Research Article

Role of Adding Hydroxychloroquine to Chemotherapy in Patients with Neoplasia

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Abstract

Cancer remains the second leading cause of death globally, with approximately 10 million deaths reported in 2020. Despite advances in treatment modalities, the toxicity and limited efficacy of conventional chemotherapy necessitate the exploration of adjuvant strategies to enhance therapeutic outcomes. Hydroxychloroquine (HCQ), a synthetic antimalarial drug derived from quinine, has emerged as a promising adjuvant agent in cancer therapy due to its autophagy-inhibiting properties and favorable safety profile. This review examines the role of adding hydroxychloroquine to chemotherapy in patients with neoplasia, focusing on its mechanisms of action and clinical potential. HCQ exerts anticancer effects through multiple pathways, including autophagy inhibition, enhancement of apoptosis, tumor vasculature normalization, reduction of cancer stem cells, and modulation of immune responses by repolarizing tumor-associated macrophages. Preclinical studies have demonstrated synergistic effects when HCQ is combined with various chemotherapeutic agents, radiation therapy, and targeted therapies. Early-phase clinical trials combining HCQ with temozolomide, temsirolimus, and vorinostat have shown tolerability and preliminary evidence of autophagy modulation, though optimal dosing and reliable biomarkers remain challenging. While HCQ presents a cost-effective and well-tolerated option for augmenting chemotherapy efficacy, further research is needed to establish its clinical benefit and identify patient populations most likely to respond to this combinatorial approach.

Keywords: Hydroxychloroquine, Neoplasia, Chemotherapy, Adjuvant therapy.

Introduction

Neoplasia is defined as a genetic disorientation of cell growth that is triggered by acquired or less commonly inherited mutations affecting a single cell or its clonal progeny .These mutations give the neoplastic cells a survival and growth advantage, resulting in excessive proliferation that is independent of physiological growth signals and control (1).

Currently, cancer is one of the illnesses that causes more deaths worldwide. According to data reported in 2020 by the World Health Organization (WHO), cancer is the second cause of death throughout the world, with 10 million deaths. Clearly, cancer is still a leading problem worldwide (2).

Cancer is a disease that begins with genetic and epigenetic alterations occurring in specific cells, some of which can spread and migrate to other tissues. Although the biological processes affected in carcinogenesis and the evolution of neoplasms are many and widely different, we will focus on 4 aspects that are particularly relevant in tumor biology: genomic and epigenomic alterations that lead to cell transformation, the cells where these changes occur, and the processes of invasion and metastasis that, to an important degree, determine tumor aggressiveness (3).

Chemotherapy aims to inhibit cell proliferation and tumor multiplication, thus avoiding invasion and metastasis. However, this results in toxic effects of chemotherapy due to its effect on normal cells. Inhibition of tumor growth can occur at several levels within the cell and its environment. Traditional chemotherapy agents primarily affect either macromolecular synthesis and function of neoplastic cells by interfering with DNA, RNA, or protein synthesis or affecting the appropriate functioning of the preformed molecule. When interference in macromolecular synthesis or function is sufficient, it leads to cell death due to the chemotherapeutic agent's direct effect or by triggering apoptosis. With traditional agents, cell death may be delayed as many cells die due to a given treatment. So, the medicine may require repeating to achieve a response. The toxicity of cytotoxic drugs is most significant during the

S phase, as it is the DNA synthetic phase of the cell cycle. Vinca alkaloids and Taxanes act in the M phase and block mitotic spindle formation (4).

Chloroquine (CQ) and its derivative hydroxychloroquine (HCQ) are synthetic analogs of a world-famous medicinal herb extract, quinine, with a few centuries of antimalarial history . They belong to a group of 4-aminoquinoline derivatives and possess the property of amphiphilic weak bases. HCQ differs from CQ by one hydroxyl group, the addition of which results in decreased toxicity with the same efficacy (5).

CQ was synthesized in 1934 by Hans Andersag and initially introduced in clinical practice in 1947 due to its significant therapeutic value as an antimalarial agent. Since then, it has been widely used as the first-line medicine for prophylactics and the treatment of uncomplicated malaria caused by a few susceptible strains of *Plasmodium* parasites. However, during the last few decades, CQ and HCQ have been probed for a variety of other diseases. These drugs have a wide therapeutic index and well-established dose safety profiles, and they are inexpensive and orally bioavailable, thus attracting the substantial interest of researchers and clinicians (6).

Our study aimed to critically evaluate the role of hydroxychloroquine as an adjuvant therapy to conventional chemotherapy in patients with neoplasia, examining its mechanisms of action, anticancer potential, and outcomes from early clinical trials.

Neoplasia

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Causes of neoplasia

Recent advance in molecular biology have demonstrated that many tumors arises as a consequence of alteration in the genetic material of cells (e.g. mutation), where the cells escape permanently from normal growth regulatory mechanisms. Tumors can result from the neoplastic transformation of any nucleated cell in the body; the transformed cells are called neoplastic cells. Several factors are known to induce neoplastic transformation of cells including physical agents (e.g. irradiation), chemical agents (e.g. tar), some chronic diseases (e.g. ulcerative colitis) and certain viruses (e.g. HIV) (7).

Classification of Neoplasm

Tumors are classified as benign and malignant, depending on the biological behavior of a tumor (8):

1. **Benign tumors:** They have relatively innocent microscopic and gross characteristics
    - Remain localized without invasion or metastasis.
    - Well-differentiated: Their cells closely resemble their tissue of origin.
    - Prognosis: It is very good, can be cured by surgical removal in most patients and the patient generally survives .
2. **Malignant tumors:** Cancer is the general term used for malignant tumors. The term “cancer” is derived from the Latin word for crab, because similar to a crab, malignant tumors adhere to any part that they seize on, in an obstinate manner.
    - Invasion: Malignant tumors invade or infiltrate into the adjacent tissues or structures.
    - Metastasis: Cancers spread to distant sites metastasize), where the malignant cells reside, grow, and again invade.
    - Exception: Basal cell carcinoma of the skin, which is histologically malignant (i.e., it invades aggressively), rarely metastasizes to distant sites. Glioma is a malignant tumor of CNS.
    - Prognosis: Most malignant tumors cause death.

Cancer Biology

During the last one hundred years, understanding of the biology of cancer increased in an extraordinary way.1-4 Such a progress has been particularly prompted during the last few decades because of technological and conceptual progress in a variety of fields, including massive next-generation sequencing, inclusion of “omic” sciences, high-resolution microscopy, molecular immunology, flow cytometry, analysis and sequencing of individual cells, new cell culture techniques, and the development of animal models, among others.

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Cancer Genomics

The genomics of cancer can be defined as the study of the complete sequence of DNA and its expression in tumor cells. Evidently, this study only becomes meaningful when compared to normal cells. The sequencing of the human genome, completed in 2003, was not only groundbreaking with respect to the knowledge of our gene pool, but also changed the way we study cancer. In the post-genomic era, various worldwide endeavors, such as the Human Cancer Genome Project, the Cancer Genome ATLAS (TCGA), the International Cancer Genome Consortium, and the Pan-Cancer Analysis Working Group (PCAWG), have contributed to the characterization of thousands of primary tumors from different neoplasias, generating more than 2.5 petabytes (10^{15}) of genomic, epigenomic, and proteomic information. This has led to the building of databases and analytical tools that are available for the study of cancer from an "omic" perspective, and it has helped to modify classification and treatment of various neoplasms (9).

Studies in the past decade, including the work by the PCAWG, have shown that cancer generally begins with a small number of driving mutations (4 or 5 mutations) in particular genes, including oncogenes and tumor-suppressor genes. Mutations in TP53, a tumor-suppressor gene, for example, are found in more than half of all cancer types as an early event, and they are a hallmark of precancerous lesions.(10)

The genomic approach has accelerated the development of new cancer drugs. Indeed, two of the most relevant initiatives in recent years are ATOM (Accelerating Therapeutics for Opportunities in Medicine), which groups industry, government and academia, with the objective of accelerating the identification of drugs, and the Connectivity Map (CMAP), a collection of transcriptional data obtained from cell lines treated with drugs for the discovery of functional connections between genes, diseases, and drugs. The CMAP 1.0 covered 1300 small molecules and more than 6000 signatures; meanwhile, the CMAP 2.0 with L1000 assay profiled more than 1.3 million samples and approximately 400 000 signatures (11).

The genomic study of tumors has had 2 fundamental contributions. On the one hand, it has allowed the confirmation and expansion of the concept of intratumor heterogeneity; and on the other, it has given rise to new classification systems for cancer. Based on the molecular classification developed by expression profiles, together with mutational and epigenomic profiles, a variety of molecular signatures have been identified, leading to the production of various commercial multigene panels. In breast cancer, for example, different panels have been developed, such as Pam50/Prosigna, Blue Print, OncotypeDX, MammaPrint, Prosigna, Endopredict, Breast Cancer Index, Mammostrat, and IHC4(12).

Currently, the genomic/molecular study of cancer is more closely integrated with clinical practice, from the classification of neoplasms, as in tumors of the nervous system, to its use in prediction, as in breast cancer.Improvement in molecular methods and techniques has allowed the use of smaller amounts of biological material, as well as paraffin-embedded samples for genomic studies, both of which provide a wealth of information. In addition, non-invasive methods, such as liquid biopsies, represent a great opportunity not only for the diagnosis of cancer, but also for follow-up, especially for unresectable tumors.(13)

Epidemiology of Cancer

Cancer is the second cause of death worldwide; today one of every 6 deaths is due to a type of cancer. According to the International Agency for Research on Cancer (IARC), in 2020 there were approximately 19.3 million new cases of cancer, and 10 million deaths by this disease, while 23.8 million cases and 13.0 million deaths are projected to occur by 2030. In this regard, it is clear the increasing role that environmental factors—including environmental pollutants and processed food—play as cancer inducers and promoters. The types of cancer that produce the greatest numbers of cases and deaths worldwide are indicated in Table 1.(14)

Table 1. Total Numbers of Cancer Cases and Deaths Worldwide in 2020 by Cancer Type (According to the Global Cancer Observatory, IARC) (2).

	Cases	
Both sexes	**Women**	**Men**
Breast (2.26 million)	Breast (2.26 million)	Lung (1.43 million)
Lung (2.20 million)	Colorectal (865 000)	Prostate (1.41 million)
Colorectal (1.93 million)	Lung (770 000)	Colorectal (1.06 million)
Prostate (1.41 million)	Cervical (604 000)	Stomach (719 000)
Stomach (1.08 million)	Thyroid (448 000)	Liver (632 000)

Cancer Treatment

cancer treatment modalities by dividing them into conventional (traditional) and advanced or novel or modern categories. In this era worldwide, over half of all ongoing medical treatment trials are focusing on cancer treatments.Entities, such as the type of cancer, its site, and severity, guide to select treatment options and its progress. The most widely used traditional treatment methods are surgery, chemotherapy, and radiotherapy, while modern modalities include hormone therapy, anti-angiogenic, stem cell therapies, immunotherapy, and dendritic cell-based immunotherapy.(15)

Chemotherapy Of Cancer

Chemotherapy aims to inhibit cell proliferation and tumor multiplication, thus avoiding invasion and metastasis. However, this results in toxic effects of chemotherapy due to its effect on normal cells. Inhibition of tumor growth can occur at several levels within the cell and its environment. Traditional chemotherapy agents primarily affect either macromolecular synthesis and function of neoplastic cells by interfering with DNA, RNA, or protein synthesis or affecting the appropriate functioning of the preformed molecule. When interference in macromolecular synthesis or function is sufficient, it leads to cell death due to the chemotherapeutic agent's direct effect or by triggering apoptosis. With traditional agents, cell death may be delayed as many cells die due to a given treatment. So, the medicine may require repeating to achieve a response. The toxicity of cytotoxic drugs is most significant during the S phase, as it is the DNA synthetic phase of the cell cycle. Vinca alkaloids and Taxanes act in the M phase and block mitotic spindle formation (4).

Combination chemotherapy is a common choice to produce adequate responses as well. They appear to prevent the development of resistant clones by promoting cytotoxicity in resting and dividing cells. Cellular mechanisms that promote or suppress cell proliferation and differentiation are intricate, involving several genes, receptors, and signal

transduction. Investigations in cancer cell biology have led to significant insight into mechanisms of apoptosis, angiogenesis, metastasis, cell signal transduction, differentiation, and growth factor modulation. Researchers are designing molecular targeted therapy on these pathways, selectively inhibiting growth, eg, targeting cell signaling or angiogenesis, blocking protein degradation, etc (16).

Chemotherapy can be administered in neoadjuvant, adjuvant, combined, and metastatic settings. Neoadjuvant therapy is a treatment given before the primary treatment. Adjuvant therapy is the treatment given in addition to the initial therapy, which can suppress or eliminate the growth of occult cancer cells. Adjuvant therapy is now the standard for breast, lung, colorectal, and ovarian cancers. Combined modalities like chemotherapy and radiation are used to shrink the tumor before the surgery or curative intent in cancers like head and neck, lung, and anal.

Combining chemotherapeutic agents is delivered cyclically based on the 3 basic principles.

1. Fraction kill hypothesis: A uniform drug dose kills a constant fraction of tumor cells rather than a constant number regardless of tumor burden.
2. Neoplastic tumor cells have a linear response between the dose and efficacy.
3. Goldie-Coldman hypothesis: Cancer cells acquire spontaneous mutations that cause drug resistance.

Henceforth, multitargeted or combination therapy is superior to single-agent therapy in most cancer treatments. Additionally, combination chemotherapy agents with different mechanisms of action and nonoverlapping toxicities can be chosen to decrease the resistance and toxicities. Curative regimens like bleomycin/vinblastine/cisplatin for testicular cancers are examples of combination chemotherapy. Combination chemotherapy is a common choice to produce adequate responses as well. They appear to prevent the development of resistant clones by promoting cytotoxicity in resting and dividing cells.

Hydroxychloroquine (HCQ)

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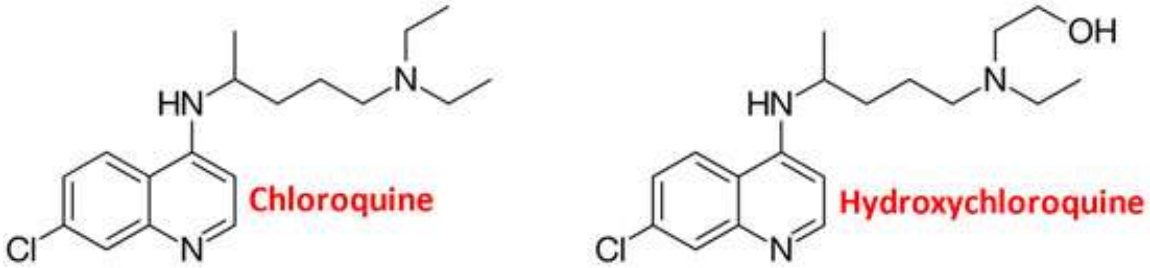

Figure 1. Chemical structure of chloroquine and hydroxychloroquine (17).

CQ was synthesized in 1934 by Hans Andersag and initially introduced in clinical practice in 1947 due to its significant therapeutic value as an antimalarial agent. Since then, it has been widely used as the first-line medicine for prophylactics and the treatment of uncomplicated malaria caused by a few susceptible strains of Plasmodium parasites. However, during the last few decades, CQ and HCQ have been probed for a variety of other diseases. These drugs have a wide therapeutic index and well-established dose safety profiles, and they are inexpensive and orally bioavailable, thus attracting the substantial interest of researchers and clinicians (6).

CQ was shown to be effective for anti-intestinal amebiasis caused by trophozoites of Entamoeba histolytica, which causes amebic dysentery. Both CQ and HCQ have been successfully used for the treatment of autoimmune diseases like rheumatic diseases and systemic lupus erythematosus. Recently, they have also been tested for the treatment and prophylactics of viral infections, including Zika virus , human immunodeficiency virus (HIV) , and COVID-19, although the obtained results were inconsistent or negligible and revealed many side effects (18).

Pharmacokinetics

Chloroquine and HCQ are well-absorbed orally, reaching peak plasma levels within 2–4.5 h . In plasma, 30–40% are bound to albumin and α1-acid glycoprotein .Their stereoisomers exhibit differential binding, metabolism and activity (19).

Chloroquine and HCQ can bind to melanin in pigmented tissues, mononuclear cells and muscles. During prolonged treatment, they accumulate with higher concentration in the heart, liver, brain, muscle and skin than in blood and their tissue concentration may correlate better with their efficacy than their blood levels (19).

In the liver, CQ is de-alkylated via cytochrome P450 (mainly CYP3A, CYP2C8 and CYP2D6) into the pharmacologically active desethyl CQ and bisdesethyl CQ metabolites . HCQ is metabolized via CYP3A4 driven dealkylation into three active metabolites: desethyl CQ, desethyl HCQ and bisdesethyl HCQ. Almost 40–50% of CQ and HCQ are excreted via the kidneys . Being amphiphilic weak bases, CQ and HCQ are partially protonated at physiological pH (7.4) and biprotonated at acidic pH (4–5). Alkalinisation increases and acidification decreases the renal excretion of CQ . CQ and HCQ have long terminal elimination half-lives (~40–50 days) (20).

Anticancer Activity of CQ and HCQ

Several studies have described the anticancer potential of CQ and HCQ when used with standard cancer therapy . CQ and HCQ elicit direct and indirect effects on cancer cells . One mechanism of action is the inhibition of autophagy. Autophagy (or self-eating) is a double-edged process, which can promote either cancer cell survival or death . As an adaptive mechanism, some cancer cells exploit autophagy to survive during stressful conditions, such as nutrient deprivation, hypoxia or cytotoxic insults triggered by cancer therapy (21).

Mimicking tumor microenvironment by co-culturing cancer cells with fibroblasts promotes autophagy . Conversely, the excessive induction of autophagy by diverse anticancer drugs has been reported to trigger autophagic cell death (or programmed cell death type II) of cancer cells . CQ and HCQ act at the late stages of autophagy by raising lysosomal pH, which inhibits the fusion between autophagosomes and lysosomes, and thereby impairs lysosomal protein degradation (22).

Palmitoyl-protein thioesterase 1 has been identified as the lysosomal target of CQ/HCQ. It is worth mentioning that the synergistic anticancer activity of CQ and temozolomide combination was abrogated with the pharmacological or genetic inhibition of early stages of autophagy . Knocking down p53 or overexpressing mutant p53 also compromised the anticancer potential of CQ-temozolomide combination. Notably, superior anticancer efficacy of CQ and erlotinib combination was maintained in the preclinical “cancer cells/fibroblasts co-culturing” setting, mimicking the tumor microenvironment (23).

Given the regulatory crosstalk between autophagy and apoptosis, the augmentation of the anticancer efficacy of chemotherapy by CQ or HCQ might be associated with increased apoptosis. Indeed, the anti-apoptotic Bcl-2 family members as Bcl-2 and Bcl-xl inhibit autophagy. CQ augmented the anticancer activity of Bcl-2 inhibitors . The overexpression of Bcl-2 or Bcl-xL compromised apoptotic cancer cell death triggered in ABT-737 (Bcl-2 inhibitor), CQ and their combination (24).

The physicochemical properties of CQ and HCQ present a critical limitation that restrains their anticancer activity . Solid tumours often develop an insufficient vasculature to supply their nutrient needs and contain regions of hypoxia. Hypoxic cancer cells depend on glycolysis, and other cancer cells may use glycolysis electively for ATP synthesis. This promotes an acidic extracellular microenvironments, which compromises the cellular uptake of CQ/HCQ . To overcome this shortcoming, a series of CQ/HCQ derivatives has been synthesized to increase their anticancer potential . For instance, Lys05, a dimeric CQ, has been reported to be a more potent inhibitor of autophagy with greater anticancer activity than HCQ . However, chronic daily treatment of mice with Lys05 was associated with Paneth cell dysfunction, although without obvious signs of gastrointestinal toxicity . Since inhibitors of autophagy have been shown to improve the effects of chemotherapy in preclinical models, they should undergo clinical evaluation (25).

CRISPR-Cas9 loss-of-function screening identified insulin-like growth factor 1 receptor (IGF1R) as a sensitizer of pancreatic ductal adenocarcinoma cells to CQ/HCQ. Co-targeting IGF1R and ERK inhibited glycolysis and augmented the dependence of pancreatic ductal adenocarcinoma cells on autophagy, and hence rendered them more vulnerable to CQ/HCQ (26).

Chloroquine has also been reported to augment the vulnerability of cancer cells to chemotherapy via autophagy-independent mechanisms, including the normalization of tumour vasculature, which decreases intratumoral hypoxia, cancer cell invasion and metastasis . Notably, CQ-induced vessel normalization was linked to activated endothelial Notch1 signaling . CQ also sensitized triple negative breast cancer cells to paclitaxel via reducing CD44+/CD24−/low cancer stem cells .

Chloroquine and HCQ have been reported to inhibit angiogenesis ; they also induced the secretion of the tumour suppressor prostate apoptosis response-4 (Par-4) from normal cells of treated mice and cancer patients, which triggered paracrine apoptosis of cancer cells and inhibited tumour metastasis . Furthermore, CQ promoted the anti-tumour immune responses via resetting tumour-associated macrophages from the M2 to the tumour-killing M1 phenotype (21).

CQ increases macrophage lysosomal pH and triggers Ca2+ release through the lysosomal Ca2+ channel mucolipin-1, which activates p38 and nuclear factor kappa B (NF-κB), thereby causing tumour-associated macrophages to adopt an M1 phenotype. The antitumor immune responses provoked by CQ were observed preclinically when used at relatively high concentration (10 μM). However, the safe plasma level/concentration of CQ is reported to be approximately 3 μM so that the antitumor immunogenic doses of CQ might not be tolerable clinically (27).

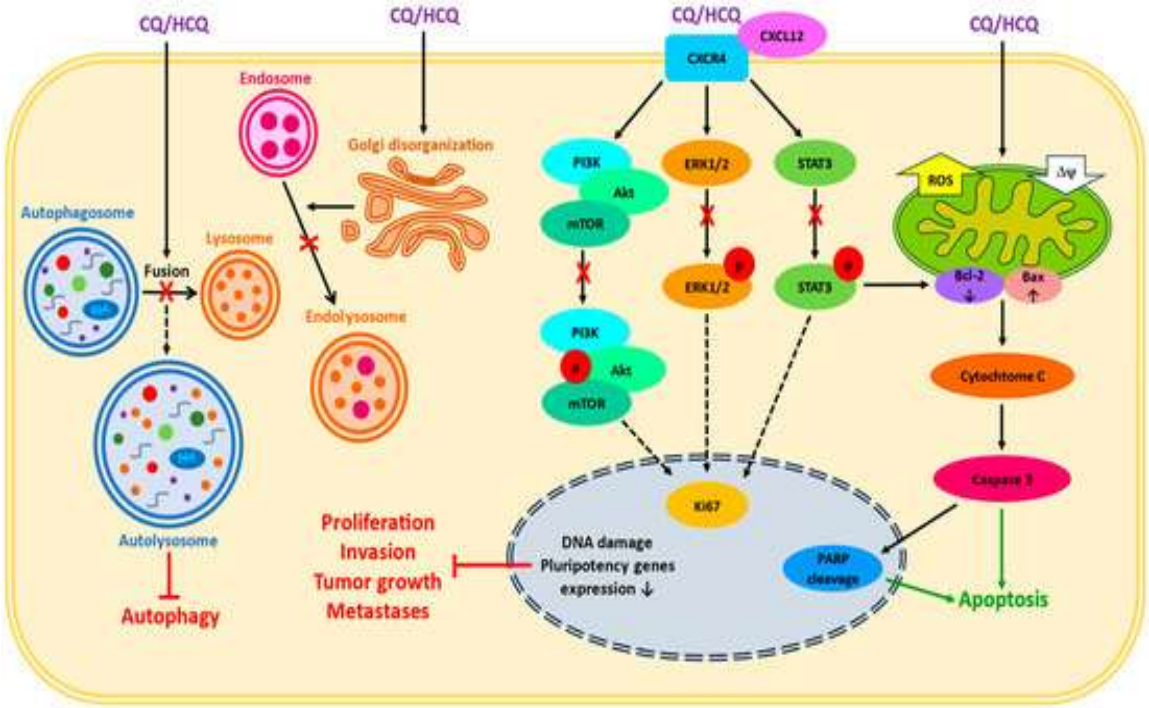

Figure 2. A simplified scheme of reported CQ/HCQ effects in cancer cells. The impact on lysosomal and endosomal systems, the disturbances in intracellular signaling, and the induction of mitochondria-dependent apoptosis are presented. X—inhibition, ROS—reactive oxygen species, Δψ—mitochondrial membrane potential, p—phosphorylation (28).

The limited clinical efficacy of immune checkpoint inhibitors as monotherapy has instigated the investigating of its inclusion in diverse combinatorial regimens . Of note, HCQ compromised the anticancer T cell immune responses triggered by anti-PD1 in syngeneic tumor mouse models. Thus, caution is warranted, since prolonged exposure to clinically approved doses of CQ and HCQ may suppress immune responses, as occurs during their use in treating autoimmune diseases (29).

Outcome Of Early Clinical Trials Of The Combination Of Hydroxychloroquine With Chemotherapy

Since autophagy can act in both a protective or a toxic fashion depending on the stimulus and the cellular target, and in some cases may have neither a cytoprotective nor cytotoxic function, the concurrent development of reliable biomarkers of autophagy assumes great importance. It is suggested from the study of vorinostat with HCQ by Mahalingam et al.(30) that analysis of peripheral blood mononuclear cells (PBMCs) may not reflect the degree of autophagy inhibition in tumors. Although the number of tumor biopsies in this study was small, there was a stark contrast in the degree of autophagy observed within the tumor specimens compared with what was seen in PBMC analysis. While markers of autophagy were identified in PBMCs in the highest dose cohort in this study, that dose proved to be intolerable to patients.

The studies of temsirolimus with HCQ by Rangawala et al.(31) and of dose intense temozolomide with HCQ also by Rangawala et al.(32) were able to achieve higher doses of HCQ administration, and increased autophagic vacuole formation was observed in PBMCs at the highest dose levels in combination with the chemotherapy treatments.

In the studies by Rosenfeld et al.(33)an increase an autophagic vacuoles in PBMCs is shown over the course of 9 wk treatment with temozolomide, radiation, and hydroxychloroquine; however, the increase did not achieve significance and the capacity of either temozolomide or radiation alone to promote autophagy was not evaluated.

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