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Chloroquine and nanoparticle drug delivery: A promising combination

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Abstract

Clinically approved cancer therapies include small molecules, antibodies, and nanoparticles. There has been major progress in the treatment of several cancer types over recent decades. However, many challenges remain for optimal use of conventional and nanoparticle-based therapies in oncology including poor drug delivery, rapid clearance, and drug resistance. The antimalarial agent chloroquine has been found to mitigate some of these challenges by modulating cancer cells and the tissue microenvironment. Particularly, chloroquine was recently found to reduce immunological clearance of nanoparticles by resident macrophages in the liver, leading to increased tumor accumulation of nanodrugs. Additionally, chloroquine has been shown to improve drug delivery and efficacy through normalization of tumor vasculature and suppression of several oncogenic and stress-tolerance pathways, such as autophagy, that protect cancer cells from cytotoxic agents. This review will discuss the use of chloroquine as combination therapy to improve cancer treatment.

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1. Introduction

Cancer is one of the most challenging diseases to treat and a leading cause of morbidity and mortality (Gao, 2008). Effective treatment of cancer requires the ability to overcome challenges such as poor drug delivery, rapid clearance, drug resistance, and patient tolerability (Gao, 2008). Although cancer therapeutics seek to interfere with biological processes that are more predominant in cancer cells, normal cells are also impacted, leading to toxic effects in patients (Gao, 2008). Additionally, conventional anti-cancer drugs are distributed throughout the body, as opposed to preferentially accumulating in cancerous tissues (Wolfram, Shen, et al. 2015). Consequently, administration of lower drug doses is often necessary, which limits therapeutic efficacy and may promote drug resistance. There is a pressing need for higher potency and site-specific delivery strategies. Multipronged solutions that reduce non-tumor tissue accumulation, modulate the tumor microenvironment for improved drug delivery, and prevent protective cancer cell processes should be developed for effective treatment.

Various nanodrugs have been developed, several clinically approved, to improve site-specific drug delivery and in many instances enhance safety and potency of therapy (Anselmo & Mitragotri, 2016; Ferrari, 2010; Gentile, et al., 2013; Wilhelm, et al., 2016; Wolfram, Zhu, et al., 2015). Nanodelivery is thought to increase site-specific accumulation of drugs from 0.001–0.01% to 1% of the systemically injected dose (Wolfram, Shen, et al., 2015). Despite the potential of nanomedicine, there are major challenges that remain (S. Shen, et al., 2017; Venuta, et al., 2017). For example, over 90% of intravenously injected nanoparticles are taken up by the liver (Bae & Park, 2011; Borrelli, et al., 2018; Zhang, et al., 2016), primarily by tissue resident Kupffer cells (Gustafson, et al., 2015; Samuelsson, et al., 2017). Certain properties of the tumor microenvironment can also limit effective drug delivery. For instance, tumors typically have abnormal and disorganized vasculature that contributes to insufficient and heterogeneous intratumoral drug delivery (Jain, 2012; Mazzone, et al., 2009). Cancer cells have also developed protective mechanisms to avoid cell death upon exposure to drugs, which remains an issue despite use of nanocarriers. For example, enhancement of autophagy in response to cell stress can have anti-apoptotic effects that desensitize cancer cells to therapy (Chen, et al., 2010; White & DiPaola, 2009).

One promising strategy to overcome some of the current limitation with cancer therapeutics involves the use of the clinically approved antimalarial agent chloroquine, which has been administered to patients for over 60 years (Wolfram, et al., 2017). In malaria infection, chloroquine inhibits heme polymerase activity in *Plasmodium* parasites, thereby leading to the buildup of free heme, a substance toxic to the parasite (Hempelmann, 2007). Chloroquine has also over the years demonstrated an ability to interfere with physiological processes in mammalian cells, a property that can be exploited for cancer therapy (Fig. 1). For example, chloroquine blocks cell autophagy through prevention of the final steps of this

process (Gonzalez-Polo, et al., 2005; Matthew Redmann, 2017), subsequently making tumor cells more susceptible to drug-induced apoptosis (Amaravadi, et al., 2007). Chloroquine also causes normalization of tumor vasculature, which improves drug delivery (Maes, et al., 2014). In regard to nanomedicines, chloroquine has more recently been shown to substantially reduce nanoparticle accumulation in the liver by affecting macrophage function (Wolfram, et al., 2017). The safety profile of chloroquine is well known due to decades of use in malaria treatment and prophylaxis. Chloroquine has also been studied in combination therapy with chemotherapeutic agents in cancer patients and has shown acceptable toxicity levels (Montanari, et al., 2014).

2. Chloroquine-induced inhibition of autophagy

Autophagy is a cellular process that involves clearance and recycling of cytoplasmic components, including protein aggregates and damaged organelles, via transport in autophagic vesicles (AVs) to lysosomes for degradation (Fig. 2) (White, 2012). This process occurs in most living cells and frequently takes place as a result of cell damage, pathogen exposure, or starvation (Amaravadi, et al., 2011). It is believed that autophagy enhances cell survival by converting cell debris into recyclable nutrients and metabolites (Cicchini, et al., 2015; Kuma & Mizushima, 2010).

Cancer cells with functional autophagy pathways have displayed increased cell viability compared to cells with defective autophagy (Amaravadi, et al., 2011). In cancer cells autophagy is enhanced to increase availability of metabolic precursors and provide protection from high-stress microenvironments within tumors, such as those with hypoxia and low levels of nutrients (Amaravadi, et al., 2011; Cicchini, et al., 2015). For example, autophagy increases metabolites for ATP production, which are required for proper DNA replication and repair.

Chloroquine is able to function as an effective autophagy inhibitor (Amaravadi, et al., 2007), as this drug is a weak base that becomes diprotonated, and entrapped in lysosomes, causing an increase in lysosomal pH (Amaravadi, et al., 2011). This pH elevation inhibits the final steps in the autophagy pathway, leading to buildup of autophagic vesicles in the cytoplasm, which prevents the production and recycling of important nutrients and metabolites and leads to cell damage and ultimately cell death (Amaravadi, et al., 2011; Gonzalez-Polo, et al., 2005). Therefore, exposure of cancer cells to chloroquine can lead to inhibition of tumor growth and apoptosis (Carew, et al., 2007; Degenhardt, et al., 2006; Ding, et al., 2009; Katayama M, et al., 2007; Torgersen, et al., 2013; White & DiPaola, 2009). Tumor types that have a V600E mutation in v-Raf murine sarcoma viral oncogene homolog B (BRAF) exploit autophagy as a metabolic advantage. For example, several central nervous system (CNS) cancer cell lines with the BRAF mutation displayed enhanced sensitivity to vemurafenib, cisplatin, or vinblastin when combined with chloroquine (Levy, et al., 2014). The same study also demonstrated that chloroquine was able to overcome drug resistance in a vemurafenib resistant cancer cell line (Levy, et al., 2014). Chloroquine has been shown to increase the therapeutic efficacy of anticancer agents that increase autophagy, such as histone deacetylase inhibitors (Torgersen, et al., 2013). For example, in Kasumi-1 acute myeloid leukemia (AML) cells, chloroquine caused an additional 30–40% reduction in the viability of cells

exposed to the histone deacetylase inhibitors valproic acid or vorinostat (Torgersen, et al., 2013). In summary, chloroquine is a promising anticancer agent for combination therapy due to the ability of this drug to improve the therapeutic efficacy of conventional cancer drugs through synergistic effects, such as prevention of undesirable therapy-induced autophagy.

However, there is currently a pressing need for a context-dependent understanding of autophagy, as this process may also have tumor suppressing effects. It has been shown that autophagy is a mediator of cell death in certain instances (Qu, et al., 2003) and has anti-inflammatory properties, as degradation of defective proteins and debris prevents inflammation (Hara, et al., 2006; Levine, et al., 2011). Thus, it is important to elucidate the role of autophagy in different tumor subtypes for optimization of cancer therapy.

3. Chloroquine-induced normalization of tumor vasculature

Tumors typically have a vascular architecture that differs from that of normal tissues, which have well-organized and homogenous vasculature (Baluk, et al., 2005; Nagy, et al., 2009; Nagy, et al., 2010). For example, tumors contain many irregularly branched tortuous blood vessels that lack pericytes, which are contractile cells that synthesize basal membrane and provide structural support for endothelial cells (Nagy, et al., 2009). As a result, there is enhanced permeability and inadequate blood flow to some tumor regions, causing hypoxia, starvation, and necrosis (Carmeliet & Jain, 2011). In addition to preventing efficient drug delivery, these vascular network abnormalities have other disadvantages, such as increased cancer cell invasion and impaired immune responses due to hypoxia (Carmeliet & Jain, 2011). Studies have shown that normalization of tumor vasculature can increase drug delivery and reduce metastasis (Chauhan, et al., 2012; Hamzah, et al., 2008; Jiang, et al., 2015; Khalid, et al., 2017; Mazzone, et al., 2009).

Chloroquine has been shown to cause tumor vasculature normalization in a dose-dependent manner in a murine model of melanoma (Maes, et al., 2014). In particular, chloroquine decreased tumor vasculature density (up to 70%) and tortuosity (up to 50%), and increased the presence of alpha-smooth muscle actin (α -SMA) (Maes, et al., 2014), which is a contractile protein characteristic of pericytes (Fig. 3 A – C) (Bergers & Song, 2005). Chloroquine treatment also promoted vessel differentiation as demonstrated by immunostaining for the endothelial cell marker cluster differentiation 31 (CD31), which displayed a more organized pattern in response to chloroquine (Fig. 3D). In addition, scanning electron microscopy (SEM) imaging revealed that the blood vessel wall had a smoother and more continuous structure following treatment (Fig. 3 E) (Maes, et al., 2014).

A major consequence of the chloroquine-induced vessel normalization process was improved intratumoral delivery of chemotherapeutic agents (Maes, et al., 2014). Chloroquine also reduced metastasis due to vasculature normalization (Maes, et al., 2014). Although this study did not investigate the effect of chloroquine on nanodelivery (Maes, et al., 2014), several other studies have shown that vasculature normalization improves intratumoral accumulation of nanoparticles (Chauhan, et al., 2012; Jiang, et al., 2015; Khalid, et al., 2017). Other clinically approved antiangiogenic drugs that normalize vasculature target the vascular endothelial growth factor (VEGF) signaling pathway, which is a major mediator of

angiogenesis and is highly activated during tumor growth (Niu & Chen, 2010; Goel, et al., 2011; Jain, 2005). Chloroquine provides an alternative mechanism to VEGF modulation for obtaining tumor vasculature normalization, which could have distinct benefits. Specifically, chloroquine upregulates the neurogenic locus notch homolog protein 1 (NOTCH1) signaling pathway (Maes, et al., 2014). NOTCH1 is a transmembrane angiogenic protein that cycles between the cell surface and endosomal compartments (Gridley, 2010). Chloroquine induces the accumulation of a NOTCH1 fragment in multivesicular bodies, leading to the release of a transcription factor that modulates vascular function (Maes, et al., 2014). In a *Notch1* endothelial cell knockout mouse model, chloroquine did not cause normalization of tumor vasculature, indicating that the NOTCH1 pathway was responsible for therapeutic efficacy (Maes, et al., 2014).

4. Chloroquine-induced reduction of hepatic nanoparticle clearance

The liver is responsible for the clearance of endogenous and exogenous materials. Hepatic tropism and clearance limits the therapeutic efficacy of nanoparticles, and the liver represents a pivotal obstacle in drug delivery. Various design strategies have been attempted to avoid immunological clearance of nanoparticles. For instance, the coating of nanocarrier surfaces with polyethylene glycol (PEG) to form a hydration layer that reduces nanoparticle interactions with resident macrophages is currently in clinical use (Parr, et al., 1994; Pasut, et al., 2015; Walkey & Chan, 2012; Wolfram, Suri, et al., 2014; Wolfram, Yang, et al., 2014). However, nanoparticle pegylation fails to extensively prevent hepatic tropism due to incomplete coverage of the surface, loss of nanoparticle integrity in the circulation, and immunological activation and destruction after repeated injections (Hatakeyama, et al., 2013; Moghimi, 2017; Yang & Lai, 2015). There are also several preclinical approaches that prolong systemic circulation of nanoparticles, such as the use of cell membrane coatings (Hu, et al., 2011; Parodi, et al., 2013) and peptides for self-recognition (Rodriguez, et al., 2013). Nevertheless, these preclinical strategies have several drawbacks, such as laborious processes for coating nanoparticles with cell surfaces and the triggering of immune responses related to the source material and processing steps. There remains a pressing need to develop improved strategies to reduce nanoparticle clearance by the liver, given the detrimental impact of hepatic uptake on circulating nanodrugs.

A complementary approach to nanoparticle surface modification strategies is modulation of the liver microenvironment to reduce the ability of resident immune cells to take up nanoparticles (Khalid, et al., 2017; Wolfram, et al., 2017). These strategies are broadly applicable to various types of drug delivery vehicles, ranging from nanosized liposomes to discoidal silicon microparticles. In a screen to identify clinically approved drugs that modulate the ability of Kupffer cells to internalize nanoparticles, chloroquine was recognized as a promising candidate (Wolfram, et al., 2017). Pretreatment of Kupffer cells with chloroquine led to reduced cellular uptake of various nanodelivery systems (Wolfram, et al., 2017). On the other hand, nanoparticle uptake in various cancer cell lines exposed to chloroquine remained unchanged, suggesting that this strategy prevents nanoparticle internalization by macrophages without affecting cancer cell uptake mechanisms (Wolfram, et al., 2017). Biodistribution studies conducted in mouse models showed that pretreatment with a clinically relevant dose of chloroquine, caused a 28.5% and 22% reduction in liver

accumulation of systemically administered liposomes and disk-shaped silicon microparticles, respectively (Fig. 4) (Wolfram, et al., 2017). The ability of chloroquine to decrease both soft (liposomes) and hard (silicon) nanomaterial deposition in the liver highlights the versatility of this strategy in improving nanodelivery of both lipid-based nanoparticles that are already approved for clinical use (Barenholz, 2012; Gentile, et al., 2013) and discoidal particles that represent the next frontier of organotropic nanomedicine (Mi, Mu, et al., 2016; Mi, Wolfram, et al., 2016; J. Shen, et al., 2015; Venuta, et al., 2017; Wolfram, Shen, et al., 2015).

A proposed mechanism for chloroquine-induced inhibition of nanoparticle uptake in macrophages is suppression of phosphatidylinositol binding clathrin assembly protein (PICALM) (Wolfram, et al., 2017). Expression of PICALM is thought to be essential for clathrin-mediated endocytosis (S. E. Miller, et al., 2015), which is one of the main internalization pathways for cellular uptake of nanoparticles (Sahay, et al., 2010). Although suppression of PICALM is likely to be a predominant mechanism by which nanoparticle internalization is inhibited, other pathways are also involved as pharmacological inhibition of clathrin-dependent endocytosis failed to prevent nanoparticle uptake to the same extent as chloroquine (Wolfram, et al., 2017). In fact, endocytic vesicles frequently fuse with the lysosome, a function which is impaired by chloroquine, potentially causing upstream processes to become affected (Wolfram, et al., 2017).

The promising benefits of chloroquine priming should spur further interest in the development of other agents that precondition the innate immune system for improved drug delivery. Nevertheless, there is concern that suppression of macrophage immune responses might impede the defense system against pathogens, which poses a safety issue (Wolfram, et al., 2017). However, there is typically a three to four-week interval between the injection of nanodrugs (Rose, 2005), and chloroquine administration would follow a similar schedule, enabling macrophage function to return to normal between dosages. Another concern with chloroquine pretreatment is potential suppression of tumor-associated macrophages (TAMs), which play a role in drug accumulation in certain types of tumors (M. A. Miller, et al., 2015; Tanei, et al., 2016). Therefore, the effect of chloroquine on drug delivery may vary, as the prevalence of TAMs differ depending on tumor type (Jung, et al., 2015). Besides having an impact on intratumoral nanoparticle accumulation, chloroquine may also display anticancer activity by interfering with the function of TAMs, which have been shown to promote cell survival, invasion, and angiogenesis (Noy, 2014).

5. Other chloroquine-induced anticancer mechanisms

Chloroquine offers additional potential uses for cancer treatment by interfering with oncogenic signaling pathways through mechanisms other than inhibition of autophagy, normalization of tumor vasculature, and suppression of liver macrophages (Table 1). For example, chloroquine has been shown to suppress pancreatic ductal adenocarcinoma (PDAC) cancer stem cells (CSCs), which are highly drug resistant and promote tumor progression and metastasis (Balic, et al., 2014). In mouse models of human PDAC, chloroquine was shown to preferentially target CSCs via inhibition of signaling pathways driven by chemokines, resulting in suppression of signal transducer and activator of

transcription 3 (STAT3) and extracellular signal-regulated kinase (ERK), which play an important role in metastatic spread (Balic, et al., 2014). Chloroquine also blocked epithelial to mesenchymal transition (EMT) in CSCs, though suppression of sonic hedgehog (SHH)-driven chemotaxis (Balic, et al., 2014). Chloroquine-mediated inhibitory effects on SHH signaling and chemokine ligand-receptor interactions suppressed the metastatic capacity of CSCs, resulting in increased cure rates in mice (Balic, et al., 2014). Chloroquine is also able to target CSCs in triple-negative breast cancer by downregulating various signaling pathways, such as STAT3, resulting in a reduction of CSCs (Choi, et al., 2014). The ability of chloroquine to eliminate CSCs makes it a promising candidate for combination therapy with cytotoxic agents for prevention of CSC-driven tumor progression (Balic, et al., 2014). Notably, other strategies to inhibit cancer stemness have shown promising results. For instance, napabucasin, a first-in-class cancer stemness inhibitor that suppresses STAT3 signaling, inhibited tumor growth when administered in combination with chemotherapeutics in a phase 1b/2 study in patients with PDAC. A disease control rate of 92% was reported, with 43% of patients displaying a 30% or greater reduction in tumor burden (Bekaii-Saab, 2017), and a pivotal phase 3 trial with napabucasin is now ongoing (). As chloroquine also interferes with the STAT3 signaling pathway in CSCs, this drug may display similar clinical efficacy as napabucasin.

Chloroquine is also able to sensitize cancer cells to therapeutic agents through modulation of non-CSC-specific signaling pathways. For instance, chloroquine treatment has been shown to improve the therapeutic efficacy of the DNA damaging agent cisplatin and the mammalian target of rapamycin (mTOR) inhibitor rapamycin in breast cancer cells (Maycotte, et al., 2012). This chemo-sensitization occurred independent of autophagy inhibition, as exposure to the autophagy inhibitor bafilomycin failed to reduce cell viability (Maycotte, et al., 2012). Additionally, knockdown of genes that promote autophagy, such as autophagy-related protein 12 (Atg12) and Beclin1, was unable to mimic the effects of chloroquine (Maycotte, et al., 2012). Potential mechanisms for this chloroquine-induced drug sensitization are thought to include DNA intercalation and activation of ataxia telangiectasia mutated (ATM) and p53 (Bakkenist & Kastan, 2003; Maycotte, et al., 2012; Wenzel, et al., 2010).

6. Conclusion

Chloroquine is an orally administered, clinically-approved drug that is used to prevent or treat malaria. This water-soluble drug can efficiently cross the cell membrane and deposit in acidic intracellular organelles, leading to antimalarial therapeutic effects. Chloroquine can also affect several mammalian physiological processes and signaling pathways. In particular, chloroquine is promising for combination cancer therapy, as this drug affects four distinct cell populations: Kupffer cells, tumor endothelial cells, cancer cells, and CSCs. Chloroquine sensitizes cancer cells to conventional therapeutic agents through prevention of autophagy; normalizes tumor vasculature, leading to improved drug delivery and decreased metastasis; reduces nanoparticle accumulation in the liver through interfering with endocytosis in Kupffer cells; suppresses cancer stem cells; and inhibits other oncogenic signaling pathways. Chloroquine has a well-known safety profile and displays acceptable toxicity when administered in combination with cancer drugs. A favorable safety profile together with

versatile anticancer effects make chloroquine a promising candidate for various combination therapy strategies with both conventional anticancer drugs and novel nanotherapeutics.

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Abbreviations:

α-SMA	alpha-smooth muscle actin
AML	acute myeloid leukemia
Atg12	autophagy-related protein 12
ATM	ataxia telangiectasia mutated
AVs	autophagic vesicles
BRAF	v-Raf murine sarcoma viral oncogene homolog B
CD31	cluster differentiation 31
CNS	central nervous system
CSCs	cancer stem cells
ERK	extracellular signal-regulated kinase
mTOR	mammalian target of rapamycin
PDAC	pancreatic ductal adenocarcinoma
PICALM	phosphatidylinositol binding clathrin assembly protein
NOTCH1	notch homolog protein 1
SHH	sonic hedgehog
SEM	scanning electron microscopy
STAT3	signal transducer and activator of transcription 3
TAMs	tumor-associated macrophages
VEGF	vascular endothelial growth factor

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Fig. 1. Chloroquine effects that can be exploited for cancer therapy. Chloroquine reduces the uptake of nanoparticles by Kupffer cells, inhibits survival-promoting autophagy, and normalizes tumor vasculature for improved drug delivery.

Fig. 2.

The autophagy processes. (A) Defective biomolecules and organelles are degraded through the autophagy pathway. A vesicle forms around particles in the cytosol, forming an autophagosome. An acidic lysosome fuses with the autophagosome to form an autolysosome in which degradation occurs. The pathway produces recyclable nutrients and metabolites that promote cell viability in stressful environments. (B) Chloroquine disrupts the autophagy pathway by decreasing the acidity of the lysosome, preventing the formation of the autolysosome. Inhibition of autophagy sensitizes cancer cells to environmental stress and increases rates of apoptosis.

Fig. 3.

The effect of chloroquine (CQ) on tumor blood vessels. B16-F10 melanoma tumor-bearing mice were treated with chloroquine (CQ) or phosphate buffered saline (PBS) (control, ctrl). (A) Tumor vessel density. (B) Percentage of tortuous tumor vessels. (C) Percentage of pericyte-covered tumor vessels. (D) Confocal microscopy images of tumors stained with CD31 (endothelial cell marker, red) and DAPI (nuclei marker, blue). (E) Scanning electron microscopy (SEM) images of tumors. Chloroquine doses of 50 mg/kg (CQ50) or 100 mg/kg (CQ100) were administered daily. Data is presented as mean $\pm$ standard error of mean. *, $P < 0.05$; ***, $P < 0.001$. Reproduced from (Maes, et al., 2014) with permission. α -SMA, alpha-smooth muscle actin; CD31, cluster differentiation 31; DAPI, 4',6-diamidino-2-phenylindole.

Fig. 4.

Effect of chloroquine on particle uptake by Kupffer cells. Kupffer cells and mice were treated with chloroquine (CQ) or phosphate buffered saline (PBS) (control, ctrl). Schematics, transmission electron microscopy (TEM) images, and charts of Kupffer cell uptake of liposomes (A) or silicon microparticles (MPs) (B). Biodistribution of fluorescently labeled liposomes (C) and radiolabeled silicon MPs (D). Data is presented as mean $\pm$ standard deviation of three replicates (A), 16 randomly selected regions (B), or $n = 5$ (C-D). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Reproduced from (Paolino, et al., 2014; Wolfram, et al., 2017; Wolfram, Shen, et al., 2015) with permission.

Table 1

Examples of anticancer effects of chloroquine mediated by mechanisms other than autophagy inhibition, tumor blood vessel normalization, and Kupffer cell suppression.

Agents	Cancer	Mechanism
Chloroquine, gemcitabine	Pancreatic ductal adenocarcinoma (PDAC)	Inhibits CXCR4/CXCL12 and sonic hedgehog signaling in cancer stem cells (CSCs), leading to anticancer activity both <i>in vitro</i> and <i>in vivo</i> (Balic, et al., 2014)
Chloroquine, cisplatin, rapamycin	Breast cancer	Autophagy-independent mechanism (potentially DNA intercalation or activation of ATM and p53) that improves therapeutic efficacy (Maycotte, et al., 2012)
Chloroquine, rapamycin, AKTi, and mTORi	Bladder cancer (FGFR3 mutant)	Inhibits cholesterol uptake resulting in enhanced permeability of the lysosomal membrane and apoptosis (King, Ganley, & Flemington, 2016)
Chloroquine and paclitaxel	Triple negative breast cancer (TNBC)	Inhibits JAK2-STAT3 signaling in CSCs, leading to anticancer activity both <i>in vitro</i> and <i>in vivo</i> (Choi, et al., 2014)

AKTi, protein kinase B inhibitor; ATM, ATM serine threonine kinase; CXCL12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C chemokine receptor type 4; FGFR3, fibroblast growth factor receptor 3; JAK2, janus kinase 2; mTORi, mammalian target of rapamycin inhibitor; p53, tumor protein p53; STAT3, signal transducer and activator of transcription 3.