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Review Article

Conquering the Bottlenecks in Cancer Therapeutics via Drug Repurposing of Antimalarials Drugs: A Review

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Abstract

Chemotherapy and radiation therapy are some of the known treatments available for treating cancer. Unfortunately, over the years patients have developed chemo resistance to the drugs. To overcome this resistance, drug repurposing is considered an effective and alternative strategy for cancer therapy. Different antimalarial drugs have exhibited anticancer properties in various cancer types. In this review, the anticancer activity of Artemether, Artesunate, Pyrimethamine, Primaquine, Quinine, Chloroquine, Mefloquine, Ferroquine, Pyronaridine, Atovaquone have been discussed with special attention to Mefloquine. Mefloquine was found to exert a potent anticancer property as compared to other antimalarial agents. Autophagy, lysosomal disruption, mitochondrial stress, endoplasmic stress, ROS/oxidative stress, drug efflux pumps and various signaling pathways such as PI3K/Akt/mTOR, NF-κB are some of the targets of Mefloquine. This review article also encapsulates therapeutic benefits of Mefloquine in breast, prostate, gastric, cervical and colorectal cancer and acute and chronic myelogenous leukemia as well as in drug-resistant cancer cell lines.

Keywords: Antimalarials; Drug repurposing; Cancer; Therapeutics; Autophagy; Drug resistance

Abbreviations

AML: Acute Myeloid Leukemia; ARS: Artemisinin; CML: Chronic Myeloid Leukemia; CQ: Chloroquine; MQ: Mefloquine; MDR: Multi Drug Resistant; NSCLC: Non- Small Cell Lung Carcinoma; P-gp: P-Glycoprotein; PND: Pyronaridine; PY: Pyrimethamine; ROS: Reactive Oxygen Species; BCR/ABL: Breakpoint Cluster Region Protein/Tyrosine-Protein Kinase ABL1; EGFR: Epidermal Growth Factor Receptor; WNT/β-catenin: Wingless-Related Integration Site/β-catenin; cIAP1: Cellular Inhibitor of Apoptosis Protein 1; cIAP2: Cellular Inhibitor of Apoptosis Protein 2; Bcl-2: BCL2 Apoptosis Regulator; CDK1: Cyclin-Dependent Kinase 1; CDK2: Cyclin-Dependent Kinase 2; CDK6: Cyclin-Dependent Kinase 6; PARP1: Poly [ADP-Ribose] Polymerase 1; COX-2 : Cyclooxygenase-2; CDC25A: Cell Division Cycle 25 A; p38 MAPK: p38 Mitogen-Activated Protein Kinases; p-IκBα: Nuclear Factor of Kappa Light Polypeptide Gene Enhancer in B-Cells Inhibitor, Alpha; p-NF-κB p65: Phospho-Nuclear Factor of Kappa Light Polypeptide Gene Enhancer in B-Cells Inhibitor P65; CXCL12/CXCR4: C-X-C Motif Chemokine Ligand 12/C-X-C Chemokine Receptor Type 4; AMPK: AMP-Activated Protein Kinase; Akt: Protein Kinase B; APL: Acute Promyelocytic Leukemia

Background

Cancer is a well-known disease that involves the abnormal growth of the cell and invading other parts of the body [1]. Some of the commonly used treatments are chemotherapy, surgery, radiation therapy. The drawback of chemotherapy is that over some time a patient tends to develop resistance to the drugs along with various side effects [2]. This is when drug repurposing comes into the picture. It involves the usage of a medication that is already approved for the treatment of one disease and trying out to see if it helps in the treatment of another disease [3]. There are three basic approaches to drug repurposing-experimental based approach, computational based approach, and clinical based approach, which include *in vitro* and *in vivo* screening. The other name for the experimental based approach is activity-based repositioning, which is based on the experimental ways, original drugs are screened for new pharmacological indications. It includes phenotypic screening and computational binding affinity. Phenotypic screening involves the identification of drug candidates from small-molecule libraries. In a computational based approach, an effective bioactive molecule is achieved *via* the molecular interaction between the protein target and drug molecule, which is performed by various cheminformatics/bioinformatics tools. Next in the pipeline is *in vitro* and *in vivo* screening. Once the successful results have been achieved in all stages, the repurposed drug candidate enters the preclinical and clinical trials for assessing its safety levels in human subjects and can be readily used when the FDA approves the drug (Figure 1) [4]. There are several published reports, which focus on different classes of drugs for repurposing purposes in cancer like antidiabetics, antihistamines, antipsychotics, antifungals including antimalarial drugs. V various antimalarial drugs are known in the market for treating infected individuals and preventing deaths from severe cases [5]. Mefloquine, Chloroquine, Pyronaridine, Primaquine, Atovaquone, Artesunate, Artemisinin, Artemether, Sulfonamides, Pyrimethamine, Quinine, Mepacrine, Doxycycline are some of the antimalarial drugs [6,7]. Quinine was isolated in the year 1820 from the bark of the Cinchona tree and represents the first classical case where a disease is treated with a pure chemical compound. In 1934, Chloroquine was synthesized, proved to be effective and since then it became an important antimalarial drug [6]. Many studies have stated

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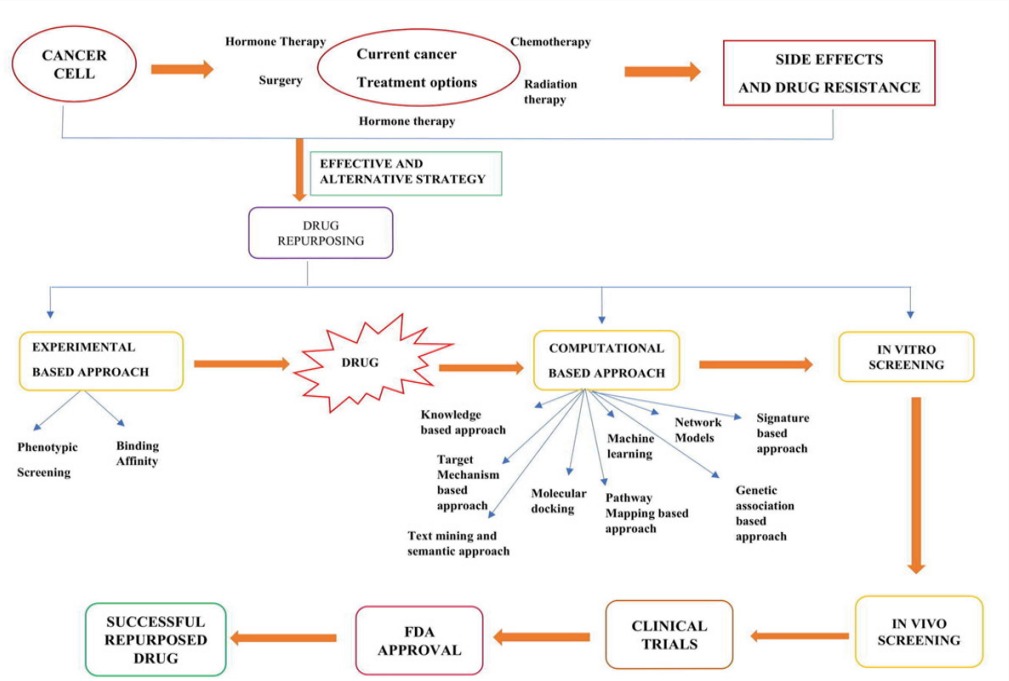

Figure 1: The above figure displays the steps involved in drug repositioning. It starts with various computational methods which involve virtual screening of large number of compounds to study binding affinities of the compounds with the target proteins, followed by *in-vitro* and *in-vivo* screening methods in order to validate the results experimentally. A lead compound is selected and allowed to undergo a series of tests known as clinical trials, for assessing its pharmacological, pharmacodynamic and adverse effects by involving human subjects. Once it gets approved by US FDA, it is authorized and distributed in the market, and is allowed for use.

that antimalarial drugs do possess the anticancer activity and due to this feature, they have been remarkably useful in treating cancer [8]. This review discusses the detailed analysis of repurposing of the antimalarial drugs in cancer.

Anticancer Effect of Antimalarial Drugs

Artemisinin compounds

Artemether and artesunate: Artemisinin (ARS) an antimalarial drug has been shown to exhibit anticancer property. Qinghao (*Artemisia annua*) is a Chinese herb from which the artemisinin is extracted. In several studies, it is stated that artemisinin and its derivatives have shown strong antineoplastic activity in drug-resistant cancer cell lines [9-11]. At the same time, several modes of action are exhibited by ARS-type drugs acting in a multi-specific manner as in ARS-type drugs, the endoperoxide moiety can be opened and form carbon-centered radical molecules and radical oxygen species. This process is promoted by iron-bound proteins (e.g. Heme) or free ferrous iron in a Fenton-type reaction. In cancer cells, a wide range of detrimental effects can be observed due to these radical molecules. The mechanism of action in cancer cells by ARS-type drugs includes the following:

1. Antioxidant response mechanisms, receptor signaling pathways, for e.g., Breakpoint Cluster Region Protein/ Tyrosine-Protein Kinase ABL1 (BCR/ABL), Epidermal Growth Factor Receptor (EGFR), Wingless-Related Integration Site/ β-catenin (WNT/β-catenin)are classified under upstream mechanisms.
2. Target site mechanisms include DNA repair and damage mechanism, alkylating target proteins, arresting cell cycle, neoangiogenesis, invasion, and ultimately metastasis.
3. Apoptotic and non-apoptotic cell death are grouped under the downstream mechanisms [10].

Artemether, an artemisinin derivative, is more lipophilic than artemisinin [11]. Published reports in different human cancer cell lines have revealed the anticancer property of Artemisinin. From the clinical evidence, it was suggested that the minimal/or reduced side-effects and good tolerability as well as the survival rates can also be improved in the cancer patients by treating them with artemether [9]. Artemether was also found to be a good anticancer agent in Non- Small Cell Lung Carcinoma (NSCLC). From the studies, it was investigated that the levels of anti- apoptotic protein Cellular Inhibitor of Apoptosis Protein 1 (cIAP1), Cellular Inhibitor of Apoptosis Protein 2 (cIAP2) and BCL2 Apoptosis Regulator (BCL2) of NSCLC cells were down- regulated and therefore apoptosis was induced significantly when artemether was used in higher concentration. At a lower concentration of artemether, it was demonstrated that the mRNA level of genes such as Cyclin-Dependent Kinase 1 (CDK 1), Cyclin-Dependent Kinase 2 (CDK2), Cyclin-Dependent Kinase 6 (CDK6), cyclin A2, cyclin B1 and cyclin D1 were down-regulated, leading to cell cycle arrest [12]. In case of Diffuse Large B Cell Lymphoma (DLBCL), artemether was found to be a successful repurposed drug for the treatment. It was investigated that the expression of proteins of the cell cycle were suppressed and therefore the proliferation of DLBCL cells was inhibited. Growth of DLBCL cells treated with artemether was arrested in the G1 phase owing to the low expression of CDK2, CDK4, Cyclin D1 and c-Myc thereby activating the cleavage of Poly [ADP-Ribose] Polymerase 1 (PARP 1) and caspase-3 resulting in cell apoptosis [13].

Artesunate, an artemisinin derivative, has shown anticancer activities in various cancer cells. It is known to mediate its anti-cancer action in gastric cancer *via* suppression of Cyclooxygenase-2 (COX-2)

and Cell Division Cycle 25 A (CDC25A) expression [8,14]. Further, Bcl-2 expression was inhibited along with increase in Bax and caspase 3 protein levels accompanied by reduction in mitochondrial membrane potential in gastric cancer cells upon incubation with artesunate [14,15]. Artesunate treatment in a triple negative breast cancer cell line led to the blockage of cell cycle in G2/M and G1 phase resulting in decrease in cell proliferation. Artesunate [8] has also exhibited inhibition of angiogenesis, metastasis as well as alteration of lysosomal mechanisms. Artesunate induced apoptosis and autophagy in colon cancer cells [15].

Pyrimethamine: Pyrimethamine (PYR), a pyrimidine derivative has been reported to induce apoptosis of cancer cells *via* cathepsin B-dependent and caspase-dependent apoptotic pathways [16,17]. In prostate cancer cells, pyrimethamine has exhibited various potential effects such as inhibition of cell proliferation, promotion of apoptosis, and suppression of p38 Mitogen-Activated Protein Kinases (p38 MAPK) [17]. Pyrimethamine exhibited its antitumor effect in ovarian cancer cells of human and its effect is attributed to cell cycle arrest, DNA damage, promoting apoptosis and further leading to increase in caspase-9 and X-Linked Inhibitor of Apoptosis Protein (XIAP) expression [18]. In Non-Small Cell Lung Cancer (NSCLC), PYR induces G1 cell cycle arrest, increase in Bak expression accompanied by reducing expression levels of Bcl-xL and Bcl-2 leading to augmented apoptosis [19].

Primaquine: Recent demonstration shows sensitization of cancer cells might be increased by antimalarial drugs in different cancer cell lines [20,21]. Drug-resistant KBV20C cancer cells can be sensitized by upregulating the inhibition of p-glycoprotein when Primaquine (PRQ) is used with mefloquine (Figure 2) as a combinatorial strategy [20]. One of the research on Acute Promyelocytic Leukemia (APL) indicated significant reduction in the expression of Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (p-IκBα), Phospho-Nuclear Factor of kappa light polypeptide gene enhancer in B-cells inhibitor p65 (p-NF-κB p65, Bcl-xL and Bcl-2 proteins in the PRQ treatment, thereby inducing apoptosis of Acute Promyelocytic Leukemia (APL) cells via the NF-κB pathway [21].

Quinine: Quinine has shown anticancer properties by inducing apoptosis and reducing cell viability in HeLa and A549 cells [22]. It is associated with the suppression of AKT (Protein Kinase B) phosphorylation at Thr-308 and Ser-473 thereby inhibiting the activation of AKT. Quinine also suppresses the Bcl-2 expression and stimulates/upregulates BAX expression for induction of apoptosis [22,23]. In laryngeal cancer cells (Hep-2), the intracellular levels of Reactive Oxygen Species (ROS) were increased significantly which eventually leads to cell death when treated with quinine in time and dose dependent manner [24].

Chloroquine: Plethora of reports have suggested the tumour inhibiting property of Chloroquine (CQ) in different cancers such as brain, colon, breast and lung cancer [25-28]. In endometrial cancer cell lines and bladder cancer, cell proliferation was suppressed by CQ. The mechanism underlying the anticancer action of CQ is attributed to suppression of autophagy and promotion of apoptosis [25,26,27]. CQ along with 5-fluorouracil as a combination therapy proved to be a promising as well as an effective strategy for the treatment of colorectal cancer [26]. Many studies have proposed that Chloroquine might exhibit its anticancer effect by influencing the TLR9/nuclear factor kappa B (NF-κB) signaling pathway, p53 pathway, the C-X-C Motif Chemokine Ligand 12/C-X-C chemokine receptor type 4 (CXCL12/CXCR4) signaling pathway [8,25,28]. Furthermore, CQ leads to inhibition of lysosome fusion with autophagosomes followed by autolysosome degradation by autophagic flux. For different types of cancer, clinical trials are being conducted for determining the antimalarial drug activity when combined with other standard treatments. The rationale behind such studies is to increase tumour cells sensitization to anticancer agents to enhance the remedial activity of chemotherapeutic drugs [8].

Ferroquine: Ferroquine (FQ) is an antimalarial drug but it has proved as a good candidate for cancer therapy. It is also an analogue of Chloroquine (CQ). Plethora of reports have stated that FQ has shown a good anticancer potential in prostate, pancreatic and breast cancer [8,29]. FQ induces cell death of the cancer cells by involving many different factors that include negative regulation of HIF-1α and Akt kinase, impairment of mitochondria, autophagic- lysosomal function inhibition and inhibition of LMP. It has also been reported that *in vivo* tumour growth in prostate cancer is strongly impaired by FQ [29].

Pyronaridine: Pyronaridine (PND) displays its cytotoxicity properties in breast cancer cells by interfering with cell cycle progression and induction of apoptosis. Previous studies have stated that apoptosis is induced *via* depolarization of mitochondria, activation of caspase 3, intercalation with cellular DNA and inhibition of the progression of cell cycle on haematological and human breast cancer cells by PND as a single agent [30]. Studies have reported that PND resulted in increased sensitivity to doxorubicin by K562/A02 and MCF-7/ADR, Multidrug Resistant (MDR) cells but it has no significant effect on the parent K562 and MCF-7 cells [30,31]. PND exhibited as a promising agent for the cancer therapy by direct inhibition of the MDR-mediated efflux process. The capability of cancer cells to survive against a broad range of anticancer drugs in cancer chemotherapy is known as Multidrug Resistance (MDR) [31,32]. PND displayed its potent anticancer activity in the Glioblastoma Multiforme (GBM) cells by arresting the cell cycle at G1 and G2/M phase and increasing the sub G0 population [33].

Atovaquone: Atovaquone shown to have anti-tumor potential in different cancers. Upon treatment of MCF-7 cells with Atovaquone, oxygen consumption was inhibited accompanied by metabolic induction of oxidative stress and aerobic glycolysis [34]. Similar study in breast cancer suggested the anti-cancer property of Atovaqunone was due to inhibition of HER2/β-catenin signalling pathway and mitochondrial complex III thereby leading to mitochondrial respiration and ATP production blockage in thyroid cancer, respectively [8,34,35]. In Acute Myeloid Leukemia (AML) cells, proapoptotic signaling is induced and the mTOR pathway is inhibited through upregulation of the *ATFA* gene and *via* suppression of oxidative phosphorylation [36]. In hepatocellular carcinoma, atovaquone inhibits the cell proliferation by arresting the cells at the S phase that was accompanied by down regulating the expression of p53 and p2. Further, the drug treatment resulted in caspase-8 and caspase-9 cleavage, suggesting that both intrinsic and extrinsic apoptosis pathway may be activated. It was also demonstrated that in hepato carcinoma when treated with atovaquone resulted in induction of double-stranded breaks which further leads to activation of Ataxia-Telangiectasia Mutated (ATM), Cycle Checkpoint Kinase-2 (CHK2) and H2AX thereby leading to cell cycle arrest [37].

Mefloquine

A proved potent anti-cancer agent. Well documented evidence has demonstrated the efficacy of Mefloquine in several cancers as

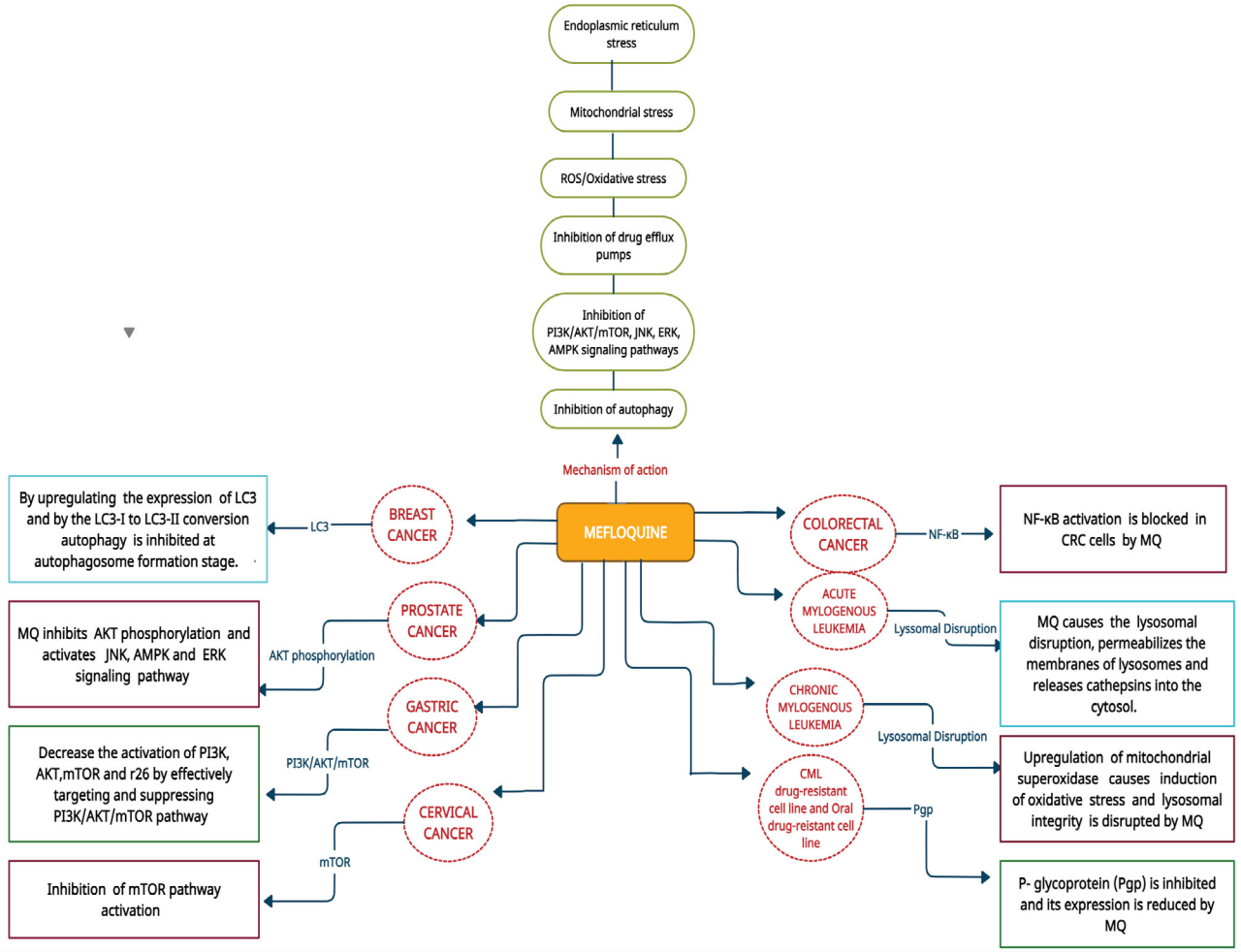

Figure 2: Schematic representation of the mechanism of action of mefloquine and its targets in various cancers.

compared to other malarial drugs that are used for the treatment of malaria [2]. MQ works as an autophagy inhibitor in different cancer cells [2,26,38]. Chloroquine, considered as a good autophagy inhibitor was tested in clinical trials to determine its efficacy as an anticancer agent. Results were promising but it displayed low anticancer activity [26,28]. MQ was identified as an efficient autophagy inhibitor with excellent antitumor efficacy and hence, it was considered more vigorous autophagy inhibitor than CQ [38].

MQ in breast cancer: The potent cytotoxic effects with respect to induction of apoptosis and inhibition of cell proliferation was reported by MQ in the estrogen receptor positive, drug-resistant and triple-negative breast cancer cells, thereby proving to be a good anticancer agent [38]. It has also proved to be a good autophagy inhibitor for hormone positive breast cancer cells MCF7, T47D and triple negative breast cancer cells MDA-MB-231 and MDA-MB-468. Autophagy was inhibited by MQ in MDA-MB-231 and MDA-MB-468 cell lines by upregulating the expression of LC3 (LC3-I to LC3-II conversion) and also at the autophagosome formation stage [2,8]. Therefore, the autophagy process was blocked by MQ similar to Chloroquine. However, MQ blocked the process at lower concentrations as compared to Chloroquine. It was also reported that nucleation of autophagosomes is assisted by Beclin-1 protein and therefore its knockdown resulted in autophagy inhibition [38]. There is a relation between endoplasmic reticulum stress and autophagy. It was stated that misfolded and aggregated proteins were accumulated due to the autophagy inhibition and this resulted in Endoplasmic Reticulum (ER) stress. However, MQ can induce ER stress only at higher concentrations. ER stress did not play an important role in MQ's cytotoxic effects and it was excluded as a crucial element of MQ-induced cytotoxicity. Survival of T47D and MDA-MB-231 cells were reduced when paclitaxel was combined with MQ and thus indicating that the chemosensitivity of paclitaxel, a chemotherapeutic drug, was increased by MQ. MCF/DOX cells which is a doxorubicin-resistant variant of MCF-7 and it was reported that at lower concentrations, MQ is effective against these cells [2,38].

MQ in prostate cancer: In cells, the primary source of Reactive Oxygen Species (ROS) is mitochondria, that contribute to cell death owing to oxidative stress, mitochondrial dysfunction etc. For the specific steps of cell death in apoptosis and necrosis, Mitochondrial Membrane Potential (MMP) hyperpolarization is required to increase the ROS production [38]. In prostate cancer, rapid cell death by Mefloquine is due to an increase in the generation of ROS and the hyperpolarization of mitochondrial membrane potential [2]. In various tumors, progression is regulated by various signaling pathways such as AMP-Activated Protein Kinase (AMPK), Protein Kinase B (Akt) and the Mitogen- Activated Protein Kinase (MAPK)

family. In tumors, cell proliferation, survival, growth is affected by Akt which is activated by phosphorylation of Ser473. Signal transduction pathways mediated by MQ which involves ROS induced signaling modulation, activated pathways such as JNK, AMPK and ERK and inhibited the phosphorylation of Akt. *In vivo* studies were conducted in the PC3 model and it was observed that the lifespan was increased when mice were treated with MQ [39]. In prostate cancer cell lines, at G1 phase cell cycle arrests due to the induction of MQ [40].

MQ in gastric cancer: Plethora of reports have stated about apoptosis being induced and the cell proliferation being inhibited effectively by MQ with the values of IC50 ranging from 0.5-0.7 micromolar, in gastric cancer cell lines [41]. In the malaria parasite, mefloquine gets accumulated in the lysosomes, killing leukemia cells when treated with MQ *via* the lysosomal disruption and inducing the production of ROS. However in the gastric cancer cells, the levels of ROS generation were not affected by MQ at lower concentrations and slightly increased the levels of ROS at higher concentrations. The cell proliferation inhibition which was observed in gastric cancer cell lines was not associated with the generation of ROS. Therefore, in gastric cancer MQ acts in a ROS-independent manner. In 40% of the cases, PI3K/Akt/mTOR signaling pathway is identified as a dominant oncogenic pathway and as an important target for cancer therapy. MQ exhibits its inhibitory effects by effectively targeting and suppressing the PI3K/Akt/mTOR pathway by decreasing the activation of PI3K, Akt, mTOR and rS6 in a dose-dependent manner [41]. Two gastric carcinoma xenograft mouse models YCC1 and SNU-1 cells were subcutaneously injected into nude mice for the *in vivo* studies. For YCC1, MQ and paclitaxel slightly inhibited the tumor growth as a single, combined agent, with tumor growth completely been suppressed by the end of the three-wk treatment period. For SNU-1, MQ moderately inhibited the tumor growth as a single agent, but the tumor growth was significantly arrested when MQ was combined with Paclitaxel which is an anticancer agent [2].

MQ in cervical cancer: MQ has exhibited different anticancer activities such as inhibiting the cell proliferation, anchorage-independent colony formation in HeLa, SiHa, and C-33A cervical cancer cell lines. Apoptosis is induced by MQ in a dose-dependent manner, followed by an intense increase in the levels of PARP cleavage. In cancer cell energy metabolism, mitochondria have an essential role and targeting it may be an effective strategy. Studies have reported that mefloquine exerts its anticancer effect in cervical cancer cells by diminishing the functions of mitochondria such as by decreasing the level of ATP, mitochondrial membrane potential, mitochondrial respiration and by increasing the level of ROS [2]. The mTOR protein is associated with the activities such as survival, growth, proliferation and cell cycle. Due to the inhibition of mitochondrial function by MQ, the mTOR signaling pathway is deactivated. Phosphorylation of mTOR and its downstream effectors rS6 and 4EBP1 is significantly decreased by MQ in HeLa cells. *In vivo* studies were conducted using HeLa derived tumor xenografts and it was revealed that the tumor growth was moderately inhibited by MQ and paclitaxel alone. The tumor growth was arrested when MQ and paclitaxel were used in combination specifying that the combination of the drugs exerted a synergistic effect in the eradication of cervical cancer cell lines [2,42].

MQ in colorectal cancer: In Colorectal Cancer (CRC), Nuclear Factor Kappa B (NF-κB) is considered as the main target, owing to its overexpression in majority of cases. It was demonstrated that the expression of target genes of NF-κB which include cyclin D1, Bcl-2 and XIAP were decreased effectively by Mefloquine [43]. Therefore, in CRC cells NF-κB activation is blocked by MQ indicating that inhibitor of NF-κB is Mefloquine in CRC cells. Apoptosis was induced by Mefloquine in CRC cells by activating the PARP cleavage and caspase-3 in dose-dependent manner and also Bcl-2, MCl-1, and XIAP that are apoptosis- inhibiting proteins were downregulated, while the proapoptotic BH3-only protein Bim was raised. It was demonstrated that when colorectal cancer cell lines were treated with doxorubicin and MQ alone, the reduction in viability was ~25% and ~50% respectively but when they were treated in combination, the viability reduction was ~80% indicating that the combination has a synergistic effect in CRC cells and the cytotoxic action of DOX was enhanced by MQ [43]. *In vivo* studies were carried out in the right flanks of nude mice by subcutaneously inoculating the HCT116 cells and it was observed that the tumor growth was reduced remarkably in the mice treated with MQ in contrast to the control group [43].

MQ in acute and chronic myelogenous leukemia (AML AND CML): Studies have stated that in AML cells, MQ has individual effect on clonogenic growth and in primary AML cells and AML progenitor cells MQ exerted selective toxicity when compared to hematopoietic progenitor cells and normal hematopoietic cells [2]. In AML cells, MQ causes lysosomal disruption, permeabilizes the membranes of lysosomes and releases cathepsins into the cytosol. To determine the anticancer effectiveness of mefloquine in leukemia treatment, *in vivo* studies were conducted in mouse lymphoma cells MDAY-D2, human AML cell line OC1-AML2 and human chronic myelogenous leukemia K562 and it was revealed that MQ significantly inhibited the tumor growth as a single agent [2,44].

For cancer therapeutics, lysosomes have become an attractive strategy. Cancer cells need to adapt to stressful environments, metabolize and proliferate and hence, they require increased function of lysosomes for such functions. Various studies that were conducted in preclinical models have shown that dysfunction of lysosomes is efficacious in the eradication of leukemia [2]. In BCR-ABL1 TKI-sensitive CML cells and TKI-resistant primary BP-CML cells, Mefloquine inhibited the growth and decreased the cell viability by targeting the lysosomes. It was also reported that in blast-phase CML CD-34+ progenitor cells, apoptosis was induced by MQ in a dose-dependent manner [2]. In CML cells, oxidative stress is induced by upregulating the mitochondrial super-oxidase and lysosomal integrity is also disrupted when cells were treated with MQ [2,45].

MQ in drug resistant cancer cell lines: MDR proteins such as P-glycoprotein (Pgp) or MDR- associated Proteins (MRP) are over expressed in the cancer cells, leading to an increase in the drug efflux, decreased uptake, and evading apoptosis subsequently leading to development of drug resistance. This is the main issue as patients initially respond to the drug but ultimately, they develop resistance to the drug. Therefore, the efficient chemosensitization of drug resistant cancer cells helps in expanding the usefulness of presently used antineoplastic drugs. MQ is an inhibitor of drug efflux pumps and causes chemosensitization of drug resistant cancer cells. From the studies it is reported that in drug-sensitive KB cells and KBV20C, an oral drug-resistant cancer cell line [2]. MQ exhibited similar biologic activity. When KBV20C cancer cells were treated with the combination of MQ and vinblastine, an antimitotic agent, the cells became highly sensitized. In drug sensitive KB cells, the viability was not inhibited by the drug combination indicating that Pgp is strongly inhibited by MQ in a time and dose-dependent manner. In K562/

DOX cells, a CML drug-resistant cell line, doxorubicin's cytotoxicity was potentiated by MQ. But in the K562, a drug sensitive parent cell line synergistic activity was exhibited by MQ in these cells. Therefore, in K562-DOXR cells, Pgp activity is inhibited and its expression is reduced by MQ. It can be concluded that in drug resistant cancer cells, drug efflux pumps are highly upregulated and it is inhibited effectively by MQ. It can chemo-sensitize the drug resistant cancer cells to standard chemotherapeutic drugs by inhibiting these pumps. Drug resistance to standard chemotherapy is a remarkable clinical problem, therefore utilizing MQ as an adjuvant along with standard chemotherapeutic drugs will proceed to greater remedial benefits [2].

Mefloquine (MQ) exhibits various pleiotropic outcomes in the cancer cells such as autophagy inhibition, disruption of lysosome, inducing mitochondrial stress, endoplasmic reticulum stress, ROS/Oxidative stress, inhibiting drug efflux pumps and inhibiting various signaling pathways. MQ is an autophagy inhibitor and it induces cell death by inhibiting autophagy. MQ inactivates AKT phosphorylation and activates various signalling pathways for its effect to be exhibited in prostate cancer. In gastric cancer, MQ suppresses PI3K/AKT/mTOR dominant oncogenic pathways. It even inhibits the activation of the mTOR pathway and causes mitochondrial dysfunction in cervical cancer. Apoptosis is greatly induced by MQ in colorectal cancer. In AML and CML cells, MQ exhibits its effect by mostly disrupting the integrity of lysosomes. In Oral and CML drug resistant cancer cell lines, Pgp is potently inhibited by MQ.

Clinical Status of Antimalarials in Cancer

Clinical trials are important to determine the safety and effectiveness of the drug. Over the years, a large number of clinical trials have been conducted for treating cancer with various repurposed drugs. Antimalarial agents were found to be effective and clinical trials of same are already in process. Some clinical trials have been completed, while some are still recruiting. Artesunate was found to be effective in Colorectal Cancer (CRC) and a phase II clinical trial is currently in the recruiting status to further explore the effect of artesunate in overall survival of stage II/III CRC (NCT03093129) [46]. For treating small lymphocytic lymphoma with pyrimethamine, clinical trial is currently in the recruiting status (NCT01066663) [47]. Phase I clinical trial has been completed for treating metastatic breast cancer with artesunate and the result were promising (NCT00764036) [48]. Atovaquone's effect and safety is currently being studied in Acute Myeloid Leukemia (AML) along with conventional chemotherapy (NCT03568994) [49]. Phase II clinical trial study is in recruiting status for investigating the effect of Hydroxychloroquine combined with other agents for inhibiting autophagy in pancreatic cancer (NCT01506973) [50]. Phase II clinical trial has been completed for treating androgen-independent prostate cancer with quinacrine (NCT00417274) [51]. Phase I clinical trial is in active status for the determining the effect of Mefloquine combined with other agents [combination therapy] in treating glioblastoma multiforme (NCT01430351) [52].

Conclusion

Well documented evidence displays the potency of anti-malarial drugs to be cytotoxic in a variety of human cancer cell lines. It has also shown to be effective in few clinical studies as well. In order to validate their safety and efficacy, anti-malarial drugs should further be explored in clinical studies and xenograft models. Plethoras of reports have elucidated the combinatorial anticancer activity of antimalarial drugs with standard chemotherapeutic drugs. Several anti-malarial medicines have been reported to intensify the activity of chemotherapeutic drugs functioning as an adjuvant in cancer therapy. However, further investigations are required to evaluate the anticancer mechanism of various combinations of repurposed drugs.

Declarations

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SN collected data, prepared original draft and figures. SS carried out reviewing and editing work. SD and SB carried out the work of editing and reviewing. JA supervised the entire study, conceptualized, managed resources, and edited. All authors have read and approved the manuscript.

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