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Investigating the use of chloroquine as antineoplastic therapy

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Investigating the Use of Chloroquine as Antineoplastic Therapy

A Review of the Literature

An Honors Program Project Presented to

the Faculty of the Undergraduate

College of Health and Behavioral Studies

James Madison University

by Alexandra Elise Mason and Catherine Elaine Herron

May 2016

Accepted by the faculty of the School of Nursing, James Madison University, in partial fulfillment of the requirements for the Honors Program.

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PUBLIC PRESENTATION

This work is accepted for presentation, in part or in full, at the School of Nursing Senior Honors Project Presentation Day at James Madison University on May 5, 2016.

CHLOROQUINE AS ANTINEOPLASTIC THERAPY

Dedication

We would like to dedicate this work to our Project Advisor, Dr. Erika Metzler Sawin, without whose support this project would not have been completed. Your compassionate service, love of learning, and adventurous spirit continuously inspire us, and we are so thankful to have been able to learn and grow under your guidance over these past two years.

Table of Contents

Preface	4
Acknowledgements	6
Introduction	7
Methods	8
Autophagy	9
Autophagy in cancer	11
Chloroquine	12
Chloroquine as antineoplastic therapy in various cancer types: A review of the literature	13
Brain cancer	14
Breast cancer	16
Colon cancer	20
Endometrial cancer	24
Lung cancer	26
Limitations	29
Nursing implications	30
Conclusion	32
Table 1: Glossary of scientific terms	33
Table 2: Levels of evidence	34
Table 3: Summarized evidence table	35
References	38

Preface

This is a collaborative Honors Program Project, co-authored by Alexandra Elise Mason and Catherine Elaine Herron. We have chosen to submit the project in the Type III format, in which both students independently submit the co-authored document including a preface delineating their contributions to the project. This submission is that of Catherine Elaine Herron, and this preface will detail the origin of our project as well as my personal contributions to the work.

The idea for this project originated when Grace Lawrence, a Ph. D. nursing candidate from George Mason University (GMU), working under the guidance of Virginia Espina, Ph.D., MT (ASCP), a Research Associate Professor at GMU's Center for Applied Proteomics and Molecular Medicine, contacted our Project Advisor, Dr. Erika Metzler Sawin. At the time, Ms. Lawrence and Dr. Espina were applying for a 4VA Grant to support their research, and wanted to work collaboratively across universities with two undergraduate nursing students from James Madison University (JMU). Dr. Espina's laboratory research focuses on how ductal carcinoma in situ (DCIS) cells, which are pre-invasive, abnormal cells located within breast milk ducts, utilize autophagy to survive in stressful environments. In addition, she is leading a clinical trial entitled Preventing Invasive Breast Neoplasia with Chloroquine (PINC), aimed at evaluating the efficacy and safety of administering chloroquine to patients with DCIS. Ms. Lawrence is leading an epidemiological study to complement the work of Dr. Espina. Ms. Lawrence's study focuses on the use of chloroquine in female healthcare workers and the prevalence of DCIS in this population.

As a part of their 4VA Grant proposal, Ms. Lawrence and Dr. Espina wanted their undergraduate partners to complete a literature review focused on the use of chloroquine as an

CHLOROQUINE AS ANTINEOPLASTIC THERAPY

antineoplastic agent in various types of cancer. It was proposed that this literature review would complement their research and be of potential use to them in their own studies. We were extremely excited at this idea for an Honors Program Project, and developed our project proposal collaboratively with our project advisor and readers at JMU, in addition to Ms. Lawrence and Dr. Espina.

Unfortunately, the research team at GMU did not receive the 4VA Grant, and therefore were unable to work as closely in collaboration with our efforts at JMU as both parties originally intended. However, Ms. Lawrence and Dr. Espina encouraged us to continue our collaborative work as undergraduate students and we chose to move forward with the originally approved project topic under the primary guidance of Dr. Sawin. Although the collaborative, inter-university aspect of this project became extremely limited following the rejection of the 4VA Grant, we still have been in intermittent contact with Ms. Lawrence and Dr. Espina regarding the progress of our project. We will submit the final project to them so that they may use it as a complement to their own research, if they so choose.

In regards to how this literature review was authored collaboratively, we each independently authored certain sections of the project. I wrote the preliminary sections titled Autophagy and Chloroquine. Additionally, I focused on reviewing the literature regarding the use of chloroquine in colon and lung cancers, including the limitations of these studies. In order to ensure that there was consistent formatting and voice throughout the independently written sections of the project, each student edited and reviewed the material written by the other student. Finally, the sections titled Dedication, Preface, Acknowledgements, Nursing implications, and Conclusion were authored collaboratively.

Acknowledgements

We would like to extend an enormous thank you our Project Advisor, Dr. Erika Metzler Sawin, for her countless hours of support on this project. Your shared laughs, reassurance, and optimism have been guiding lights to us over the past two years. Thank you so much for keeping us motivated, even in our moments of extreme doubt, and helping us develop a project we are proud to have authored.

Additionally, we would like to thank our reader, Professor Christina Lam, for her guidance on this project. We greatly appreciate your patience, and your kind words of encouragement along the way.

We would also like to thank our reader, Dr. Linda Sobel, for her assistance in the development of this project. Your patience was greatly appreciated, and we are thankful to have had your input.

Also, we would like to thank Carolyn Schubert for her assistance in navigating the databases and locating articles to include in our literature review. We greatly appreciate your guidance.

Furthermore, we would like to thank Grace Lawrence and Dr. Virginia Espina from GMU for their initial guidance on this project. Without their original desire for an inter-university collaboration, this project would not have been developed. We are thankful to have been introduced to you both as well as this groundbreaking potential cancer treatment, and have learned so much through the completion of this literature review.

Finally, we would like to thank our friends and family for their loving support over the past two years. We could not have completed this project without this incredible team behind us, and to you all we are tremendously grateful.

Introduction

Chloroquine (CQ) is an oral lysosomotropic agent routinely used as an anti-malarial drug (Espina & Liotta, 2013). In recent years, it has been discovered that CQ also possesses anticancer effects, potentially due to the drug's inhibition of autophagy (Kimura, Takabatake, Takahashi, & Isaka, 2012). Autophagy is a normal cellular pathway that allows for the degradation of cytoplasmic contents. In cancer cells autophagy can also serve as a pro-survival pathway under stressful metabolic conditions, ultimately promoting the survival of malignant cells (Sui et al., 2013). Therefore, in recent years CQ has been speculated as a potential antineoplastic therapy. When administered in conjunction with typical chemotherapeutic agents, CQ also has the potential to decrease acquired drug resistance, enhance the efficacy of chemotherapeutic agents, and prevent pre-invasive cells from transitioning to invasive cells (Espina & Liotta, 2013). However, this potential antineoplastic effect has been observed to vary somewhat between cancer types and phases of tumorigenesis, and the precise antineoplastic mechanism of CQ is not clearly understood (Sui et al., 2013).

The purpose of this literature review was to evaluate the current evidence in order to compare the efficacy and safety of the use of CQ as an antineoplastic agent in various types of cancer. Additionally, the conjectured antineoplastic effects of CQ discussed in each reviewed study were compared in order to gain greater understanding of the probable mechanism of action of CQ in cancer cells. Definitions for bolded terms can be found in Table 1.

Methods

The literature review was completed using CINAHL, PubMed, Google Scholar and Cochrane Library databases to locate articles published between 2010 and 2015 that discussed the use of CQ as **antineoplastic** therapy in the treatment of various types and stages of cancer. Search terms included *chloroquine*, ***neoadjuvant***, *antineoplastic*, *autophagy*, and *cancer*. The inclusion criteria for articles were, a) the article had to have been published in English between 2010 and 2015, b) publication in a peer-reviewed research journal, c) the article had to contain a methods section, and d) the article had to report statistical evidence or a review of statistical evidence from other studies. Any opinion pieces or articles not reporting original research were excluded from the review.

Using the search terms, initial results were obtained from each database. Each article was analyzed to determine if it met the inclusion criteria. Of the initial results from the databases, seven articles met the inclusion criteria and are included in this review. These were then studied, organized, and rated based on Fineout-Overholt, Melynck & Schultz's hierarchy of evidence (2005). The articles were then summarized and arranged into an evidence table (see Table 2 for definitions of the levels of evidence and Table 3 for article summaries).

Autophagy

Autophagy is a normal, “self protective cellular mechanism” that allows cells to degrade cytoplasmic materials (Zou et al., 2013). During homeostasis, autophagy is used to rid cells of damaged organelles and proteins. Under stressful conditions, such as starvation and **hypoxia** caused by decreased blood supply, autophagy can be induced at higher rates in order to degrade larger amounts of protein for cellular energy. This upregulation of autophagy allows cells to survive even under stressful conditions (Kimura et al., 2012).

There are four steps in the process of autophagy, 1) initiation, 2) elongation, 3) fusion to lysosomes, and 4) degradation. In the first step of initiation, a single membrane vesicle is formed in the endoplasmic reticulum. During elongation, this membrane expands to form an **autophagosome**. The autophagosome engulfs the cytoplasmic materials to be degraded, and in the third step fuses to lysosomes. In the fourth and final step, this fusion allows for the degradation of engulfed cytoplasmic materials by lysosomal enzymes (Fukuda et al., 2015).

Autophagy-associated proteins regulate the process of autophagy and are used to measure levels of autophagy in cells. The most commonly measured protein is LC3. In its cytoplasmic form, this protein is referred to as LC3-I. During the second phase of autophagy, LC3-I is converted from its cytoplasmic form to its autophagosome-associated form, known as LC3-II. By measuring the levels of LC3-I and LC3-II in cells, autophagy rates can be determined by comparing the quantity of LC3-I versus LC3-II. As levels of LC3-II increase in a cell, it indicates that the rate of autophagy is also increasing (Zou et al., 2013).

The ability to measure autophagy rates is important in cancer research because it has been observed that tumor cells utilize the process of autophagy to survive and flourish in nutrient deprived environments. For this reason, greater amounts of research have focused on the

potential use of autophagy inhibitors as antineoplastic therapy in recent years. One of the greatest challenges to this research though is the dual role that autophagy can serve within cells (Sui et al., 2013).

Autophagy in cancer

Autophagy serves a dual purpose in cancer cells: promotion and suppression. In the role of promoter, autophagy supports the survival of cancer cells. In contrast, as a suppressor, autophagy causes cancer cell degradation. Precisely when and why autophagy serves this dual role in cancer cells is poorly understood. Furthermore, it is believed autophagy's role is affected by a myriad of factors, including cell cycle, type of cancer, stage of **tumorigenesis**, metabolic conditions, and the type of antineoplastic therapy being used to treat the malignancy (Kimura et al., 2012).

In the promoter role, autophagy allows cells to produce energy in unfavorable environments, including conditions of hypoxia, increased acidity, and nutrient deprivation. Such environments are not compatible with healthy cells, but autophagy allows cancer cells to flourish within these settings. Therefore, in these situations malignant cells are naturally selected over normal, non-malignant cells that are unable to use autophagy to survive under these conditions (Sui et al., 2013).

In the suppressor role, autophagy can induce autophagic cell death among cancer cells. Autophagic cell death is a form of physiological cell death different from **apoptosis**, and it is poorly understood why an increase in autophagy can sometimes lead to this cytotoxic effect. However, it is clear that in these situations inhibiting autophagy prevents tumor cell suppression, ultimately resulting in tumor cell growth (Sui et al., 2013).

In order to fully explore the potential use of medications known as autophagy inhibitors and inducers as antineoplastic therapy, it is extremely important that researchers gain a better understanding of when and why cancer cells utilize these dual roles of autophagy.

Chloroquine

Chloroquine (CQ) is an autophagy inhibitor. Although routinely and effectively used as an anti-malarial drug, this oral **lysosomotropic** agent has also been used to treat various other conditions due to its anti-inflammatory effects, including rheumatoid arthritis, systemic lupus erythematosus, and Sjögren's syndrome (Fukuda et al., 2015). CQ has very few side effects when administered at clinical doses, typically between 100 and 500 mg/day. In situations where large doses of the drug are administered over extended periods of time, side effects can include visual disturbances, gastrointestinal upset, electrocardiographic changes, headache, and pruritus (Kimura et al., 2012).

CQ is currently being investigated in pre-clinical and clinical trials as a potential antineoplastic therapy because of its inhibitory effects on autophagy and relatively low risk profile. CQ interferes with autophagy in the third step of the process, fusion to lysosomes. By raising the lysosomal pH, autophagosomes are unable to fuse with lysosomes, thus inhibiting degradation of cytoplasmic material contained within autophagosomes (Espina & Liotta, 2013). This disruption of autophagy directed cell survival is a key therapeutic method for using chloroquine as an antineoplastic agent. Supplementation of chloroquine with traditional chemotherapy may provide an innovative therapeutic regimen for those with various types of cancer.

Chloroquine as antineoplastic therapy in various cancer types: A review of the literature

Seven pre-clinical and clinical studies were analyzed for this literature review.

Summaries of the articles and subsequent findings can be found in Table 2. In total, one study focused on brain cancer, two studies on breast cancer, two studies on colon cancer, one study on endometrial cancer, and one study on lung cancer. Multiple facets of study design were compared, including sample, type and stage of cancer studied, and outcome measures. The findings are discussed in the following sections, followed by a comprehensive discussion of the limitations, nursing implications, and conclusion of this literature review.

Brain cancer

Rojas-Puentes et al. (2013) completed a randomized, double-blind, placebo controlled clinical study examining the effect of CQ in combination with **whole brain irradiation (WBI)** on brain metastases. The researchers stated that the results of several other studies suggest combination and neoadjuvant CQ therapy can potentiate the effects of chemotherapy, conventional surgery, and conventional therapy in primary brain tumors. Therefore, this study aimed to determine if the same was true for CQ therapy in combination with WBI in patients with brain metastases.

The study was completed at Instituto Nacional de Cancerología in Mexico City. In order to participate in the study patients had to have greater than 3 brain metastases, and an uncontrolled primary tumor. A total of 73 patients were included in the study, and all patients were randomly divided into two separate arms; the CQ arm (39 patients) and the control arm (34 patients). Patients in the CQ arm received 30 Gray (Gy) of WBI in ten daily fractions along with a daily dose of 150mg CQ one hour prior to radiation for all ten days, and once daily thereafter for twenty-eight days (totaling four weeks of CQ treatment). Patients in the control arm also received 30 Gy of WBI in ten daily fractions along with a daily placebo one hour prior to radiation for all ten days and once daily thereafter for twenty-eight days. In addition to the WBI, patients also received treatment for their primary tumor and non-brain metastases (Rojas-Puentes et al., 2013).

Efficacy of treatments between arms of the study was measured in a number of ways. The researchers compared overall response rate, toxicity, progression free survival of brain metastases, overall survival, event free survival, and quality of life. Of these measures, progression free survival of brain metastases was the only one that showed significant difference

between study arms. One year after the treatment, the progression free survival of brain metastases was 83.9% for the CQ arm, and 55.1% for the control arm. Even when multivariate analysis was performed, CQ treatment was the only variable shown to be associated with better progression free survival of brain metastases. There was no difference observed between arms in regards to overall survival, overall response rate, or event free survival. No toxicity was observed in either study arm, nor were any unique or increased side effects noted in the CQ arm (Rojas-Puentes et al., 2013).

The results of this study show that CQ can improve the progression free survival of brain metastases when administered in combination with WBI. CQ causes this **radiosensitization** by inhibiting autophagy, activating p53-mediated apoptosis, initiating oxidative stress, and activating **capase** within tumor cells (Rojas-Puentes et al., 2013). When WBI was performed following CQ treatment, the tumor cells targeted by the radiotherapy were less resistant due to the fact that CQ had interfered with these numerous cellular functions that typically allow the tumor to thrive and become more resistant in stressful environments. It is surprising that improved progression free survival did not correlate positively with improved overall survival. The researchers hypothesized that this relationship was not present in their results because many of the patients in the study still died due to metastatic tumor progression outside of the brain. Thus, even though the combination CQ therapy was successful at preventing further progression of brain metastases, the patient's primary tumors and other metastases were not as well controlled with conventional treatment and may have contributed to the lack of significance in survival. However, the results of this study are still important to review due to the fact that progression free survival of brain metastases can help to improve patient quality of life (Rojas-Puentes et al., 2013).

Breast cancer

DCIS is a non-invasive breast cancer that arises within the intraductal niche (Espina & Liotta, 2011). DCIS is classified as a non-obligate precursor to invasive carcinoma, meaning that DCIS may never progress to invasive, metastatic cancer. However, there is evidence to support the idea that DCIS often serves as a precursor to malignant breast carcinomas (Espina, Wysolmerski, Edmiston, & Liotta, 2013).

Prior to the pre-clinical trials completed by Espina and Liotta, the survival mechanisms of DCIS were poorly understood (2011). The intraductal niche is a hypoxic, nutrient deprived environment. In order to survive under these conditions, the cells must find an alternate way to create energy than what is used during homeostasis (Espina et al., 2013). In order to determine what alternative energy pathway is utilized by DCIS, Espina and Liotta studied DCIS cell cultures and mouse xenograft models. The results of these pre-clinical trials demonstrated that DCIS uses the process of autophagy in order to survive within the high-stress environment of the intraductal niche. Additionally, it was theorized that in using this survival pathway DCIS cells actually promote replication of the invasive, malignant phenotype. This supported the idea that DCIS is most often a precursor to invasive breast carcinomas, and raised the question of whether neoadjuvant therapy could be created to target these potential malignancy precursor cells (Espina & Liotta, 2011).

In response to the results of these pre-clinical trials, Espina and Liotta are currently conducting a clinical trial investigating the potential use of CQ as anti-autophagy therapy in DCIS. This trial is entitled “Preventing Invasive Neoplasia with Chloroquine,” and is also referred to as the PINC trial. The goal of the trial is to determine the efficacy and safety of CQ when used as neoadjuvant therapy in the treatment of DCIS (Espina & Liotta, 2011).

Neoadjuvant therapy indicates therapeutic strategies are being performed prior to primary treatment for the neoplasm. In the PINC trial, participants are randomly assigned to receive a dose of 250mg or 500mg of CQ on a weekly basis for one month prior to surgical removal of the DCIS lesion. Prior to enrollment in the study and before surgical removal of the lesion, each participant undergoes magnetic resonance imaging (MRI) to visualize the lesion and allow for comparison of lesion size before and after CQ treatment. The removed lesion is then analyzed to test for the presence of protein markers, including LC3-II, that indicate autophagy inhibition and induction of apoptosis. Additionally, all removed lesions are genetically tested to determine if there is any abnormal tissue present (Espina et al., 2013).

The PINC trial is currently accepting participants, therefore there are not currently any published results from this study. Based on the results of the pre-clinical trials performed by Espina and Liotta, it has been hypothesized that routine administration of CQ to DCIS patients for four weeks prior to surgical removal of the lesion will result in decreased lesion size. Additionally, it is predicted that lesions exposed to CQ will have decreased expression of protein markers that indicate the presence of autophagy and apoptosis when compared to lesions not exposed to CQ. Finally, it is anticipated that after treatment with CQ, DCIS lesions will not contain genetically abnormal cells (Espina et al., 2013). The PINC trial is set to close in September of 2016, and results should be available at some point after this date.

DCIS is not the only type of breast cancer in which the antineoplastic effects of CQ have been investigated. In 2012, Maycotte et al. completed an *in vitro* study exploring the mechanism through which CQ sensitizes primary breast tumor cells to chemotherapy. The goal of this study was to gain greater understanding as to whether the antineoplastic effects of CQ are due to autophagy inhibition.

CHLOROQUINE AS ANTINEOPLASTIC THERAPY

Two mouse breast cancer cell lines (67NR and 4T1) were cultured and grown *in vitro*. The cells were then treated with 10 μ M of CQ and one chemotherapy agent; cisplatin, rapamycin, or LY294002. Various outcomes were measured including autophagic flux, short and long term survival assays, and induction of apoptosis. The results of this study showed that each of the chemotherapy drugs used induced autophagy in the breast cancer cells. Additionally, when treated with CQ the breast cancer cells became more sensitive to the chemotherapy drugs (Maycotte et al., 2012).

Most notable were the results produced when the researchers blocked autophagy prior to autophagosome formation. Typically, CQ inhibits autophagy at the third stage of the process, when the autophagosome fuses to the lysosome. By blocking autophagy prior to this point in the process, the researchers were able to observe if CQ exhibited sensitizing effects on the breast cancer cells through a process other than autophagy inhibition. The results showed that CQ did still sensitize the cells to chemotherapy treatment, indicating that the antineoplastic effects of CQ are independent of autophagy in these mouse breast cancer cell lines. Furthermore, this implies that mechanisms other than autophagy inhibition also need to be considered when discussing the antineoplastic effects of CQ in human breast cancer cells (Maycotte et al., 2012).

Given these intriguing results, the researchers delved further into discovering if autophagy played any role in the sensitization of mouse breast cancer cells to cisplatin, rapamycin, and LY294002. Three extremely important results were observed. First, sensitization to the chemotherapy drugs was observed in mouse breast cancer cells in which the genes that regulate autophagy were silenced. Surprisingly, short and long term assays showed that CQ sensitized the cells to the chemotherapy drugs despite the fact that autophagy was not occurring in these gene-silenced cells. Secondly, it was observed that the **chemosensitization** effects

elicited by CQ in mouse breast cancer cells without autophagy-associated proteins were similar to those results seen in mouse breast cancer cells with autophagy-associated proteins. Thirdly, short and long term assays showed that BafA, another autophagy inhibitor that prevents autophagy at the same stage as CQ, was unable to produce sensitization to the chemotherapy drugs in these mouse breast cancer cell lines. All together, these results further supported the idea that CQ sensitization to cisplatin, rapamycin, and LY294002 in these mouse breast cancer cells is independent of autophagy inhibition (Maycotte et al., 2012).

Given that these findings directly contradict what is widely believed to be the mechanism behind the antineoplastic effects of CQ, it is hard to understand these results. Furthermore, it is difficult to comprehend how researchers such as Espina and Liotta have produced contradictory results in similar *in vitro* studies (2011). What is extremely important to remember when evaluating these and other results is that the protective and non-protective effects of autophagy in tumor cells are poorly understood and believed to vary greatly between cancer types, among other factors. As conjectured by Maycotte et al. (2012), in order to better understand the potential for antineoplastic therapy through use of CQ and other autophagy inhibitors, more selective autophagy inhibitors may need to be developed. This would allow for more specific influence of autophagy and improved comprehension of how this cellular process affects the proliferation or death of cancer cells, and its interaction with other cellular processes, such as apoptosis (Maycotte et al., 2012).

Colon cancer

Despite the increased survival rates due to treatment of colon cancer with chemotherapy and radiation, there are many incidences of colon cancer that remain significantly noncompliant to antineoplastic treatment. Selvakumaran, Amaravadi, Vasilevskaya, and O'Dwyer (2013) completed a study that investigated the effects of autophagy inhibition on colon cancer cells *in vitro* and the effects of bevacizumab and oxaliplatin therapy in combination with CQ *in vivo*. The goal of this study was to determine the role autophagy plays in the protection of colon cancer cells from the effects of chemotherapy. Also, the study aimed to establish if CQ can enhance the **cytotoxicity** of bevacizumab and oxaliplatin, chemotherapy drugs commonly used to treat colon cancer.

In the *in vitro* study arm, eight colon cancer cell lines were cultured. Half of the colon cancer cells were grown in normal growth medium, and half were grown in medium treated with oxaliplatin. All colon cancer cells were grown under hypoxic conditions meant to stimulate the hypoxic stress cancer cells experience when proliferating in nutrient poor environments. After cells were allowed to proliferate, outcome measures included cytotoxicity and inhibition of autophagy (Selvakumaran, 2013).

The results of the *in vitro* study arm showed that hypoxic conditions cause induction of autophagy in colon cancer cells. Furthermore, autophagy induction rates were shown to increase in cells grown in oxaliplatin treated media, indicating that cancer cells use autophagy to survive under stressful conditions. This is important to understand because if autophagy allows colon cancer cells to resist chemotherapy drugs, perhaps inhibiting autophagy would allow for increased sensitivity to chemotherapy drugs (Selvakumaran, 2013).

In the *in vivo* study arm, mice were inoculated with one of the colon cancer cell lines. The cancer cells were allowed to proliferate until a tumor size of 400mm was reached, at which point the mice were separated into eight study groups. Each group received a different treatment combination; 1) control group, thus no treatment applied; 2) bevacizumab only; 3) oxaliplatin only; 4) combination of bevacizumab and oxaliplatin; 5) chloroquine alone; 6) combination of bevacizumab and chloroquine; 7) combination of oxaliplatin and chloroquine; 8) combination of bevacizumab, oxaliplatin, and chloroquine. All dose quantities and frequencies were kept constant among groups, and all concentrations used were clinically relevant, meaning that the doses used *in vitro* are comparable to that which would be administered to patients clinically. Outcome measures included tumor size and rates of autophagy inhibition (Selvakumaran, 2013).

The results of the *in vivo* study show that CQ potentiates the cytotoxic effects of bevacizumab and oxaliplatin when used in combination with these chemotherapy drugs. When tumor sizes were compared among treatment groups, mice that received combination therapy of CQ with either or both bevacizumab and oxaliplatin showed the greatest reduction in tumor size. Additionally, mice in these combination treatment groups showed the greatest rates of autophagy inhibition, as indicated by lower levels of LC3-II within the tumor cells (Selvakumaran, 2013).

Overall, the results from the *in vitro* and *in vivo* study arms suggest that CQ causes increased sensitivity of colon cancer cells to bevacizumab and oxaliplatin through the inhibition of autophagy. While this certainly is part of the antineoplastic mechanism of CQ, Selvakumaran et al. state that this study design did not allow for the distinction between autophagy inhibition and other potential unknown cytotoxic effects of CQ. Therefore, further research needs to be completed in order to investigate these other potential cytotoxic effects of CQ in colon cancer (2013).

The results of a study by Choi, Yoon, Won, Park, and Lee (2012) are an interesting complement to the results found by Selvakumaran et al.(2013). Choi et al. investigated the anti-cancer effects of 5-fluorouracil (5-FU) and CQ combination therapy in colon cancer cells in an *in vitro* study. The goal of this study was to determine the effects of autophagy inhibition on the sensitivity of colon cancer cells to 5-FU, a typical chemotherapy agent used to treat colon cancer (2012).

One colon cancer cell line was cultured and grown *in vitro*. Cells were allowed to proliferate, and were then separated into six treatment groups. Four experimental groups were treated with four varying doses of CQ, and two groups served as controls. One control group was treated with just 5-FU, and one with just CQ. After the experimental groups were treated with CQ, the cells were exposed to 5-FU. Outcome measures included growth inhibition, cell cycle distribution, induction of apoptosis, and disruption of autophagy (Choi, 2012).

The results of the study showed that autophagy was induced at greater rates when colon cancer cells were treated alone with 5-FU, indicating that this drug induces autophagy. However autophagy rates were shown to decrease when treated with 5-FU and CQ combination therapy, indicating that CQ inhibited autophagy among these cells despite treatment with 5-FU (Choi, 2012).

Similar to Selvakumaran et al. (2013), Choi et al. speculated whether there were cytotoxic effects of CQ aside from autophagy inhibition causing increased sensitivity of colon cancer cells to 5-FU that were not being measured by the study design. Therefore, Choi et al. evaluated the cell cycle distribution among colon cancer cells treated with 5-FU and CQ. The results showed that combination therapy with the two drugs caused an increase in colon cancer cells arrested during the G1 cell cycle. Apoptosis occurs in the sub-G1 cell cycle; therefore CQ

combination therapy was not causing an increase in apoptosis, but rather an increase in G1 cell cycle arrest. Thus, it was deduced that the antineoplastic effects of CQ in colon cancer cells might be due to the arrest of the G1 cell cycle (2012).

These results regarding the potential cytotoxic effects of CQ aside from autophagy inhibition are interesting for two major reasons. Firstly, in the conclusion of their 2013 study, Selvakumaran et al. stated that it could not be determined if CQ potentiated the cytotoxic effects of bevacizumab and oxaliplatin solely through autophagy inhibition because their study design did not allow for distinction between autophagy inhibition and other potential unknown cytotoxic effects of CQ.

Secondly, Zou et al. performed a study focused on the antineoplastic effects of CQ in lung cancer in which CQ's inhibition of autophagy was found to induce increased rates of apoptosis (2013). The results presented here by Choi et al. somewhat contradict these findings due to the fact that Choi et al. state the rates of apoptosis were not found to be significantly different between cells treated with 5-FU only and cells treated with combination 5-FU and CQ (2012). However, it is important to remember that the antineoplastic mechanism of CQ is not well understood and is believed to work differently in various cancer types. While it is interesting to draw these comparisons between studies, it must be understood that this research is currently evolving. Overall it can be concluded that in order to further investigate all of the potential cytotoxic effects of CQ in colon cancer cells, more pre-clinical and clinical research is necessary.

Endometrial cancer

The role of autophagy in endometrial cancer has not been studied extensively; therefore it is unclear how autophagy inhibitors, such as CQ, affect this cancer type. Fukuda et al. (2015) investigated the effects of CQ in endometrial cancer cells in an *in vitro* study. The purpose of this study was threefold; 1) to determine the antineoplastic effects of CQ in endometrial cancer, 2) to determine if CQ causes autophagy inhibition in endometrial cancer cells, and 3) to determine if combination therapy with CQ can cause increased sensitivity in cisplatin-resistant endometrial cancer cells.

Six endometrioid adenocarcinoma cell lines were cultured and grown *in vitro*. After this period, the cells were continuously exposed to varying doses of CQ for 14 hours. The CQ concentrations used were clinically relevant at 0.2-100 μ M (Fukuda et al., 2015).

Various outcome measurements were used to analyze the results of this experiment, including the number of colonies formed, cell cycle distribution, induction of apoptosis, and disruption of autophagy. In general, it was observed that as the dose of CQ increased, the number of colonies formed decreased. When analyzed in combination with the cell cycle distribution results, it was determined that CQ inhibited growth of endometrial cancer cells through the induction of apoptosis during the sub-G1 phase of the cell cycle, the phase that occurs directly before proteins and mRNA are synthesized within the cell. Additionally, a decrease in LC3-II was observed following the addition of CQ, and this decrease in LC3-II correlated inversely with increased doses of CQ. Overall, these results show that CQ suppresses the growth of endometrial cancer cells (Fukuda et al., 2015).

To better understand the mechanism through which CQ causes endometrial cancer cell growth suppression, the researchers used gene silencing to create a collection of cells that did not

contain the autophagy-associated proteins necessary to regulate the process of autophagy. When these cells incapable of autophagy were then treated with CQ to measure its anti-proliferative effects, a decrease in suppression was seen. This result indicates that CQ relies, at least in part, on autophagy inhibition to suppress the growth of endometrial cancer cells (Fukuda et al., 2015).

In order to investigate the effects of CQ in endometrial cancer further, the researchers created a line of resistant cells. These cells were cultured in a medium containing the chemotherapy agent cisplatin for three months. After confirming resistance, LC3-II expression was measured in the cisplatin resistant cells as compared to the non-cisplatin resistant cells. The results showed that LC3-II expression was much greater in resistant cells, indicating that resistant cells use the autophagy pathway under periods of stress to become and remain resistant to cisplatin. Intrigued, the researchers then treated the resistant cells with CQ in varying doses. The results showed that CQ and cisplatin combination therapy caused cisplatin resistant cells to become re-sensitized to cisplatin. However in non-resistant cells, combination therapy with CQ had no effect on the efficacy of cisplatin (Fukuda et al., 2015).

The results of this study provide a strong foundation regarding the effects of CQ in endometrial cancer. Of greatest interest is the idea that the anti-proliferative effects of CQ are in some amount dependent on autophagy inhibition. The researchers speculate that induction of apoptosis may be a second contributing factor affecting the anti-proliferative effects of CQ. The relationship between autophagy and apoptosis is extremely complex, and more research is needed to determine exactly how these two processes interact (Fukuda et al., 2015).

Lung cancer

In 2013, Zou et al. designed a study performed *in vitro* and *in vivo* to investigate why some non-small cell lung cancer (NSCLC) tumors are innately resistant to epidermal growth factor receptor (EGFR) **tyrosine kinase inhibitors**. It is understood that NSCLC tumors with wild-type EGFR are innately resistant to EGFR tyrosine kinase inhibitors, while NSCLC tumors with activating EGFR mutations are sensitive to this class of chemotherapeutic drug. Previously the reasons for this innate resistance of NSCLC cells with wild type EGFR had not been thoroughly examined, although it was speculated that autophagy played a role in the acquisition and continuation of resistance to traditional chemotherapy. Thus, the aim of the study was to properly assess the role of autophagy in EGFR tyrosine kinase inhibitor-mediated cytotoxicity (Zou et al., 2013).

In the *in vitro* study arm, four NSCLC cell lines were cultured and treated with assorted concentrations of erlotinib, an EGFR tyrosine kinase inhibitor currently approved for treatment of NSCLC tumors, CQ, or combinations of the two drugs. Two of the NSCLC cell lines used had wild type EGFR (erlotinib-resistant), and two had activating EGFR mutations (erlotinib-sensitive). After the cells were allowed to proliferate for a week, various outcome measures were assessed, including cell proliferation, cell cycle analysis, induction of apoptosis, and disruption of autophagy (Zou et al., 2013).

The results of the *in vitro* study arm showed that combination therapy with erlotinib and CQ caused the greatest enhancement of erlotinib cytotoxicity in both erlotinib-resistant and sensitive NSCLC cells. Specifically, when treated with combination therapy of the two drugs a 70-93% reduction in colony size was seen in erlotinib-resistant cells and an 80-95% reduction was seen in erlotinib-sensitive cells (Zou et al., 2013).

Given these significant results, the researchers also investigated the mechanism through which CQ caused enhanced cytotoxicity of erlotinib. In order to determine this mechanism, autophagy induction levels and rates of apoptosis were compared among cells treated with erlotinib only, CQ only, or a combination of erlotinib and CQ. The rates of autophagy in cells treated with CQ alone compared to cells treated with erlotinib and CQ were surprisingly very similar, indicating that CQ does not enhance the cytotoxicity of erlotinib through the inhibition of autophagy. Additionally, it was observed that when both lines of NSCLC cells were treated with a combination of the two drugs apoptosis was induced at higher rates than when cells were treated with either drug alone (Zou et al., 2013).

These results indicate that CQ increases NSCLC cell sensitivity to erlotinib through the enhancement of apoptotic pathways. In order to test this final hypothesis, the researchers cultured a line of NSCLC cells in which the genes that regulate autophagy were silenced. Through these methods, researchers were able to observe that apoptosis was also induced at increased rates in gene-silenced cells. These results indicate that the inhibition of autophagy by CQ allows for the induction of an “apoptotic cascade” by erlotinib, ultimately resulting in an increased sensitivity of NSCLC cells to the chemotherapy drug (Zou et al., 2013).

In the *in vivo* study arm, mice were inoculated with two of the NSCLC cell lines. One of the cell lines used had wild type EGFR (erlotinib-resistant), and one had activating EGFR mutations (erlotinib-sensitive). After seven days, tumor size was evaluated and the mice were divided into four groups. The first group served as the control, the second received only erlotinib, the third received only CQ, and the fourth received combination therapy of erlotinib and CQ. Medications were administered at regular intervals over the course of three weeks and all doses

were considered clinically relevant. Outcome measures included mouse weight and tumor size (Zou et al., 2013).

The results of the *in vivo* study arm support the previously discussed results of the *in vitro* study; combination therapy of erlotinib and CQ causes a significantly greater increase in the cytotoxic effects of erlotinib in erlotinib-resistant and sensitive cells than either drug can elicit alone. The mechanism through which CQ causes enhanced cytotoxicity of erlotinib cannot be attributed to the inhibition of autophagy alone, and may potentially be due to an increased induction of apoptosis (Zou et al., 2013).

Overall, the results of this study are highly clinically relevant because the researchers were able to elicit sensitivity to erlotinib among innately resistant NSCLC cells (Zou et al., 2013). With further clinical research to help explain the safety of CQ combination therapy in patients, combination therapy of erlotinib and CQ could prove a groundbreaking new treatment option for patients with tumors that are resistant to traditional treatment options.

Limitations

Overall, the greatest limitation of this literature review was the great variance between the study designs of the reviewed articles. Each study had dissimilar samples, methods, and outcome measurements, thus making drawing comparisons among them difficult. Also, the literature regarding the use of chloroquine as antineoplastic therapy is still rather limited, as is emphasized in the results of this review. Despite these overarching limitations, the reviewers made every effort to ensure that all discussions were comprehensive and inclusive of each article that met the inclusion criteria.

A full list of limitations of the seven articles included in the review can be seen in Table 3. Overall, common limitations included small sample size (Rojas-Puentes et al., 2013), results produced only *in vitro*, thus limiting their potential application *in vivo* (Espina et al., 2013; Choi et al., 2012; Fukuda et al., 2015; Zou et al., 2013), and the potential for unknown and therefore immeasurable cytotoxic effects of CQ influencing its antineoplastic effects (Selvakumaran et al., 2013; Maycotte et al., 2012).

Nursing implications

As CQ is further investigated as a potential antineoplastic therapy, it is important for nurses to remain abreast of its evolving use. CQ is currently being studied in pre-clinical and clinical trials to determine its safety and efficacy when used alone and in combination with traditional chemotherapy to treat various types and stages of cancer. According to 2012 United States Cancer Statistics (USCS), the types of cancer analyzed in this review are among the top ten most prevalent cancer types in the United States (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, & National Cancer Institute, 2015). Furthermore, cancer is the leading cause of morbidity and mortality both within the United States and worldwide (World Health Organization, 2015). It is essential that nurses are educated on this drug and current research about its use because as greater understanding is gained about the antineoplastic mechanism of CQ and its safety in human use, it is possible that nurses will begin to see more widespread use of the drug to treat these deadly cancers. Thus, nurses should remain informed regarding the current research surrounding CQ so that they understand the benefits, indications, contraindications, and side effects of this likely antineoplastic therapy.

Additionally, by remaining informed of the current research surrounding chloroquine nurses will better understand how to organize care for patients receiving this therapy. In particular, nurses should be aware of the important educational, assessment, and interventional needs for patients receiving chloroquine. Education is essential prior to beginning this treatment due to the fact that it is extremely new and experimental. Nurses must be able to disseminate the research in a manner that can be understood by patients and families who are not familiar with the medication. Also, nurses must be mindful of the benefits and risks associated with

CHLOROQUINE AS ANTINEOPLASTIC THERAPY

chloroquine treatment for each individual patient in order to allow for a fully informed consent for treatment.

When assessing patients receiving chloroquine as antineoplastic therapy, nurses must be aware of common side effects in order to assess for the presence of complications. While some side effects are benign, such as gastrointestinal upset, rash, and headache, others can have long-term negative effects, including ophthalmic degradation (Espina & Liotta, 2013). Nurses must understand what to look for during an assessment in order to intervene properly to promote patient comfort and prevent long-term negative effects. Ultimately, informed nurses will be best able to adequately educate, care, and advocate for patients receiving chloroquine as antineoplastic therapy.

Conclusion

The potential use of CQ as antineoplastic therapy in various cancer types and at different stages of tumorigenesis is an extremely exciting discovery. The drug has been found to 1) increase sensitization of breast, colon, endometrial, and lung cancers to traditional chemotherapy (Maycotte et al., 2012; Selvakumaran et al., 2013; Choi et al., 2012; Fukuda et al., 2015; Zou et al., 2013); 2) reduce proliferation of pre-invasive breast neoplasms (Espina et al., 2013); and 3) hinder the progression of brain metastases (Rojas-Puentes et al., 2013). Given these results, it is believed that CQ could be used as combination therapy to improve the anti-proliferative effects of traditional chemotherapy (Maycotte et al., 2012; Selvakumaran et al., 2013; Choi et al., 2012; Fukuda et al., 2015; Zou et al., 2013; Rojas-Puentes et al., 2013). Additionally, CQ is hypothesized to prevent the progression of pre-invasive neoplasms to invasive carcinomas, and to decrease the presence of genetically abnormal precursor cells (Espina et al., 2013).

The greatest question still surrounding CQ's use as antineoplastic therapy is the exact mechanism through which it elicits these cellular responses. Some results support the idea that the antineoplastic effects of CQ are primarily due to its inhibition of autophagy (Espina et al., 2013; Zou et al., 2013; Choi et al., 2012); while others show that the drug's antineoplastic effects are only either partially due to (Selvakumaran et al., 2013; Fukuda et al., 2015) or completely independent of autophagy inhibition (Maycotte et al., 2012). In order to gain better understanding of the antineoplastic effects of CQ, further research must focus on the protective and non-protective cellular functions of autophagy and how this cellular process interacts with apoptosis (Maycotte et al., 2012).

Table 1: Glossary of scientific terms

Term	Definition
Antineoplastic	Inhibition of tumor development
Apoptosis	Normal process of cell death which plays an important role in healthy body development
Autophagosome	A vesicle which fuses with lysosomes during autophagy
Capase	Protease enzymes that are essential in programmed cell death
Chemosensitization	Treatment of a tumor with medicine to make it more sensitive to radiation
Cytotoxicity	Toxic to living cells
Hypoxia	Diminished amount of oxygen
<i>In vitro</i>	“In glass,” Study performed with microorganisms outside of their natural biological environment
<i>In vivo</i>	“Within the living,” Studies performed on whole, living organisms, including humans or animals
Lysosomotropic agent	Type of drug which accumulates in the lysosomes of the cells
Neoadjuvant	Administration of therapeutic agents before the onset of a main treatment
Radiosensitization	Act of sensitizing tumor cells to radiation therapy
Tumorigenesis	Process of tumor formation
Tyrosine kinase inhibitor	Type of drug which inhibits tyrosine kinases, enzymes responsible for the activation of various proteins
Whole brain irradiation (WBI)	Type of treatment for patients with whole brain metastases

Table 2: Levels of evidence*

Level of evidence	Definition
1	Systematic review or meta-analysis of all relevant randomized controlled trials (RCTs); Evidence-based clinical practice guidelines based on systematic reviews of RCTs
2	At least one well-designed RCT
3	Well-designed controlled trials without randomization
4	Well-designed case-control and cohort studies
5	Systematic reviews of descriptive and qualitative studies
6	Single descriptive or qualitative study
7	Expert opinion

* Fineout-Overholt et al., 2005

Table 3: Summarized evidence table

Author, Year, Level of Evidence (LOE)	Type of cancer, Tumor stage & Type of study	Sample	Intervention	Outcomes Assessed	Results	Limitations
Rojas-Puentes, L., Gonzalez-Pinedo, M., Crismatt, A., Ortega-Gomez, A., Gamboa-Vignolle, C., Nunez-Gomez, R., Dorantes-Gallareta, Y., et al., 2013 LOE: 2	<ul style="list-style-type: none"> Brain Metastases <i>In vivo</i> 	N=73 patients between 18-80 years old	Two study arms; one arm (39 patients) received chloroquine in addition to whole brain radiation, and the other arm (34 patients) received a placebo and whole brain irradiation	<ul style="list-style-type: none"> Overall response rate Toxicity Progression free survival of brain metastases Overall survival Event free survival Quality of life 	The use of chloroquine with whole brain irradiation for treatment of brain metastases increases the chance of the patient's progression free survival	Small sample size, No outcome measure used to assess the reason for CQ's antineoplastic effect
Espina, V., Wysolmerski, J., Edmiston, K., Liotta, L. A., 2013 LOE: 2	<ul style="list-style-type: none"> Breast Pre-invasive lesions <i>In vitro & In vivo</i> 	<p><i>In vitro</i>: DCIS cells harvested and grown <i>in vitro</i></p> <p><i>In vivo</i>: Currently recruiting clinical trial; Participants must be diagnosed with either ER- or ER+ DCIS</p>	<p><i>In vitro</i>: Cultured cells treated with chloroquine alone</p> <p><i>In vivo</i>: Patients randomly assigned to receive 250mg or 500mg of CQ once a week for one month prior to surgical removal of DCIS lesion</p>	<p><i>In vitro</i>:</p> <ul style="list-style-type: none"> Autophagy levels Induction of apoptosis Disruption of autophagy <p><i>In vivo</i>:</p> <ul style="list-style-type: none"> Reduction in DCIS lesion volume Pathologic regression Reduction or elimination of genetically abnormal tumorigenic DCIS stem-like cells Suppression of cellular proliferation Induction of apoptosis Disruption of autophagy 	<p><i>In vitro</i>: CQ therapy alone caused DCIS cell death in less than one week of treatment</p> <p><i>In vivo</i>: Still being completed</p>	<p><i>In vitro</i>: The cells tested cannot be formally classified as "cancer stem cells," thus limiting the potential application of these results <i>in vivo</i></p> <p><i>In vivo</i>: Still being completed</p>

CHLOROQUINE AS ANTINEOPLASTIC THERAPY

<p>Maycotte, P., Aryal, S., Cummings, C. T., Thorburn, J., Morgan, M. J., Thorburn, A., 2012</p> <p>LOE: 3</p>	<ul style="list-style-type: none"> • Breast • Primary tumor cells • <i>In vitro</i> 	<p>Two mouse breast cancer cell lines (67NR and 4T1) cultured and grown <i>in vitro</i></p>	<p>Cells treated with one chemotherapy drug (either cisplatin, rapamycin, or LY294002) and CQ</p>	<ul style="list-style-type: none"> • Autophagic flux • Short and long term survival assays • Induction of apoptosis 	<p>CQ sensitizes breast cancer cells to the chemotherapy drugs cisplatin, rapamycin, and LY294002 through a method other than autophagy inhibition</p>	<p>Using the selected outcome assessments, it was unable to be determined through which mechanism other than autophagy inhibition CQ was able to cause sensitization to chemotherapy</p>
<p>Choi, J., Yoon, J. S., Won, Y., Park, B., Lee, Y., 2012</p> <p>LOE: 3</p>	<ul style="list-style-type: none"> • Colon • Primary tumor cells • <i>In vitro</i> 	<p>A single colon cancer cell line was cultured and grown <i>in vitro</i></p>	<p>Cells were treated first with 5-fluorouracil, then with one of four varying doses of CQ</p>	<ul style="list-style-type: none"> • Growth inhibition • Cell cycle distribution • Induction of apoptosis • Disruption of autophagy 	<p>Combination therapy with CQ caused sensitization of colon cancer cells to 5-fluorouracil</p>	<p>Only one colon cancer cell line was studied, Results were evaluated <i>in vitro</i> only, thus limiting the potential application of these results <i>in vivo</i></p>
<p>Selvakumaran, M., Amaravadi, R. K., Vasilevskaya, I. A., & O'Dwyer, P. J., 2013</p> <p>LOE: 3</p>	<ul style="list-style-type: none"> • Colon • Primary tumor cells • <i>In vitro & In vivo</i> 	<p><i>In vitro</i>: Eight colon cancer cell lines were cultured and grown <i>in vitro</i></p> <p><i>In vivo</i>: Mice were inoculated with one of the colon cancer cell lines</p>	<p><i>In vitro</i>: Two study arms; one arm served as the control, and the other arm was exposed to oxaliplatin and CQ</p> <p><i>In vivo</i>: Eight study arms received various single and combination therapy treatments. Medications used included CQ, bevacizumab, and oxaliplatin</p>	<p><i>In vitro</i>:</p> <ul style="list-style-type: none"> • Cytotoxicity • Disruption of autophagy <p><i>In vivo</i>:</p> <ul style="list-style-type: none"> • Tumor size • Disruption of autophagy 	<p><i>In vitro</i>: Combination therapy with CQ increased sensitivity to oxaliplatin in all of the cell lines tested</p> <p><i>In vivo</i>: Combination therapy with CQ caused significantly increased tumor growth delay</p>	<p>Outcome assessments used <i>in vitro</i> and <i>in vivo</i> cannot distinguish between antineoplastic effects caused by autophagy inhibition vs. potentially unknown cytotoxic effects of CQ</p>

CHLOROQUINE AS ANTINEOPLASTIC THERAPY

<p>Fukuda, T., Oda, K., Wada-Hiraike, O., Sone, K., Inaba, K., Ikeda, Y., Miyasaka, A., et al., 2015</p> <p>LOE: 3</p>	<ul style="list-style-type: none"> • Endometrial • Primary tumor cells • <i>In vitro</i> 	<p>Six endometrioid adenocarcinoma cell lines were cultured and grown <i>in vitro</i></p>	<p>Cells treated with cisplatin only until resistance was established, then treated with clinically relevant doses of CQ (0.2-100 μM) for fourteen days</p>	<ul style="list-style-type: none"> • Number of colonies formed relative to control • Cell cycle distribution • Induction of apoptosis • Disruption of autophagy 	<p>Treatment of cisplatin resistant endometrial cancer cells with CQ partially restored sensitivity to cisplatin, at least partly due to autophagy inhibition</p>	<p>Measurement of autophagy inhibition done entirely <i>in vitro</i>, unclear how to measure effectively <i>in vivo</i>, thus limiting the ability to replicate these methods <i>in vivo</i></p>
<p>Zou, Y., Ling, Y., Sironi, J., Schwartz, E., Perez-Soler, R., Piperdi, B., 2013</p> <p>LOE: 3</p>	<ul style="list-style-type: none"> • Lung • Primary tumor cells • <i>In vitro & In vivo</i> 	<p><i>In vitro</i>: Four wild-type EGFR non-small cell lung cancer cells were cultured and grown <i>in vitro</i></p> <p><i>In vivo</i>: Mice were inoculated with the wild-type EGFR non-small cell lung cancer cells</p>	<p><i>In vitro</i>: Cells treated with erlotinib alone or erlotinib and CQ</p> <p><i>In vivo</i>: Two study arms; one arm was treated daily with 150mg of erlotinib alone, and the other arm was treated daily with 800mg total of erlotinib and CQ</p>	<p><i>In vitro</i>:</p> <ul style="list-style-type: none"> • Cell proliferation • Cell cycle analysis • Induction of apoptosis • Disruption of autophagy <p><i>In vivo</i>:</p> <ul style="list-style-type: none"> • Mouse weight • Tumor size 	<p><i>In vitro</i>: CQ can partially overcome resistance to erlotinib in wild-type EGFR non-small cell lung cancer cells through inhibiting autophagy and inducing increased rates of apoptosis</p> <p><i>In vivo</i>: Tumor growth was reduced significantly in mice treated with combination erlotinib and CQ</p>	<p>Results in both <i>in vitro</i> and <i>in vivo</i> studies limited to very specific lung cancer cell line, cannot be determined if these results would hold true in other lung cancer cell types</p>

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