), pp.281-286.

11. Al-Shammari, A.M., Allak, W.J.K., Umran, M., Yaseen, N.Y. and Hussien, A., 2015. Angiogenesis factors associated with new breast cancer cell line AMJ13 cultured in vitro. Advances in Breast Cancer Research, 4(04), p.100.
12. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. and Jemal, A., 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 68(6), pp.394-424.
13. Bhattacharya, S., Das, A., Datta, S., Ganguli, A. and Chakrabarti, G., 2016. Colchicine induces autophagy and senescence in lung cancer cells at clinically admissible concentration: potential use of colchicine in combination with autophagy inhibitor in cancer therapy. Tumor Biology, 37(8), pp.10653-10664.
14. Al-Shammari, A.M., Jalill, R.D.A. and Hussein, M.F., 2020. Combined therapy of oncolytic Newcastle disease virus and rhizomes extract of Rheum ribes enhances cancer virotherapy in vitro and in vivo. Molecular Biology Reports, 47(3), pp.1691-1702.
15. Al-Shammari, A.M., Salman, M.I., Saihood, Y.D., Yaseen, N.Y., Raed, K., Shaker, H.K., Ahmed, A., Khalid, A. and Duiach, A., 2016. In vitro synergistic enhancement of Newcastle Disease Virus to 5-fluorouracil cytotoxicity against tumor cells. Biomedicines, 4(1), p.3.
16. Al-Shammari, A.M., Abdullah, A.H., Allami, Z.M. and Yaseen, N.Y., 2019. 2-Deoxyglucose and Newcastle disease virus synergize to kill breast cancer cells by inhibition of glycolysis pathway through glyceraldehyde3-phosphate downregulation. Frontiers in molecular biosciences, 6, p.90.
17. Al-Ziaydi, A.G., Al-Shammari, A.M., Hamzah, M.I., Kadhim, H.S. and Jabir, M.S., 2020. Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. Virusdisease, 31(3), pp.341-348.
18. Arora, R., Schmitt, D., Karanam, B., Tan, M., Yates, C. and Dean-Colomb, W., 2015. Inhibition of the Warburg effect with a natural compound reveals a novel measurement for determining the metastatic potential of breast cancers. Oncotarget, 6(2), p.662.
19. Mantena, S.K., Sharma, S.D. and Katiyar, S.K., 2006. Berberine, a natural product, induces G1-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells. Molecular cancer therapeutics, 5(2), pp.296-308.
20. Miller, K.D., Nogueira, L., Mariotto, A.B., Rowland, J.H., Yabroff, K.R., Alfano, C.M., Jemal, A., Kramer, J.L. and Siegel, R.L., 2019. Cancer treatment and survivorship statistics, 2019. CA: a cancer

journal for clinicians, 69(5), pp.363-385.

21. Mohammed, M.S., Al-Taee, M.F. and Al-Shammari, A.M., 2019. Caspase dependent and independent anti-hematological malignancy activity of AMHA1 attenuated newcastle disease virus. International Journal of Molecular and Cellular Medicine, 8(3), p.211.
22. Mualla, F.H. and Al-Alwan, N.A., 2014. Promoting clinical breast examination as a screening tool for breast cancer in Iraq. Iraqi National Journal of Nursing Specialties, 27(1).
23. Wang, X., Zhang, H. and Chen, X., 2019. Drug resistance and combating drug resistance in cancer. Cancer Drug Resistance, 2(2), p.141.
24. W. Humphrey, R., M. Brockway-Lunardi, L., T. Bonk, D., Dohoney, K.M., Doroshow, J.H., Meech, S.J., Ratain, M.J., Topalian, S.L. and M. Pardoll, D., 2011. Opportunities and challenges in the development of experimental drug combinations for cancer. Journal of the National Cancer Institute, 103(16), pp.1222-1226.
25. Yu, Q., Liu, L., Wang, P., Yao, Y., Xue, Y. and Liu, Y., 2017. EMAP-II sensitize U87MG and glioma stem-like cells to temozolomide via induction of autophagy-mediated cell death and G2/M arrest. Cell Cycle, 16(11), pp.1085-1092.
26. Pandrangi, S.L., Raju Bagadi, S.A., Sinha, N.K., Kumar, M., Dada, R., Lakhanpal, M., Soni, A., Malvia, S., Simon, S., Chintamani, C. and Mohil, R.S., 2014. Establishment and characterization of two primary breast cancer cell lines from young Indian breast cancer patients: mutation analysis. Cancer cell international, 14(1), pp.1-20.
27. Rodea-Palomares, I., Petre, A.L., Boltes, K., Leganés, F., Perdigón-Melón, J.A., Rosal, R. and Fernández-Piñas, F., 2010. Application of the combination index (CI)-isobologram equation to study the toxicological interactions of lipid regulators in two aquatic bioluminescent organisms. water research, 44(2), pp.427-438.
28. Siegel, R.L., Miller, K.D. and Jemal, A., 2019. Cancer statistics, 2019. CA: a cancer journal for clinicians, 69(1), pp.7-34.
29. Commons RJ, Simpson JA, Thriemer K, Humphreys GS, Abreha T, Alemu SG, et al. The Effect of Chloroquine Dose and Primaquine on Plasmodium Vivax Recurrence: A WorldWide Antimalarial Resistance Network Systematic Review and Individual Patient Pooled Meta-Analysis. Lancet Infect Dis (2018) 18(9):1025–34.
30. Skrzypek R, Callaghan R. The “Pushmi-Pullyu” of Resistance to Chloroquine in Malaria. Essays Biochem (2017) 61(1):167–75.
31. Thomé R, Lopes SC, Costa FT, Verinaud L. Chloroquine: Modes of Action of an Undervalued Drug. Immunol Lett (2013) 153(1-2):50–7.

32. Aguiar A, Murce E, Cortopassi WA, Pimentel AS, Almeida M, Barros D, et al. Chloroquine Analogs as Antimalarial Candidates With Potent In Vitro and In Vivo Activity. Int J Parasitol Drugs Drug Resist (2018) 8(3):459–64.
33. Pasquier B. Autophagy Inhibitors. Cell Mol Life Sci (2016) 73(5):985–1001.
34. Mindell JA. Lysosomal Acidification Mechanisms. Annu Rev Physiol (2012) 74:69–86.
35. Martinez GP, Zabaleta ME, Di Giulio C, Charris JE, Mijares MR. The Role of Chloroquine and Hydroxychloroquine in Immune Regulation and Diseases. Curr Pharm Des (2020) 26(35):4467–85.
36. Amaravadi RK, Lippincott-Schwartz J, Yin XM, Weiss WA, Takebe N, Timmer W, et al. Principles and Current Strategies for Targeting Autophagy for Cancer Treatment. Clin Cancer Res (2011) 17(4):654–66.
37. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A Systematic Review on the Efficacy and Safety of Chloroquine for the Treatment of COVID-19. J Crit Care (2020) 57:279–83.
38. . Pascolo S. Time to Use a Dose of Chloroquine as an Adjuvant to Anti-Cancer Chemotherapies. Eur J Pharmacol (2016) 771:139–44.
39. Kaneno R, Shurin GV, Kaneno FM, Naiditch H, Luo J, Shurin MR. Chemotherapeutic Agents in Low Noncytotoxic Concentrations Increase Immunogenicity of Human Colon Cancer Cells. Cell Oncol (Dordr) (2011) 34(2):97–106.
40. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological Aspects of Cancer Chemotherapy. Nat Rev Immunol (2008) 8(1):59–73.
41. Zitvogel L, Kroemer G. Anticancer Immunochemotherapy Using Adjuvants With Direct Cytotoxic Effects. J Clin Invest (2009) 119(8):2127–30.
42. Vanmeerbeek I, Sprooten J, De Ruysscher D, Tejpar S, Vandenberghe P, Fucikova J, et al. Trial Watch: Chemotherapy-Induced Immunogenic Cell Death in Immuno-Oncology. Oncoimmunology (2020) 9(1):1703449.
43. Kaneno R, Shurin GV, Tourkova IL, Shurin MR. Chemomodulation of Human Dendritic Cell Function by Antineoplastic Agents in Low Noncytotoxic Concentrations. J Transl Med (2009) 7:58.
44. Zamame RJ, Romagnoli GG, Falasco BF, Gorgulho CM, Sanzochi FC, Dos SD, et al. Blocking Drug-Induced Autophagy With Chloroquine in HCT-116 Colon Cancer Cells Enhances DC Maturation and T Cell Responses Induced by Tumor Cell Lysate. Int Immunopharmacol (2020) 84:106495.
45. Kim S, Han Y, Kim SI, Kim HS, Kim SJ, Song YS. Tumor Evolution and Chemoresistance in Ovarian Cancer. NPJ Precis Oncol

(2018) 2:20.

46. Sakthivel KM, Hariharan S. Regulatory Players of DNA Damage Repair Mechanisms: Role in Cancer Chemoresistance. BioMed Pharmacother (2017) 93:1238–45.
47. Nagel ZD, Kitange GJ, Gupta SK, Joughin BA, Chaim IA, Mazzucato P, et al. Dna Repair Capacity in Multiple Pathways Predicts Chemoresistance in Glioblastoma Multiforme. Cancer Res (2017) 77(1):198–206.
48. Hwang JR, Kim WY, Cho YJ, Ryu JY, Choi JJ, Jeong SY, et al. Chloroquine Reverses Chemoresistance Via Upregulation of p21(WAF1/CIP1) and Autophagy Inhibition in Ovarian Cancer. Cell Death Dis (2020) 11(12):1034.
49. Yue D, Zhang D, Shi X, Liu S, Li A, Wang D, et al. Chloroquine Inhibits Stemness of Esophageal Squamous Cell Carcinoma Cells Through Targeting Cxcr4-STAT3 Pathway. Front Oncol (2020) 10:311.
50. Varisli L, Cen O, Vlahopoulos S. Dissecting Pharmacological Effects of Chloroquine in Cancer Treatment: Interference With Inflammatory Signaling Pathways. Immunology (2020) 159(3):257–78.
51. Compter I, Eekers D, Hoeben A, Rouschop K, Reymen B, Ackermans L, et al. Chloroquine Combined With Concurrent Radiotherapy and Temozolomide for Newly Diagnosed Glioblastoma: A Phase IB Trial. Autophagy (2020), 1–9.
52. Zhou W, Wang H, Yang Y, Chen ZS, Zou C, Zhang J. Chloroquine Against Malaria, Cancers and Viral Diseases. Drug Discovery Today (2020) 25(11):2012–22.