Review

Chloroquine and Chemotherapeutic Compounds in Experimental Cancer Treatment

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Chloroquine and Chemotherapeutic Compounds in Experimental Cancer Treatment

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Abstract. Chloroquine (CQ) and its derivate hydroxychloroquine (HCQ), the compounds with recognized ability to suppress autophagy, have been tested in experimental works and in clinical trials as adjuvant therapy for the treatment of cancers of different origin to increase the efficacy of cytotoxic agents. Such strategy can be effective to overcome the resistance to standard chemotherapy or anti-angiogenic therapy. This review presents the results of combined application of CQ/HCQ with conventional chemotherapy drugs (doxorubicin, paclitaxel, platinum-based compounds, gemcitabine, tyrosine kinases and PI3K/Akt/mTOR inhibitors, and other agents) for the treatment of different malignancies obtained in experiments on cultured cancer cells and on animal xenograft models, with a few examples of clinical trials. The effects of such approach on viability of cancer cells and tumor growth, as well as autophagy-dependent and independent molecular mechanisms underlying cellular responses of cancer cells to CQ/HCQ are summarized. Although the majority of experimental studies *in vitro* and *in vivo* have shown that CQ/HCQ can effectively sensitize the cancer cells to cytotoxic agents and increase the potential of chemotherapy, the results of clinical trials are often inconsistent. Although pharmacological suppression of autophagy remains a promising tool for increasing the efficacy of standard chemotherapy, the development of more specific compounds is required.

Keywords: chloroquine; hydroxychloroquine; autophagy; chemotherapy; cultured cancer cells; animal xenografts; clinical trials

1. Introduction

Chloroquine (CQ) and its derivative hydroxychloroquine (HCQ) are synthetic analogs of a world-famous medicinal herb extract quinine known for a few-centuries antimalarial history [1–3]. They belong to a group of 4-aminoquinoline derivatives and possess the property of amphiphilic weak bases. HCQ differs by one hydroxyl group which addition results in decreased toxicity with the same efficacy. CQ was synthesized in 1934 by Hans Andersag and initially introduced in the clinical practice in 1947 due to its significant therapeutic value as an antimalarial agent. Since then, it was widely used as the first-line medicine for the prophylactics and treatment of uncomplicated malaria caused by a few susceptible strains of *Plasmodium* parasites until 1980s. CQ and HCQ have a wide therapeutic index and well-established dose safety profiles, they are inexpensive and orally bioavailable, thus attracting a substantial interest among researchers and clinicians [4]. During the last decades, these drugs have been probed for a variety of other diseases. CQ was shown to be effective as anti-intestinal amebiasis caused by trophozoites of *Entamoeba histolytica* which causes amebic dysentery [5]. Both CQ and HCQ have been successfully used for treatment of autoimmune diseases like rheumatic diseases [2,6] and systemic lupus erythematosus [6–8]. Recently, they have also been tested for the treatment and prophylaxis of viral infections including Zika virus [9,10], human immunodeficiency virus (HIV) [11,12], and COVID-19, although the obtained results are inconsistent or negligible and revealed many side effects [13,14].

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Most important, CQ and HCQ have been intensively investigated as the potential tools for treatment of cancers of various origins [3,4,15,16]. Anti-tumor CQ/HCQ activity as a single agent or as adjuvant therapy in combination with widely used cytotoxic compounds has been probed in a long list of malignancies. This review provides a summary of a series *in vitro* and *in vivo* experimental findings and a few examples of clinical trials which applied CQ or HCQ as additives to conventional chemotherapeutic drugs. For a more comprehensive review of the clinical trials that tested CQ and HCQ in the treatment of various cancers, the readers are referred to other recent works [17–19]. The effects of CQ/HCQ on the cultured cancer cells, on various animal xenografts, and examples of clinical trials are summarized in the Tables. In experimental settings, CQ application outnumbered HCQ (Tables 1 and 2), while the majority of clinical trials were conducted as combination therapy with HCQ (Table 3) due to its lower toxicity.

2. Cellular chloroquine effects

The major molecular mechanism believed to underly anti-tumor CQ and HCQ effects and making them potential tools for cancer therapy is their ability to suppress autophagy [3,15,16]. Autophagy is an evolutionarily conserved intracellular process necessary for the maintenance of cellular homeostasis and providing the selective recycling of damaged proteins, macromolecular complexes or whole organelles into lysosomes. Under conditions of nutrient deprivation or stress, autophagy is stimulated to supply the cells with an alternative energy source thus promoting a temporary survival [20,21]. A key process of autophagy is a transient generation of phagophores, the sequestering structures which engulf unwanted cellular material and mature into double-membrane autophagosomes. Further delivery to and fusion with lysosomes allows the cargo degradation and turnover. The major molecular players of autophagy are Beclin-1, p62/SQSTM1 degrading scaffold protein, marker of autophagosomes LC3-II, and ATG (autophagy-related) proteins, which phosphorylate autophagy-related proteins, form the phagophores and autophagosomes.

Autophagy was implicated in the progression of cancers of different origin, with its higher levels closely correlating with lower overall survival. However, its roles in these malignancies are complicated, it can work as either a promoter or suppressor of cell death depending on the stage and type of cancer [22–24]. By recycling the accumulated metabolites and positively regulating the metabolism of cancer cells, autophagy can function as a self-protective response against the antitumor compounds, thus being the critical factor in development of resistance to chemotherapy. On the other hand, recent studies indicate that a series of mutations such as RAS, BRAF and p53 can alter the vulnerability of cancer cells to death and their sensitivity to cytotoxic drugs. Thus, chemotherapy-induced autophagy is emerged as a promising critical target. It is believed that its suppression leads to accumulation of autophagosomes which can compromise cell viability and trigger apoptosis.

CQ and HCQ are lysosomotropic agents which suppress the final step of autophagy by inhibiting the fusion of late endosomes with lysosomes. After entering the cells, they passively diffuse into subcellular structures responsible for protein synthesis and recycling - Golgi vesicles, endosomes, and lysosomes. In acidic lysosomes they undergo protonation and remain trapped inside, thus causing alkalinization which inhibits the ability of enzymes to degrade unwanted material and blocks the survival mechanisms that allows cancer cells to proliferate [3,4].

However, CQ/HCQ are not the specific autophagy inhibitors, they can affect other cellular processes beyond autophagy. Among their reported therapeutic effects on cancer cells are autophagy-independent induction of apoptosis, modifications in tumor microenvironment, normalization of tumor-associated vascularization, prevention of pro-thrombotic processes, activation of anti-tumor immune responses, inhibition of tumor-promoting intermediates by tumor-associated macrophages, negative modulation of cancer-associated fibroblasts, modulation of metabolic responses, alteration of intracellular calcium balance, disruption of membrane stability [2–4].

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3. Chloroquine as a single treatment

In the majority of *in vitro* and *in vivo* (Tables 1 and 2) studies, CQ/HCQ have been shown as effective single agent able to activate the cellular anti-tumor mechanisms leading to both induction of apoptosis and suppression of autophagy. CQ inhibited the growth of orthotopic U87MG glioblastoma in mice model, whereas the decreased viability of cultured glioma cells was accompanied by the stimulation of caspase-3, pro-apoptotic protein Bax and p53 death pathway [25]. Lakhter et al. [26] showed that CQ reduces the growth of melanoma SKMe123 cells and mice melanoma xenografts by lysosome-independent induction of apoptosis and prevention of PUMA protein degradation. The diminished tumorigenicity of primary pancreatic duct adenocarcinoma cells (PDAC) induced by CQ was a result of its inhibition of chemokine receptors CXCL12/CXCR4 and hedgehog signaling pathways accompanied by downregulation of pluripotency-related genes, which led to depletion of cancer stem cells (CSCs) pool, although CQ had no effect on the growth of primary patient-derived pancreatic cancer xenografts *in vivo* [27]. Moreover, CQ did not increase LC3-II level in primary PDAC, although inhibited autophagy in Panc1, 8988 T and BxPC3 cells [27]. CQ treatment of liver HepG2 cancer cells *in vitro* induced G0/G1 cell cycle arrest, DNA damage, activation of caspase-3 and pro-apoptotic protein Bim, PARP cleavage, loss of mitochondrial membrane potential, while injection of CQ to mice bearing HepG2-GFP human liver cancer cells suppressed tumor growth [28]. In pancreatic neuroendocrine neoplasm (PanNEN) culture, CQ treatment induced ER stress and unfolded protein response via activation of PERK-eIF2α-ATF4 pathway, resulting in expression of pro-apoptotic protein CHOP. In Men1 heterozygous-deficient (Men1+/ΔN3-8) mice, a mouse PanNEN model, HCQ administration decreased tumor size and accelerated apoptosis, although proliferative activity was unchanged [29]. In patient-derived glioblastoma stem cell lines with or without p53 mutations, CQ-suppressed proliferation was accompanied by decreased activity of ATM (ataxia-telangiectasia mutated) and HIPK2 kinases (homeodomain-interacting protein *kinase)* functioning as modulators of p53-mediated transcription [30]. However, the efficacy of CQ for survival of mice bearing glioblastoma xenografts greatly depended on the p53 mutations [30]. In human cervical cancer HeLa cells and osteosarcoma U2OS cells, CQ treatment induced autophagy-independent disorganization of the Golgi systems [31]. The compromised mammosphere-forming efficiency of triple-negative breast cancer (TNBC) cells Hs578t, MDAMB231 and SUM159PT following CQ exposure *in vitro* and anti-metastasizing CQ effects in mice TNBC xenograft model was associated with reduction in tumorigenic $CD44^{+}/CD24^{-/low}$ stem cells population accompanied by inhibited Jak2 and STAT3 phosphorylation, global DNA hypomethylation and damage, oxidative stress, mitochondrial membrane depolarization and release of cytochrome C to cytosol [32,33]. In a few cultured cell lines of Adult T-cell leukemia/lymphoma (ATT) and mice Su9T01 tumor xenograft, CQ or HCQ exerted a pronounced anti-tumor effect by rescuing p47 protein, a negative regulator of NF-κB pathway, from autophagy-lysosomal degradation, and by downregulation of CADM1 (cell adhesion molecule 1) [34].

The direct effect of CQ/HCQ on autophagy was confirmed in a series of other works. Thus, increased number of autophagosomes and late endosomes, as well as upregulation of LAMP, p62 and LC3-II proteins have been reported in HeLa, U2OS [31] and TNBC cells [32,33]. Compromised proliferation and colony formation of endometrial adenocarcinoma cell lines with or without p53 mutations and increased population of apoptotic cells following CQ treatment was also accompanied by accumulation of autophagosomes, endosomes, LC3 and p62 [35]. In the human bladder cancer cell lines (RT4, 5637, and T24), CQ or HCQ inhibited proliferation and clonogenic formation by not only DNA fragmentation, increased apoptosis, stimulation of caspases 3/7 and PARP, but also by suppression of lysosome fusion and accumulation of p62 and LC3-II [36]. The similar inhibition of autophagy and stimulation of apoptosis was shown in other tumors such as brain [30,37], ovarian [38], breast [39,40], thyroid [41] and ATT [34].

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Table 1. The effects of single CQ treatment or combination with chemotherapy drugs on cultured cancer cells of different origin.

Agent	Experimental system	Treatment regime	Effects	Molecular markers	Reference
CQ	Glioma U87MG, U251, G120, G130 and G44 cells	10-40 μg/ml for 24-72 h	↓ Cell growth ↓ Viability	↑ Caspase 3 ↑ p53 ↑ Bax	[25]
CQ	Melanoma SK-MEL23 and VMM39 cells	25-50 μM for 5-28 h	↓ Viability, ↓ Lysosomal activity ↑ Apoptosis ↓ Autophagy	↑ Caspase 3 ↑ PUMA ↑ p62 ↑ LC3	[26]
CQ	Primary pancreatic cancer cells	10 μM for 7 days	↓ CSCs number ↓ Sphere-forming ability ↓ CSCs pool in spheres ↓ Invasiveness	↓ CXCL12/CXCR4 signaling ↓ Hedgehog signaling ↓ p-ERK and p-STAT3 ↓ Expression of pluripotency-related genes *OCT4*, *SOX2*, *NANOG*, *cyclins D1 and E1*	[27]
CQ	HepG2 and Huh7 human liver cancer cells	10-30 μM for 24-72 h	↓ Proliferation, ↑ Apoptosis G0/G1 cell cycle arrest	DNA damage, ↑ Caspase-3, cleaved PARP, Bim ↓ Mitochondrial membrane potential	[28]
CQ	Human cervical cancer HeLa cells	100 μM for 2-5 h	↓ Autophagy	↑ Autophagosomes Disorganization of Golgi and endo-lysosomal systems	[31]
CQ	Osteosarcoma U2OS cells	100 μM for 2-5 h	↓ Autophagy	Disorganization of Golgi and endo-lysosomal systems ↑ LC3-II, p62/SQSTM1, LAMP	[31]
CQ	Pancreatic neuroendocrine neoplasm		↑ ER stress ↑ Apoptosis	↑ PERK, eIF2α, ATF4, CHOP	[29]
CQ	TNBC Hs578t, MDAMB231 and SUM159PT cells	1 μM for 48 h	↓ Mammosphere-forming efficiency ↓ $CD44^{+}/CD24^{-/low}$ stem cells population ↓ Autophagy ↓ DNA methylation	↑ Autophagosomes ↑ LC3, p62, caspase-3 ↓ STAT3 and Jak2 phosphorylation, ↓ DNMT1	[32]

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CQ	TNBC Hs578t, MDAMB231 and SUM159 cell lines	10-20 μM for 48 h	↓ Autophagy ↓ CD44+ /CD24−/low CSCs number Mitochondrial damage Cristae vacuolization DNA damage	Mitochondrial membrane depolarization Cytochrome c release ↑ LC3 and p62 ↑ Superoxide ↓ Cytochrome c oxidase, NQO1 ↑ γ-H2AX	[33]
CQ	Endometrial cancer AN3CA, KLE and Ishikawa cells	0.5-20 μM for 24-72 h	↓ Proliferation ↓ Colony formation ↓ Autophagy ↑ Apoptosis Cell cycle arrest	↑ Cleaved caspase-3 ↑ LC3-I, LC3-II, p62 ↑ Autophagosomes and endosomes	[35]
CQ, HCQ	Bladder cancer RT4, 5637, and T24 cells	CQ 25 μM, HCQ 20 μM for 24-72 h	↓ Viability ↓ Clonogenic ability ↓ Autophagy ↑ Apoptosis	↑ Caspase3/7 activity, ↑ Cleaved PARP ↑ LC3-II and p62 ↓ Lysosome fusion DNA fragmentation	[36]
CQ	Vemurafenib-resistant brain tumor 794R and AM38R cells	CQ 5 or 10 μM for 6 or 96 h		↑ LC3-II	[37]
CQ	Epithelial ovarian CSCs	10-50 μM for 72 h or 2-10 μM for week	↓ Viability ↓ Adhesion ↓ Spheroid cell viability and diameter		[38]
CQ	Breast cancer MCF-7 cells	16-256 μM for 48 h	↓ Viability and growth		[39]
CQ	Breast cancer MCF-7 cells	32.5 μM for 48 h	↓ Viability and growth ↑ Apoptosis ↓ Autophagy	DNA damage ↑ Autophagosomes ↑ Bax, p53, cytochrome C ↑ Caspases 3 and 9 mRNA	[40]
CQ/HCQ	Adult T-cell leukemia/lymphoma (ATLL) cell lines	CQ 50 μM or HCQ 25 μM for 6-24 h	↓ Viability and growth ↓ Autophagy ↑ Apoptosis	↑ Caspase-3, LC3 ↑ Autophagosomes ↑ p47, IκBα ↓ NEMO, CADM1	[34]
CQ	Thyroid cancer TPC1, ATC1 and KTC1 cells	50 μM for 48 h	↓ Viability ↓ Autophagy ↑ Apoptosis	↑ LC3 and p62 DNA damage	[41]
CQ	Patient-derived glioblastoma stem cell	CQ 30 μM for 24-72 h	↓ Proliferation ↓ Viability	↓ Ki67 ↑ SubG1 fraction ↑ p53, p21, caspase-3	[30]

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	lines #993, G112SP and #1095			↓ HIPK2 and ATM ↓ p-Akt ↑ LC3-II, p62	
CQ + DOX	Hepatocellular cancer HepG2, Huh7, SNU387 and SNU449 cells	DOX 0.25-1 μg/mL + CQ 20 μM for 48 h	↑ DOX cytotoxicity ↓ Viability ↓ Autophagy	↑ LC3 and p62	[47]
CQ + DOX	Melanoma SK-MEL-5, SK-MEL-28, A-375 cells	DOX 1-2.5 μM + CQ 20 μM for 24 h	↑ Pyroptosis ↓ Autophagy ↓ Viability	↑ Cleaved caspase-3 ↑ N-DFNA5	[48]
CQ + DOX	Breast cancer MCF-7 cells	DOX 0.05-0.2 μM + CQ 16-64 μM for 48 h	↑ Sensitivity to DOX ↓ Viability and growth		[39]
CQ + DOX	Breast cancer MCF-7 cells	DOX 0.17 μM + CQ 16-256 μM for 48 h	↓ Viability and proliferation	↓ Viability ↓ PPT1 expression	[49]
CQ + DOX	Breast cancer MCF-7 cells	DOX 3.38 + μM CQ 32.5 μM for 48 h	↑ Sensitivity to DOX ↓ Viability and growth ↑ Apoptosis ↓ Autophagy	DNA damage, ↑ Autophagosomes ↑ Bax, p53, caspases 3 and 9 ↑ Beclin-1, ATG7, LC3-II and p62 Cytochrome C release, ↓ PI3K, Akt, mTOR, Bcl-2	[40]
CQ + DOX	Cervical cancer HeLa cells	DOX 40 nM + CQ 40 μM	↑ Sensitivity to DOX ↑ Apoptosis ↓ Autophagy	↑ p62, LC3-II, caspase-3, PARP1 ↓ LAMP-2, Syntaxin 17, Rab 5, Rab 7	[51]
CQ + DOX	Human umbilical vein endothelial cells (HUVECs)	DOX 01-1 μM + CQ 0.25-32 μM for 48 h	↑ Anti-angiogenic effect of DOX		[54]
CQ + SpHL-DOX	Cervical cancer HeLa cells	SpHDL-DOX 3.22 μM + CQ 20 μM for 4 h	↓ Viability ↑ Apoptosis		[60]
CQ + DOX@FP-MoS_2	Cervical cancer HeLa-R cells	DOX 5 μg/mL + CQ 5 μg/mL + FP-MoS_2 40 μg/mL for 48 h	↓ Viability ↑ Transfer and accumulation in tumor cells		[61]
CQ + DOX·HCl in DC-DIV/C	DOX-resistant MCF-7/ADR and K562/ADR cells	DOX 5 μg/mL + CQ 10 μg/mL for 24-48 h	↑ Sensitivity to DOX ↑ Apoptosis ↓ Autophagy	↑ Autophagosomes ↑ LC3-II and p62	[62]
CQ + PTX	Breast cancer MCF-7 cells	PTX 1.5-3 nM + CQ 32-64 μM for 48 h	↓ Viability and growth		[39]
CQ + PTX	TNBC Hs578t, MDAMB231 and SUM159PT cells	PTX 5 nM + CQ 1 μM for 48 h	↑ Sensitivity to PTX ↓ Autophagy ↓ $CD44^+/CD24^-$	↑ Autophagosomes ↑ Cleaved caspase-3 ↑ LC-3II and p62 ↓ p-STAT3 and p-Jak2	[32]

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			$^{/low}$ stem cells population ↓ Sphere-forming capacity ↓ DNA methylation	↑ SOCS1, SOCS3 ↓ DNMT1	
CQ + CIS	CIS-resistant endometrial cancer Ishikawa cells	CIS 0.01-100 μM + CQ 1 μM for 72 h	↑ Sensitivity to CIS		[35]
CQ + CIS	Epithelial ovarian cancer SKOV3 and hey cells	CIS 2.5-10 μM + CQ 5-10 μM for 24-48 h	↓ Viability, migration and invasion ↑ Apoptosis	↑ Autophagosomes ↑ Bax, LC3-II/LC3-I ↑ Cleaved caspase-3 and PARP ↓ Bcl-2, Bcl-XL	[72]
CQ + CIS	Thyroid TPC1, ACT1, KTC1 cells	CIS 2 μM + CQ 50 μM for 48 h	↑ Apoptosis ↓ Autophagy	↑ LC3 and p62	[41]
CQ + CIS	Human neuroblastoma SH-SY5Y	CIS 2 μM + CQ 15 μM for 48 h	↑ Apoptosis ↑ CIS sensitivity	↑ LC3-II/LC3-I and p62	[71]
HCQ + CIS	Human neuroblastoma SH-SY5Y	CIS 0.5-2 μM + HCQ 1 μg/mL for 24-48 h	↑ Apoptosis ↓ Autophagy	↑ LC3-II ↑ ROS	[75]
CQ + CPT	TNBC SUM159 SCSs	CPT 10 μM + 10 μM CQ for 48 h	Additive CQ effect ↓ $CD44^{+}/CD24^{-/low}$ DNA damage	↓ Rad50, Rad51 ↑ Cleaved PARP, Bcl-2	[33]
CQ + OXP	Hepatocellular carcinoma HepG2 transfected with *ATG7* shRNA	OXP 18 μM + CQ 80 μM for 12-48 h	↑ Apoptosis	↑ AVOs ↑ LC3 ↑ caspase-3	[78]
CQ + OXP	Colon cancer HT29	OXP 0.95-1.6 μM + CQ 1-5 μM for 24 h	↑ Sensitivity to OXP ↓ Autophagy	↓ LC3 staining	[79]
TH-NP with HCQ+OXP	Hepatocellular carcinoma HepG2, Huh-7 and HCCLM3 cells	OXP 20 μM + HCQ 10 μM for 24 h	↓ Autophagy ↓ Proliferation ↓ Colony formation ↓ Invasion and migration	↑ LC3-I, LC3-II, p62 ↑ E-cadherin, Paxillin, PARP ↑ Autophagosomes	[80]
CQ + GEM	Gallbladder cancer cell lines GBC-SD, SGC-996 and NOZ	GEM 20 μM + CQ 10 μM for 48 h	↑ Anti-tumor GEM effect ↑ Apoptosis ↓ Viability ↓ Colony formation Cell cycle arrest	↑ Bax, LC3-II/LC3-I and p62 ↓ Bcl-2, PARP ↓ p-Akt, p-mTOR	[82]

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CQ + GEM	Pancreatic cancer PANC-1 cells	GEM 20 μM + CQ 10 μM for 72 h	↓ Viability		[83]
PDGL-GEM@CAP/CQ	PDAC Pan 02 cells	GEM 0.5 μg/mL + CQ 2.5 μg/mL for 48 h	↓ Viability ↓ Migration or invasion ↓ Proliferation	↑ LC3-II/LC3-I and p62 ↑ Autophagosomes ↓ Degradation of paxillin and MMP-2	[25]
CQ + IMA	CML K562 cells,	IMA 0.25-0.5 μM + 25 μM CQ for 48 h	↑ IMA-induced cell death ↓ Autophagy	↑ LC3-II	[89]
CQ + IMA	IMA-resistant BaF3/E255K and BaF3/T315I lymphoid cells	IMA 5-10 μM + 25 μM CQ for 48 h	↑ IMA-induced cell death ↓ Autophagy	↑ LC3-II	[89]
CQ + IMA	CML K562 cells	IMA 5 μM + CQ 25 μM for 24 h and up to 5 days	↑ Sensitivity to IMA ↓ Viability ↓ Autophagy ↑ Necrosis Cell shrinkage	↓ Beclin-1 ↑ LC3 Nuclei fragmentation	[90]
CQ + IMA	GIST-T1 cells	IMA 1 μM + CQ 50 μM for 72 h or IMA 0.1 μM + CQ 5 μM for 14 d	↓ Cell growth ↓ Colony formation ↑ Apoptosis	↑ Caspases 3/7 ↑ CC-3 staining	[91]
CQ + IMA	GIST GIST882 cells	IMA 0.5-5 μM for 48 h	↓ Cell growth ↑ Apoptosis ↓ Viability	↓ p-ERK/ERK and p-Kit/Kit ↓ LC3-II/LC3-I ↑ Caspases 3/7	[92]
CQ + Lenvatinib	Papillary thyroid cancer K1 and BCPAP cells	Lenvatinib 10-25 μM + CQ 50 μM for 24 h	↑ Inhibitory effect of Lenvatinib ↑ Apoptosis ↓ Viability and proliferation ↓ Angiogenesis	↑ LC3-I, LC3-II ↓ VEGFA level	[96]
CQ + Apatinib	Anaplastic thyroid cancer KHM-5M and C643 cells	Apatinib 20 μM + CQ 10 μM for 24 h	↓ Autophagy ↑ Apoptosis	↑ LC3-II/LC3-I, p62 ↑ Cleaved PARP ↓ p-mTOR, p-Akt ↓ Autophagosomes	[99]
CQ + Apatinib	Esophageal squamous cell carcinoma ECA-109 and KYSE-150 lines	Apatinib 25 μM + CQ 10 μM for 24 h	↑ Apoptosis ↓ Autophagy ↓ Viability and proliferation ↓ Formation of ESCC clones	↑ LC3-II/LC3-I, p62 ↑ Bax, ↓ Bcl-2, p-Akt, p-mTOR ↓ Autophagosomes	[100]
CQ + RAPA	Osteosarcoma MG63 cells	RAPA 20 μM + CQ 20 μM for 24 h	↑ Effects of RAPA ↑ Apoptosis	↑ LC3-I/II and p62 ↑ Cleaved caspases 3 and 9, PARP	[104]

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			↓ Proliferation ↓ Autophagy	↑ Autophagosomes	
CQ + RAPA	Human well differentiated liposarcoma 93T449 cells	RAPA 6 μM + CQ 80 μM for 24 h	↓ Viability DNA damage	↑ Autophagosomes ↑ LC3-II ↑ TUNEL-positive cells	[105]
CQ + Ipatasertib	MDAMB231, MDAM468, MCF7, SKBR3 breast cancer cell lines	Ipatasertib 1-10 μM + CQ 1-10 μM	↑ Apoptosis ↓ Autophagy ↓ Proliferation ↓ Clonogenic capacity ↓ Spheroid-forming capacity	↑ Cleaved PARP ↑ LC3-II and p62 ↑ Autophagosomes	[119]
CQ + Taselisib	MDAMB231, MDAM468, MCF7, SKBR3 breast cancer cell lines	Taselisib 1-10 μM + CQ 1-10 μM	↑ Apoptosis ↓ Autophagy ↓ Proliferation ↓ Clonogenic capacity ↓ Spheroid-forming capacity	↑ Cleaved PARP ↑ LC3-II and p62 ↑ Autophagosomes	[119]
CQ + Salidroside	Hepatocellular cancer HepG2 and 97H cells	Salidroside 80 μM + CQ 5-20 μM for 48 h	↑ Apoptosis ↓ Viability ↓ Autophagy Changes in cell morphology Chromatin condensation	↑ ROS ↓ mitochondrial membrane potential ↑ Bax, cleaved caspase-3 ↓ Bcl-2, Beclin-1 ↑ p62, p-mTOR/mTOR, p-PI3K/PI3K, p-Akt/Akt	[107]
Lys05 + Dactolisib	Lung cancer A549 cells	Dactolisib 0.05 μM + Lys05 3.19 μM	↓ Autophagy ↑ Apoptosis ↓ Proliferation	↓ *ATG4B*, *LC3A*, *LC3B*, *KI67* genes ↑ *CASP3* ↑ LC3B/LC3A and p62	[109]
CQ + Everolimus	Renal adenocarcinoma A498, RXF393, 769P and SN12C cells	Everolimus 1.3-19.3 μM + CQ 2.4-19.3 μM for 72 h	Synergic growth inhibition ↑ Apoptosis ↓ Autophagy	↓ Bcl-2 ↓ Beclin-1/Bcl-2 complex formation ↓ p-4EBP1, ERK1/2 ↑ Caspases 3 and 9	[110]
CQ + Pd(II) complex	Prostate cancer PC-3 and LNCaP cells	Pd (II) complex 12.5 μM + CQ (5 μM for 12-48 h	↓ Viability ↑ Apoptosis ↓ Autophagy ↑ ROS	↑ Caspases 3/7 ↓ Atg5, Beclin-1, LC3 and p62 ↓ p-Akt/p-mTOR, p-STAT5 and p-CREB	[113]
CQ + Tamoxifen	Antiestrogen-resistant breast carcinoma MCF7-RR, LCC9 cells	1 μM CQ,	↓ Cell growth ↓ Autophagy ↑ Cell death	↑ Autophagosomes ↑ LC3-II, p62	[114]

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		10-1000 nM Tamoxifen for 6 days			
CQ + Faslodex	Antiestrogen-resistant breast carcinoma MCF7-RR, LCC9 cells	1 μM CQ, 10-1000 nM Faslodex for 6 days	↓ Cell growth ↓ Autophagy ↑ Cell death	↑ Autophagosomes ↑ LC3-II, p62	[114]
CQ + Vemurafenib	Glioblastoma 794 and AM38 cells	Vemurafenib 1 μM + CQ 5 μM	↓ Clonogenic growth		[37]
CQ + Trametinib	Glioblastoma 794 and AM38 cells	Trametinib 7.5-30 nM + CQ 5 μM	↓ Growth ↓ Clonogenic growth		[37]
CQ + Vemurafenib	Patient-derived glioblastoma cells	Vemurafenib 1-2 μM + CQ 10-20 μM for 72 h	↓ Autophagy ↓ Tumor growth	↑ LC3B-II, p-ERK/ERK ↑ Caspases 3/7 ↓ p-Akt, pS6	[37]
HCQ + Temozolomide	Glioblastoma U-87 Mg cells	TMZ 100 μg/mL + HCQ 1 μg/mL for 24 h	↑ Apoptosis ↓ Autophagy	↑ LC3-II ↑ ROS	[75]
CQ + IR	Glioblastoma #993, #1095 and G112SP cells	CQ 30 μM + IR 2.5 Gy for 72 h	↓ Proliferation, ↑ Cell death Cell cycle arrest	↑ LC3B-II, p62, ↓ Akt, Ki67 ↑ SubG1 population	[30]
CQ + Sorafenib	Thyroid cancer TPC1, ACT1 and KTC1 cell lines	Sorafenib 100 nM + 50 μM CQ for 48 h	↑ Apoptosis ↓ Autophagy	↑ LC3B-II, p62	[41]
CQ + PTX + Apatinib	Esophageal carcinoma ECA-109 and KYSE-150 cells	PTX 5 μM + CQ 10 μM + Apatinib 25 μM for 24-72 h	↑ Sensitivity to PTX ↑ Apoptosis ↓ Proliferation ↓ Colony formation	↑ Bax, cleaved caspase-3 ↓ Bcl-2, p-Akt, p-mTOR	[100]

Abbreviations: DOX – doxorubicin, PTX – paclitaxel, CIS – cisplatin, CPT – carboplatin, OXP – oxaliplatin, GEM – gemcitabine, IMA – imatinib, RAPA – rapamycin, IR – irradiation, CSCs – cancer stem cells, TNBC – triple negative breast cancer, GSCs – glioblastoma stem-like cells, HUVECs - human umbilical vein endothelial cells, PDAC – pancreatic duct adenocarcinoma cells, CML – chronic myeloid leukemia, GIST - gastrointestinal stromal tumor cells.

4. Chloroquine and chemotherapy drugs

4.1. Chloroquine and doxorubicin (DOX)

Doxorubicin (DOX), a member of Anthracyclines family, is widely used in chemotherapy against a variety of malignancies such as breast, genitourinary and ovarian cancers, Hodgkin's and non-Hodgkin's lymphomas, Ewing and soft tissue sarcoma, lymphocytic and myelogenous leukemias, gastrointestinal, liver, thyroid cancers and neuroblastoma [42,43]. The molecular mechanisms of DOX action on cancer cells include intercalation into DNA–topoisomerase II complex that causes DNA damage followed by p53-mediated cell cycle arrest, alterations in the redox state due to ROS accumulation and iron-dependent lipid peroxidation, dysregulation of calcium binding proteins and channels, increased production of interleukins and interferons facilitating immune-driven clearance of tumor cells. However, severe DOX cardiotoxicity leading to the death of cardiomyocytes and endothelial cells by autophagy, ferroptosis, necroptosis or pyroptosis limits the benefits of DOX therapy [44]. Besides, autophagy was suggested to be linked with DOX resistance acquired during

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long-term therapy allowing the tumor cells to adapt to changing environment, therefore it was proposed as a potential clinical target to overcome DOX resistance [45,46].

Combined application of CQ or HCQ with DOX in *in vitro* and *in vivo* studies have confirmed the effectiveness of DOX-induced autophagy suppression (Tables 1 and 2). In human hepatocellular carcinoma cells, an addition of non-toxic CQ dose potentiated DOX cytotoxicity by diminishing DOX IC50 and preventing DOX-induced autophagy with increased LC3-II/LC3-I ratio and p62 expression [47]. Co-treatment with CQ significantly sensitizes the melanoma cells to DOX *in vitro* by suppression of autophagy and enhancement of pyroptosis accompanied by generation of plasma membrane-targeting DFNA5-N fragment of gasdermin family protein DFNA5 [48]. In the cultured MCF-7 human breast cancer cells and MCF-7 xenograft mice model, CQ increased the sensitivity to DOX treatment and suppressed cell growth and aggressiveness, with reduced expression of Ki67 protein, nuclear marker of active proliferation, PPT1 enzyme involved in lysosomal degradation, and downregulated PI3K/AKT/mTOR signaling pathways [39,40,49]. In TNBC HCC1806 cells, however, although DOX/CQ co-treatment reduced DOX doses and potentiated growth inhibitory effect, such exposure also suppressed apoptotic cell death, which indicated the alternative death pathways [50]. Bano et al. [51] showed an ability of CQ to enhance anticancer effects of DOX in cervical cancer HeLa cells, where synergistic effect was associated with cleavage of procaspase-3 and PARP1, upregulation of p62 and LC-3II, but decreased expression of LAMP-2, Syntaxin17, Rab5 and Rab7 proteins that play critical roles in the fusion of autophagosomes to lysosomes. In human adenocarcinoma alveolar basal A549 cells, CQ accelerated DOX-induced apoptosis mediated by oxidative stress, and led to dephosphorylation of ERK kinases [52]. DOX/CQ administered to mice inoculated with Ehrlich ascites carcinoma cells partially prevented disruption of alveolar structure, reduced the levels of antioxidant enzymes, but increased the level of neutrophil gelatinase-associated lipocalin (NGAL) playing an important role in bacterial defense and inflammation [53]. Besides, CQ therapy enhanced the anti-angiogenic effect of DOX in HUVECs [54]. However, in thyroid cancer cell lines (TPC1, ACT1 and KTC1) CQ failed to enhance the efficacy of DOX [41].

Since DOX is well known for its high toxicity and development of resistance, DOX/CQ was also tested in a series of new formulations proposed to decrease their doses and overcome prominent hydrophobicity [3,55,56]. One of such compounds is PEGylated (poly(ethylene glycol)-coated) liposomal DOX (PLD) with a prolonged circulation time, increased microvascular permeability, and no apparent cardiac toxicity [42,57]. Combination of CQ with PLD and pulse-wave ultrasound hyperthermia (pUH), the scheme developed to enhance the delivery of drugs to subcutaneous 4T1 breast cancer explant in BALB/c mice, induced long-term suppression of tumor growth, in contrast to CQ monotherapy or PLD+pUH treatment [58,59]. In HeLa cells, CQ enhanced the cytotoxicity of DOX encapsulated in pH-sensitive liposomes (SpHL-DOX) created to accelerate the drug delivery in acidic environment [60]. DOX/CQ co-loading in polyglycerol functionalized MoS_2 nanosheets (DOX/CQ-FPMoS_2), designed for targeted delivery and chemo-photothermal therapy, enhanced anticancer effect on multidrug-resistant HeLa (HeLa-R) cells after laser irradiation [61]. Delivery of simultaneously encapsulated DOX·HCl and CQ in pH-responsive cholesteryl hemisuccinate self-assembled nanovesicles (DC-DIV/C) to DOX-resistant K562/ADR and MCF-7/ADR cells or nude mice bearing drug-resistant K562/ADR xenograft led to much stronger anti-tumor efficacy, accompanied by apoptosis and blockage of autophagosomes and lysosomes fusion [62].

4.2. Chloroquine and paclitaxel (PTX)

Paclitaxel, a tricyclic diterpenoid belonging to taxanes and found in the bark and needles of *Taxus brevifolia*, is one of the most successful natural chemotherapeutic compounds [63,64]. Due to minimal toxicity, high efficiency and broad-spectrum antitumor activity, PTX is widely used for the therapy of ovarian, cervical, breast, colorectal, esophageal, lung and prostate cancer, either alone or in combination with other agents. The major mechanism of its activity is a capacity to disrupt microtubule assembling dynamics and induce cell cycle arrest at G2/M phase leading to apoptosis. However, as for other chemotherapeutic drugs, a major problem for PTX application is a development of chemoresistance due to protective autophagy [65].

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By inhibiting autophagy, CQ and PTX in combination were found to be synergic in suppressing the viability and growth of MCF-7 human breast tumor cells [39] and three TNBC cell lines [32]. Moreover, CQ increased the sensitivity to PTX and reduced lung metastases, tumor growth and recurrence in orthotopic murine MDAMB231 and SUM159PT tumor models, as well as diminished $CD44^{+}/CD24^{-/low}$ CSC population in clinical trial [32]. Co-exposure of esophageal carcinoma EC109 cells to CQ and PTX was found to enhance the suppressive effect of PTX by inhibiting autophagy through Akt/mTOR pathway [66]. The phase II clinical trial, which recruited the patients with advanced or metastatic breast cancer (of HR+/HER2− and TNBC types) who previously did not benefit from anthracycline-based chemotherapy, has shown that CQ in combination with taxane or taxane-like agents (paclitaxel, docetaxel, nanoparticle (NP) albumin-bound nab-paclitaxel, and ixabepilone) increases the objective response rate in comparison to expected for PTX-based therapy itself, with good tolerance and low rate of adverse effects [67] (Table 3).

4.3. Chloroquine and platinum-based anticancer drugs

The cohort of clinically approved platinating derivatives includes cisplatin (CIS), carboplatin (CPT) and oxaliplatin (OXP). The major mechanism of their action is DNA damage followed by inhibition of transcription, but they are also able to exert cytoplasmic effects such as mitochondrial damage, ER stress, suppression of ribosome biogenesis and elevation of micro-RNA activity [68,69]. They are widely used as a first-line chemotherapy compound for ovarian, cervical, testicular, bladder, esophageal, lung, head and neck cancers, brain tumors and neuroblastoma. However, the resistance and many side effects (nephrotoxicity, neurotoxicity and hepatotoxicity) of these agents are reported, which drives the necessity to reduce their toxicity [70].

Cisplatin. CQ enhanced the sensitivity to CIS treatment of endometrial adenocarcinoma cells [35], thyroid cancer cell lines (TPC1, ACT1 and KTC1) [41] and SH-SY5Y cells [71]. In all these cells, CQ effects were associated with suppression of autophagy accompanied by increased LC3 and p62 expression. In epithelial ovarian cancer SKOV3 and hey cells, CQ alone had no effect on tumor migration and invasion capacities, but alleviated CIS-induced autophagy with upregulation of apoptosis-related proteins [72]. In mice bearing gastric cancer xenograft, CQ enhanced CIS chemosensitivity and anti-tumor effect by downregulation of multidrug resistance gene MDR1/P-gp and activation of caspase-3, as well as by inhibition of CIS-triggered autophagy [73]. In a mouse hepatocarcinoma xenograft model, CIS or CQ alone were able to reduce the tumor growth, however, their combination significantly augmented anti-tumor effect and impaired proliferation of tumor cells by causing higher level of apoptosis [74]. The inhibition of autophagy with HCQ and CIS enhanced apoptosis and potentially therapeutic oxidative stress in neuroblastoma SH-SY5Y [75].

Carboplatin. In combination with CPT, CQ exerted an additive anti-tumor effect in TNBC SUM159 stem cells and effectively reduced the growth of mice CPT-resistant SUM159 orthotopic xenografts proved to be linked with inhibition of CPT-induced autophagy [33]. The effectiveness of CQ/CPT combination was confirmed in experiments on epithelial ovarian tumor cells from the patients and mice xenograft, in which such treatment decreased CSCs pool with surface co-expression of CD117 (c-Kit) and CD44, and suppressed their tumorigenic potential and spheroid-forming ability [38]. In heavily pretreated patients with advanced solid tumors of different origin (GIST, neck and head, colorectal, urothelial, esophageal, etc.), combination of CQ or HCQ with CPT increased progressive-free disease and overall survival (OS), although some side effects were reported [76]. Importantly, in the exosomes obtained from blood plasma of patients which received such treatment both LC3-B isoforms were detected at advanced time points of the second and third cycles [77].

Oxaliplatin. Apoptotic cell death induced by OXP was significantly enhanced by CQ treatment in hepatocellular carcinoma HepG2 cells with ATG7 knockdown due to inhibition of autophagy [78]. Application of CQ sensitized a few colon cancer cell lines to OXP under both oxic and hypoxic conditions and showed a synergistic interaction in suppressing the growth of mice HT29 xenografts with reduced number of autophagosomal cells [79]. Recently, biomimetic nanoparticles

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encapsulating both HCQ and OXP were shown to reduce the tumor capacities of hepatocellular carcinoma cells *in vitro* and *in vivo* by blocking or reversing autophagy [80].

4.4. Chloroquine and gemcitabine (GEM)

Gemcitabine is a nucleoside metabolic inhibitor which active metabolites function as deoxycytidine analog able to replace the building blocks of nucleic acids during DNA elongation, thus preventing DNA synthesis, arresting tumor growth and promoting apoptosis [81]. Although GEM was initially approved for the treatment of pancreatic cancer, it is currently used as adjunct therapy of various solid tumors such as ovarian cancer, non-small cell lung carcinoma, metastatic breast cancer. However, the resistance to GEM remains a serious problem among a noticeable rate of patients. It is not surprising that CQ was tested as potential synergist to GEM.

In vivo CQ and GEM co-exposure more effectively eliminated tumors and improved overall survival of mice bearing pancreatic patient-derived PDAC xenografts by inhibition of CXCL12/CXCR4 with reduced phosphorylation of downstream effectors ERK and STAT3, and inhibition of hedgehog signaling [27]. The addition of CQ to anti-tumor therapy strengthened the cytotoxic effects of GEM on human gallbladder cancer cells (GBC) *in vitro* and inhibited the growth of GBC xenografts in mice *in vivo*, with upregulation of LC3-II/LC3-I ratio and Bax, downregulation of Bcl-2 and PARP, and inhibition of AKT/mTOR pathway [82]. GEM/CQ combination significantly reduced the viability of human pancreatic cancer PANC-1 cells, although CQ alone did not exhibited any effect [83]. The addition of CQ or HCQ to GEM therapy increased OS of patients with advanced solid tumors of different types previously received other treatment regimens [76].

As for other chemotherapy drugs, new delivery strategies with enhanced penetration ability have been developed. Administration of poly lactic-co-glycolic acid (PLGA) nanoparticles loaded with CQ, created as the carriers to reduce its doses, in combination with GEM to mice bearing orthotopic pancreatic cancer xenograft diminished tumor progression and suppressed the density of activated tumor cells at lower CQ doses [84]. Chen et al. [85] designed the pH-sensitive PDGL-GEM@CAP/CQ particles consisting of GEM loaded in 6PA-modified DGL and co-precipitated with CQ and calcium phosphate. Administration of this system to cultured pancreatic Pan 02 cells or mice bearing Pan 02 xenografts intensified anti-tumor GEM/CQ effects by inhibition of proliferation, tumor growth, metastases and fibrosis, suppression of autophagy, and decrease in the number of activated fibroblasts. In contrast to GEM monotherapy, adjuvant autophagy inhibition with HCQ significantly increased the median OS and DFS, especially in the patients with high-risk PDAC, and correlated with increased LC3II level [AlMasri 2021].

Table 2. The effects of single CQ treatment or combination with chemotherapy drugs on animal tumors models.

Agent	Experimental system	Concentration	Effect	Molecular markers	Reference
CQ	Glioblastoma U87MG xenografts of NMRI nude mice	CQ 30 mM/day intracranially for 17 days	↓ Tumor growth, ↓ Cell viability, ↓ Number of mitotic cells		[25]
CQ	Melanoma SKMel23 cells xenografts of NOD-SCID mice	CQ 25 mg/kg (IP) twice/week for 3 weeks	↓ Tumor growth ↓ Autophagy		[26]
CQ	Athymic nude mice with orthotopic MDAMB231 breast cancer tumor	CQ 10 mg/kg daily (IP) for 2 2 weeks	↓ Tumor growth ↓ Lung metastasis	↓ $CD44^{+}/CD24^{-/low}$ stem cells number	[32]
CQ	Liver cancer HepG2-GFP xenograft of nude mice	CQ 80 mg/kg twice daily 3-d-	↓ Tumor growth and weight ↓ Proliferation	↓ Ki-67 ↑ cleaved PARP	[28]

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		on/2-d-off (SC) for 25 days			
CQ	Immunocompromised mice implanted with patient-resected PDAC cells	CQ 50 mg/kg (IP) for 21 days	↓ CSCs-driven metastases ↓ Tumorigenicity	↓ CD133+ cells number ↓ *ALK4* ↓ Nodal/Activin ↓ Self-renewal genes	[27]
CQ	Female BALB/c mice with MCF-7 xenograft	CQ 50 mg/kg (IP) 2 once/3 days for 43 days	↓ Viability and growth ↑ Apoptosis ↓ Autophagy DNA damage	Cytochrome C release ↑ Bax, p53 ↑ Caspases 3 and 9	[40]
CQ/HCQ	Immunodeficient NOD/Shi-scid/IL-2Rγnull (NOG) mice transplanted with ATLL MT2 or Su9T01 cells	CQ 50 mg/kg/day (IP) or HCQ 6.5-60 mg/kg/day (OR) for 21 days	↑ Survival ↓ Tumor growth and weight Degeneration and necrosis of tumor cells	↑ Caspase-3 Condensed hyperchromatic or fragmented nuclei with shrunk cytoplasm	[34]
CQ + DOX	Female BALB/c mice with MCF-7 xenograft	DOX 2 mg/kg (IP) + CQ 50 mg/kg (IP) once/3 days for 43 days	↓ Tumor growth, ↑ Apoptosis ↓ Autophagy DNA damage	↑ Autophagosomes Cytochrome C release ↑ Bax, p53, caspases 3 and 9, Beclin-1, ATG7, LC3-II, p62 ↓ PI3K, Akt, mTOR, Bcl-2	[40]
CQ + DOX	Female mice injected with Ehrlich ascites carcinoma (EAC) cells	DOX 1.5 mg/kg and 3 mg/kg + CQ 25 mg/kg and 50 mg/kg (IP) on 2, 7 and 12 days	↓ Disruption of alveolar structure ↓ Oxidative stress	↓ MDA, CAT, GPx, SOD, iNOS, eNOS ↑ NGAL	[53]
CQ + PEG-DOX+ pUH	BALB/c mice subcutaneously injected with 4T1 breast tumor cells	PEG-DOX 10 mg/kg (IV) + CQ 50 mg/kg + 15-min on-tumor pUH on day 5 after tumor implantation up to 60 days	↓ Viability, ↓ Tumor growth ↑ Animal survival DNA damage	↑ LC3-II ↑ TUNEL-positive cells	[58, 59]
CQ + DOX·HCl in DA-DIV/C nanovesicles	Female BALB/c nude mice subcutaneously inoculated with DOX-resistant K562/ADR cells	DOX-HCl 5 mg/kg + CQ 10 mg/kg (IV) at 0, 2, 4 and 6 days	↓ Tumor volume and weight ↓ Autophagy ↓ Cell density ↑ Necrosis DNA damage	↓ Ki67 ↑ TUNEL-positive cells ↑ LC3-II	[62]

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CQ + PTX	Athymic nude mice with orthotopic MDAMB231 and SUM159PT tumors	PTX 15-30 mg/kg (IP) weekly + CQ 10 mg/kg daily for 2 weeks or twice/week for 4 weeks	↑ Sensitivity to PTX ↓ Tumor growth ↓ Lung metastasis ↓ Tumor recurrence ↓ PTX-induced CSCs population	↓ $CD44^{+}/CD24^{-/low}$ CSCs	[32]
CQ + Taselisib	Female NOD/SCID athymic mice injected with TNBC MDAMB231 cells	Taselisib 5 mg/kg (OR) 5 days/week + CQ 30 mg/kg (OR) 5 days/week for 2 weeks	↑ Anti-tumor PTX effect ↓ Tumor growth		[119]
CQ + CIS	Nude BALB/C female mice with gastric cancer SGC7901 xenograft	CIS 5 mg/kg + CQ 45 mg/kg every three days 10 times	↓ Tumor weight	↓ LC3II/I ratio, Beclin-1 ↓ MDR1/P-gp ↑ caspase-3	[73]
CQ + CIS	Nude mice with ovarian cancer SKOV3 xenograft	CIS 5 mg/kg/6 days + CQ 60 mg/kg/day (IP) for 21 days	↓ Tumor volume and weight	↑ Cleaved caspase-3 ↓ Ki-67-positive cells	[72]
CQ + CIS	BALB/C nude mice with hepatocarcinoma SMMC-7721 xenograft	CQ 60 mg/kg + CIS 3 mg/kg (IP) thrice/week for 2 weeks	↓ Tumor volume and weight ↑ Apoptosis ↓ Proliferation	DNA damage ↓ Ki-67-positive cells	[74]
CQ + CPT	Immunodeficient SCID-Beige mice with TNBC SUM159 xenograft	CPT 24 mg/kg weekly + CQ 30 mg/kg every 3 days for 3 weeks	↓ Tumor volume ↓ Viability ↑ Apoptosis	↓ Mitochondrial metabolic activity ↓ Bcl-2, Rad50, Rad51 ↑ LC3B-II, p62	[33]
CQ + CPT	Immunodeficient NSG mice injected with $CD45^{-}CD44^{+}$ epithelial ovarian tumor cells	CPT 50 mg/kg + CQ 100 mg/kg every 2 days weekly for 16 weeks	↓ Tumor volume	↓ $CD44^{+}/CD117^{+}$ cells population ↓ Ki67	[38]
CQ + OXP	Immunodeficient C/.B.17 SCID mice injected with colon cancer HT29 cells	OXP 5 mg/kg (IP) per week for 2 weeks + CQ 3.5 mg.kg daily for 21 days	↓ Tumor growth and volume ↓ Autophagosomal cells	↓ LC3 staining	[79]
TH-NP with HCQ+OXP	Nude mice with hepatocellular carcinoma HCCLM3 xenograft	OXP 10 mg/kg + HCQ 20 mg/kg (IV) every three days for 30-49 days	↓ Tumor growth ↓ Metastases ↓ Autophagy	↑ Cleaved caspase 3 and PARP ↓ Ki67 ↓ Autophagosomes/autolysosomes	[80]

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CQ + GEM	Immunocompromised mice implanted with patient-resected PDAC	GEM 125 mg/kg (IP) for 52 days + CQ 50 mg/kg (IP) for 21 days	↓ Tumor growth ↑ Survival rate	↓ CD133+ CSCs ↓ Nodal/Activin pathway	[27]
CQ + GEM	Male BALB/c nude mice injected with gallbladder cancer SGC-996 cells	GEM 20 mg/kg (IP) + CQ 60 mg/kg (IP) twice/week for 22 days	↑ Sensitivity to GEM ↓ Tumor growth		[82]
PDGL-GEM@CAP/CQ	Mice bearing pancreatic cancer Pan 02 xenografts and Orthotopic pancreas Pan 02 tumor	GEM 3 mg/kg (IV) + CQ 15 mg/kg (IV) every other day 4 times	↓ Tumor growth ↓ Metastases ↑ Tumor necrosis ↓ Number of activated fibroblasts ↓ Fibrosis ↓ Autophagy	↑ Autophagosomes ↑ LC3II/LC3I ratio and p62 ↓ MMP-2, IL-6 ↓ Collagen ↑ Paxillin ↓ αSMA	[85]
CQ-loaded PLGA nanoparticles + GEM	BALB/c AJcl nu/nu female mice orthotopically transplanted with immortalized patient-derived pancreatic stem cells with SUIT-2 cancer cells	GEM 40 mg/kg (IV) at days 10, 17, 24 + Nano-CQ 30 mg/kg (IV) at days 10, 17, 24	↓ Density of activated cancer stem cells ↑ Sensitivity to GEM ↓ Tumor volume and weight	↓ αSMA	[84]
CQ + IMA	NOD/SCID male mice implanted with IMA-sensitive and resistant GIST882 cells	IMA 150 mg/kg (OR) twice/day + CQ 60 mg/kg (IP) daily for 28 days	↓ Autophagy No effect on tumor growth	↑ LC3II ↓ p-ERK/ERK	[92]
CQ + IMA	Female athymic nude NMRI nu/nu with heterotopic GIST-T1 xenograft	IMA 50 mg/kg (OR) twice/day + CQ 60 mg/kg (IP) daily for 15 days	↑ Apoptosis No effect on tumor growth	↑ CC-3 staining	[91]
CQ + Lenvatinib	Nude mice injected with thyroid cancer K1 cells	Lenvatinib 30 mg/kg + CQ 50 mg/kg for 14 days	↑ Anti-cancer LEN effect ↓ Tumor growth ↓ Angiogenesis	↓ VEGFA, CD31, C-Myc	[96]
CQ + Lenvatinib	Nude BALB/c mice injected with hepatocellular carcinoma HCCLM3 cells	Lenvatinib 5-10 mg/kg (IP) + HCQ 50 mg/kg (IP)	↓ Tumor growth ↓ Lung metastases ↑ Overall survival		[97]
CQ + Apatinib	Male BALB/c nude mice injected with KHM-5M thyroid cancer cells	Apatinib 50 mg/kg (OR) daily + CQ 60 mg/kg (OR)	↓ Tumor volume and weight ↓ Proliferation ↑ Apoptosis	↑ Cleaved caspase-3 ↑ TUNEL-positive cells	[99]

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		daily for 26 days		↓ Ki67	
CQ + Apatinib	Male BALB/c nude mice injected with esophageal carcinoma ECA-109 cells	Apatinib 60 mg/kg OR) daily + CQ 60 mg/kg (OR) daily for 4 weeks	↓ Tumor volume and weight ↓ Proliferation ↑ Apoptosis	↑ Cleaved caspase-3 ↑ TUNEL-positive cells ↓ Ki67-positive cells	[100]
CQ+RAPA	Athymic nude male mice injected with patient-derived dedifferentiated liposarcoma	RAPA 1 mg/kg/day (IP) + CQ 100 mg/kg/day (IP) for 15 days	↓ Tumor growth ↓ Cancer cells density ↑ Apoptosis	↑ TUNEL-positive cells	[106]
CQ + Salidroside	Female BALB/c mice subcutaneously injected with HepG2 cells	Salidroside 80 mg/kg (IP) + CQ 5 mg/kg (IP) every other day for 4 weeks	↓ Tumor growth ↓ Number of tumor cells	↑ Bax ↓ Bcl-2	[107]
CQ + 5-FU	BALB/c nude mice with hepatocarcinoma SMMC-7721 xenograft	5FU 30 mg/kg (IP) + 60 mg/kg CQ (IP) trice/week for 2 weeks	↑ Sensitivity to 5-FU ↑ Apoptosis ↓ Proliferation, ↓ Tumor growth	↑ TUNEL-positive cells ↓ Ki67-positive cells	[74]
CQ + Tamoxifen	Athymic nude mice injected with breast cancer MCF7-RR or LCC9 cells	Tamoxifen 32 mg/kg/d or + CQ 1-2 mg/mouse/d (OR) for 5 weeks	↓ Tumor growth ↑ Angiogenesis ↓ Macrophage activation	↑ CD31-positive cells ↑ pVEGFR2 ↑ CD68-positive cells	[114]
CQ + Faslodex	Athymic nude mice with breast cancer MCF7-RR or LCC9 xenografts	Faslodex 0.5 mg/mouse/w (SC) + CQ 1-2 mg/mouse/d (OR) for 5 weeks	↓ Tumor growth ↑ Angiogenesis	↑ CD31-positive cells ↑ pVEGFR2	[114]
CQ +	Female Nu/nu mice subcutaneously injected with cisplatin-resistant ovarian cancer OVCAR3 cells	CQ 30 mg/kg + nelfinavir 250 mg/kg + RAPA 2.24 mg/kg + dasatinib 4 mg/kg + metformin 150 mg/kg in 50% PEG400 for 7 days	Tumor remission	↑ LC3B-II, Grp78	[121]
CQ + Apatinib + PTX	Nude BALB/c mice injected with esophageal carcinoma ECA-109 cells	Apatinib 60 mg/kg (OR) daily + CQ 60 mg/kg (OR) daily + PTX 15 mg/kg (IP)	↓ Tumor volume and weight ↑ apoptosis ↓ Proliferation ↑ Apoptosis	↑ Cleaved caspase-3 ↑ TUNEL-positive cells ↓ Ki67	[100]

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		twice/week for 4 weeks		
CQ + Taselisib + PTX	Female NOD/SCID athymic mice injected with TNBC MDAMB231 cells	Taselisib 5 mg/kg (OR) 5 days/week + CQ 30 mg/kg (OR) 5 days/week + PTX 10 mg/kg IP once/week for 2 weeks	↑ Anti-tumor effect of PXT and Taselisib ↓ Tumor volume and weight	[119]
CQ + IR	Female NMRI immunodeficient mice injected with GBCs #993, #1095 and G112SP cells	CQ 14 mg/kg IP IR 2.5 Gy for 6 days	↑ Survival ↑ Sensitization to IR	[30]

Abbreviations: IP-intraperitoneally, SC – subcutaneously, OR - orally, IV - intravenously.

4.5. Chloroquine and tyrosine kinase inhibitors

Imatinib (IMA). Imatinib is a small molecule tyrosine kinase inhibitor targeting numerous enzymes like CSF1R, c-KIT, FLT3, and platelet-derived growth factor receptor PDGFR-β, but reasonable selective to BCR-ABL fusion protein. It binds to ATP pocket at kinase active site thus preventing downstream phosphorylation of target proteins. IMA is the most common first-line cytotoxic agent for the treatment of chronic myeloid leukemia (CML) and gastrointestinal stromal tumor (GIST) in the systemic therapy, but CML stem cells are intrinsically resistant to IMA [87,88].

An important role of autophagy in resistance of CML cells to IMA was established in K562 cells, in which CQ or IMA alone did not change the rate of death, while CQ/IMA co-treatment enhanced the sensitivity to IMA and accelerated apoptotic cell death. Moreover, the combination of drugs produced the same effects in IMA-resistant lymphoid cell lines [89]. CQ improved IMA-induced cytotoxicity and reduced long-term viability of K562 cells due to inhibition of autophagy initiation and autophagosome turnover [90]. In GIST-T1 cells, CQ as a single agent or in combination with IMA prevented the growth, decreased viability and increased LC3-II, furthermore, in a mouse GIST-T1 xenograft model, treatment with IMA/CQ increased apoptosis [91]. Although CQ or IMA alone did not or weakly inhibit the growth of GIST882 IMA-resistant cells, CQ addition enhanced the suppressive effect of IMA on cell proliferation and promoted apoptosis by blocking autophagy and altering the level of ERK phosphorylation [92]. The phase II clinical trial, however, did not reveal any pronounce differences in long-lasting (12 and 24 months) "success" rates after 48-weeks administration of IMA/CQ, although authors noticed some molecular responses [93].

Lenvatinib. Lenvatinib is a potent tyrosine kinase inhibitor targeting PDGFRα, vascular endothelial growth factor receptors VEGFR1-3, fibroblast growth factor receptors FGFR1-4, tyrosine kinase receptor c-Kit and RET proto-oncogene. It is widely used for the treatment of thyroid cancer and hepatocellular carcinoma [94,95]. Although the resistance and side effects following its application are common, the data on Lenvatinib and CQ therapy are scarce. The effectiveness of CQ/Lenvatinib co-exposure was shown in thyroid cancer K1 and BCPAP cells, with suppression of Lenvatinib-induced autophagy leading to inhibition of proliferation and angiogenesis, increased apoptosis and reduced VEGFA levels, while co-treatment of mice bearing K1 xenograft diminished tumor growth accompanied by decrease in VEGF markers VEGFA and CD31, and proliferation marker c-Myc [96]. Combined HCQ/Lenvatinib therapy led to increased overall survival, inhibition of tumor growth and lung metastases in mice hepatocellular carcinoma xenograft model [97].

Apatinib. Apatinib is a tyrosine kinase inhibitor that selectively inhibits VEGFR2 and has mild activity towards c-Kit and c-SRC tyrosine kinases [98]. The major anti-cancer effect of Apatinib is blockage of angiogenesis, namely VEGF-mediated endothelial cell migration and proliferation,

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leading to suppression of new blood vessel formation in tumor tissue. Inhibition of Apatinib-induced autophagy with CQ *in vitro* increased apoptosis in thyroid cancer KHM-5M and C643 cells through downregulation of p-AKT and p-mTOR, while Apatinib/CQ therapy augmented tumor suppression in mice thyroid cancer xenograft *in vivo* [99]. In ECA-109 and KYSE-150 esophageal squamous carcinoma cells, CQ administration enhanced anticancer effects of Apatinib *in vivo* and *in vitro* by inhibiting autophagy via IRE-1α–AKT–mTOR pathway and enhancing apoptosis by stimulation of Bax and caspase-3, but decreasing the levels of Bcl2, p-AKT and p-mTOR [100].

4.6. Chloroquine and PI3K/Akt/mTOR inhibitors

PI3K/Akt/mTOR (phosphoinositide 3-kinase/Akt/mammalian target of rapamycin) cascade is one of the most crucial signaling pathways which control key cellular functions such as proliferation, growth, metabolism and survival. Since its abnormal activation is a frequent oncogenic event in many human malignancies, while the suppression leads to upregulation of autophagy, the combination of PI3K/Akt/mTOR and autophagy inhibitors was suggested to have a higher therapeutic benefit [101–103]. To date, more than 40 different agents targeting this pathway have been developed and tested in various stages of clinical trials, but only a few of them have been approved for cancer therapy.

In MG63 osteosarcoma cells, CQ enhances apoptotic cell death promoted by mTOR inhibitor rapamycin (RAPA) by blocking the activity of downstream molecules of the Akt/mTOR pathway 4E-BP1 and p70S6k, increasing the expression of autophagy-related proteins LC3-II and Atg12-Atg5 complex, but decreasing p62 level [104]. Although CQ was not effective as a single treatment, CQ/RAPA exposure induced apoptosis by overaccumulation of autophagosomes in well differentiated human liposarcoma 93T449 cells (WDLS) [105] and arrested the growth of dedifferentiated liposarcoma in mice patient-derived orthotopic xenograft (DDLS PDOX) model [106].

The addition of CQ to Salidroside, a glycoside isolated from the root of *Rhodiola rosea L.*, enhanced the sensitivity of hepatocellular cancer HepG2 and 97H cells to this compound and exerted synergic effect on the growth of mice HepG2 xenograft by suppressing the invasion and metastasis of cancer cells through PI3K/Akt/mTOR pathway, promoting mitochondrial dysfunction and altering the ratio between expression of pro- and anti-apoptotic proteins [107,108]. The combination of imidazoquinoline derivative Dactolisib, dual inhibitor of PI3K/*mTOR*, and dimeric CQ Lys05 exerted a significant additive effect on the cultured lung cancer A549 cells by stimulation of apoptotic genes, downregulation of proliferative gene marker *KI67* and blocking the expression of autophagic genes [109]. Grimaldi et al. [110] applied Everolimus, RAPA analog approved for second-line therapy, with CQ to a few renal cancer cell lines and found synergistic effects in suppressing cell viability, inhibition of autophagy and shift to apoptosis via intrinsic mitochondrial pathway associated with decrease in Beclin-1/Bcl-2 complex, although the tested cell lines had different sensitivity to such treatment. A phase I/II clinical trial which included the patients with previously treated clear-cell renal carcinoma (ccRCC) has shown that combined therapy with HCQ and Everolimus is safe and tolerable, leading to partial response and prolonged stable disease in a subset of patients, although activating mutations in mTOR signaling pathway were associated with shorter survival [111]. A significant anti-tumor capacity due to modulation of autophagy was reported in a phase I clinical trial with HCQ and Temsirolimus, an intravenous RAPA analog, in patients with solid tumors and melanoma [112].

4.7. Chloroquine and other agents

In PC-3 and LNCaP prostate cancer cell lines, the combined treatment of Palladium (Pd)(II) complex and CQ caused pyknotic nuclei and induced apoptosis accompanied by increased activity of caspase 3/7, moreover, in PC-3 cells such exposure suppressed the expression of autophagy proteins Atg5, Beclin-1 and LC3, pro-survival PI3K/AKT/mTOR-related protein and Jak/STAT5, while p38 were highly phosphorylated, which might have contributed to enhanced cytotoxicity [113]. The study of Cook [114] has shown that CQ in combination with estrogen receptor-α (ERα)-targeted agents such as Tamoxifen or Faslodex augmented the sensitivity of breast cancer cells resistant to endocrine therapies both *in vitro* (in MCF7-RR, LCC9 and ZR-75-1/ICI-R cells) and *in vivo* (in mice

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xenografts models), with this effect linked with alterations in the immune response. CQ addition suppressed autophagy and enhanced the efficacy of anticancer therapeutics Sorafenib in TPC1, ACT1 and KTC1 thyroid cancer cell lines [41]. The suppression of autophagy with CQ was able to improve the responses to chemotherapy with MEK inhibitor Trametinib of the cultured brain tumor cells resistant to BRAF blockers and, more importantly, reduced the metastases of brain glioblastoma in the patients with BRAF mutations [37]. HCQ enhanced apoptosis and potentially therapeutic oxidative stress in glioblastoma U-87 cells treated with Temozolomide which possesses an ability to alkylate/methylate DNA triggering its damage and death of tumor cells [75]. The combination of 5-FU with CQ significantly reduced the viability of human pancreatic cancer PANC-1 cell line in comparison to single exposure, although CQ alone did not exhibited any effect [83]. In a mouse xenograft hepatocarcinoma model, CIS or CQ alone were able to reduce the tumor growth, however, the combination of 5FU and CQ significantly augmented anti-tumor effect and impaired proliferation of tumor cells by causing higher level of apoptosis [74]. A few randomized clinical trials attempted to use CQ as adjuvant for conventional chemotherapy and radiotherapy of the patients with glioblastomas (GBM) reported an enhanced response to antineoplastic treatment and improved mid-term survival [115,116]. Recent meta-analysis of clinical trials allowed the authors to conclude that CQ supplementation led to significantly improved survival or remission time and decreased mortality, with low incidence of adverse effects and seizures, thus showing some effectiveness in improving the treatment for glioblastoma [117]. A broad range of responses, from minor to good partial, and stable disease were reported in the study evaluating the effects of combined therapy for the patients with relapsed or refractory myeloma with HCQ and Bortezomib, reversibly inhibitor of chymotrypsin-like subunit of the 26S proteasome [118].

4.8. Chloroquine in multi-drug combinations

The development of chemoresistance and existence of mutations have forced the search for new treatment combinations consisting of drugs acting on different cellular targets. In many of such combination, CQ was added to suppress the cytoprotective autophagy. In TNBC MDAMB231 or MDAMB468 cells, CQ potentiated the antitumor effect of PI3K/Akt/mTOR inhibitors Ipatasertib and Taselisib in combination with PTX with the features of reduced autophagic flux and enhanced apoptosis [119]. In breast cancer MDAMB231 and MCF-7 cells, triple combination of CQ, DOX and Ixazomib, which *binds β5 subunit of 20S proteasome thus inhibiting its chymotrypsin-like activity*, synergistically suppressed cell growth and increased the sensitivity to chemotherapy [120]. Using COAST (Combination of Autophagy Selective Therapeutics: CQ, Nelfinavir, RAPA, Dasatinib and Metformin in 50% PEG400), Delaney et al. [121] have shown that this drugs cocktail effectively arrested the growth of three types of mice xenografic ovarian cancers resistant to CIS-Docetaxel chemotherapy, with residual tumors exhibited enhanced levels of LC3-II and ER stress marker GRP78. The combined addition of Apatinib and CQ enhanced anti-proliferative effect of PTX on esophageal squamous carcinoma cells ECA-109 and KYSE-150 *in vitro* or intensified tumor suppression *in vivo* [100]. Modest improvement in the clinical responses (higher ORR and PFS) following combined HCQ/CPT/PTX therapy was observed in the patients with newly diagnosed stage IV non-small cell Kras-mutated lung cancer [122]. Pre-operative HCQ plus GEM/nab-PTX chemotherapy in the patients with potentially resectable pancreatic adenocarcinoma demonstrated an improved Evans Grade histopathological response, decreased CA19-9 tumor marker level correlated with enhanced OS, and increased immune cells infiltration within the tumor [123], as well as led to a significant response rate of PDAC tumors in patients with loss of tumor suppressor SMAD4, although no significant OS was reported Fei et al. [124]. However, addition of HCQ to conventional chemotherapy for the patients with metastatic PDAC improved the response rate but not OS [125].

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Table 3. CQ or HCQ and chemotherapy drugs in clinical trials.

	Tumor type	Concentration	Effects	Reference
CQ + Carmustine + IR	Glioblastoma multiforme (GBM)	Carmustine 200 mg/L once every 6 weeks + CQ 150 mg daily from 1 day after surgery + radiotherapy 6000 Gy	Longer survival Tumor remission	[115]
CQ + Carmustine + IR	Glioblastoma multiforme (GBM)	Carmustine 200 mg/L + CQ 150 mg daily from 5 day after surgery for 12 months + 6000 Gy, 4 cycles	Improved mid-term survival	[116]
HCQ + Temsirolimus	Melanoma, colorectal carcinoma, head and neck cancer, breast cancer	TEM 25 mg (IV) + HCQ 200-1200 mg/day (OR) daily for 4-6 weeks	Stable disease	[112]
HCQ + Bortezomib	Relapsed/refractory myeloma	2-week HCQ 100-1200 mg (OR) + Bortezomib 1-1.3 mg/m^2 on days 1, 4, 8 and 11 of 21-d cycle	Partial response Minor response Stable disease	[118]
HCQ + Everolimus	Advanced renal cell carcinoma	Everolimus 10 mg for 1 week + HCQ 600 mg/twice daily for 35-28 days	Partial response Stable disease ↑ PFS	[111]
CQ + IMA	Chronic-phase-CML	IMA 400-800 mg + CQ 400-800 mg (OR) daily for 48 weeks	No significant effect	[93]
HCQ+GEM	Pancreatic carcinoma	Preoperative GEM 1500 mg/m^2 + HCQ for 31 days until surgery	↑ OS and PFS Partial histopathological response ↓ CA19-9 level	[86]
HCQ + GEM/nab-PTX	Metastatic pancreatic cancer	HCQ 600 mg/twice daily (OR) for 28 days + standard chemotherapy	No improvement of OS Partial response	[125]
HCQ+GEM/nab-PTX	Pancreatic carcinoma	Two preoperative cycles of GEM 1000 mg/L + nab-PTX 125 mg/L on days 1, 8 and 15 + HCQ 1200 mg/day from day 1	Improved OS ↑ Evans grade histopathologic tumor response, ↑ Tumor immune infiltration index	[123]
HCQ + CPT/PTX+/- bevacizumab	Untreated metastatic non-small cell lung cancer	PTX 200 mg/m^2 (IV) on day 1 + CPT 6 AUC on day 1 +/- Bevacizumab 15 mg/kg (IV) on day 1 + CQ 200 mg (OR) on days 1-21 for 6 cycles	Modest improvement in RR ↑ ORR and PFS in patients with Kras-mutations	[122]
CQ + PTX or nab-PTX or Docetaxel or Ixabepilone	Advanced or Metastatic Anthracycline-refractory Breast Cancer	CQ 250 mg (OR) daily + PTX 80-175 mg/m^2 (IV) every 3 weeks, or docetaxel 75-100 mg/m^2 (IV) every 3 weeks, or nab-PTX 100-260 mg/m^2 (IV) every 3 weeks, or Ixabepilone 40 mg/m^2 iv	Increase in ORR	[67]

		every 3 weeks. Maximum 6 cycles.		
HCQ+GEM or HCQ+GEM+nab-PTX	Pancreatic carcinoma	1 month of pre-operative GEM + HCQ 1200 mg/day or 2 months of GEM/nab-PTX + HCQ 600 mg twice daily	↑ Evans grade histopathological responses in patients with SMAD4 loss. Improvement of biochemical markers	[124]
CQ or HCQ + Carboplatin-Gemcitabine	Phase I trial, refractory advanced solid tumors	CQ 50 mg/day or HCQ 100-150 mg/day (OR) on 7-21 days + CPT 5 AUC (IV) on day 1 + GEM 1000 mg/day (IV) on days 1 and 8 for 21 days, 4 cycles	PR SD PD Improved PFS and OS	[76]

Abbreviations: OS – overall survival, ORR – objective response rate, PFS - progression-free survival (PFS).

5. Conclusions

Together, these data show that in the majority of experimental works the addition of CQ or HCQ to chemotherapy drugs significantly enhanced their cytotoxic effects, especially in cultured cancer cells. Therefore, these agents can be suggested as effective adjuvant therapy sensitizing tumor cells to chemotherapy, offering more efficient elimination of tumors and improvement of clinically relevant curative rates. However, the clinical trials were not always successful, with the "partial response" being the most frequent finding, and in some cases did not reveal the significant improvement in overall surviving rates, probably, due to enrollment of the patients with advanced stages of diseases or existence of undetected mutations. Moreover, long CQ and HCQ exposure is known to be associated with serious adverse effects such as allergic reaction, irreversible retinal toxicity, gastrointestinal discomfort, cardiomyopathy symptoms, neuromyotoxicity, and bone marrow suppression [126]. The moderate side effects linked with their application have been observed in almost all clinical trials listed in the Table 3. Finally, the effects of CQ and HCQ appear to be cancer-specific, and they do not exclusively inhibit autophagy, which raises some pessimism regarding their use. Nevertheless, they should be further tested in experimental and clinical settings with the malignancies of different origin to reveal the types of tumors most sensitive to such treatment, and the most effective chemotherapeutic combinations. To more precisely target autophagy and diminish the side effects, the development of new more specific and potent autophagy inhibitors is required.

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