

Review

Low Intensity Ultrasound as an Antidote to Taxane/Paclitaxel-induced Cytotoxicity

Celina Amaya¹, Elizabeth R. Smith², Xiang-Xi Xu^{1,3}✉

1. Department of Radiation Oncology, University of Miami Miller School of Medicine, Miami, FL 33136
2. Department of Obstetrics, Gynecology and Reproductive Science, University of Miami Miller School of Medicine, Miami, FL 33136
3. Sylvester Comprehensive Cancer Center, University of Miami, Miller School of Medicine, 1120 NW 14th Street, Miami, FL, USA

✉ Corresponding author: xxu2@med.miami.edu

© The author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>). See <http://ivyspring.com/terms> for full terms and conditions.

Received: 2022.01.20; Accepted: 2022.04.04; Published: 2022.04.18

Abstract

The taxane family of compounds, including Taxol/paclitaxel and Taxotere/docetaxel, are surprisingly successful drugs used in combination or alone for the treatment of most major solid tumors, especially metastatic cancer. The drugs are commonly used in regimen with other agents (often platinum drugs) as frontline treatment, or used as a single agent in a dose dense regimen for recurrent cancer. The major side effects of taxanes are peripheral neuropathy, alopecia, and neutropenia, which are grave burden for patients and limit the full potential of the taxane drugs. Especially in the current treatment protocol for peripheral neuropathy, taxane dosage is reduced once the symptoms present, resulting in the loss of full or optimal cancer killing activity.

Substantial efforts have been made to address the problem of cytotoxic side effects of taxanes, though strategies remain very limited. Following administration of the taxane compound by infusion, taxane binds to cellular microtubules and is sequestered within the cells for several days. Taxane stabilizes and interferes with microtubule function, leading to ultimate death of cancer cells, but also damages hair follicles, peripheral neurons, and hemopoietic stem cells. Currently, cryo-treatment is practiced to limit exposure and side effects of the drug during infusion, though the effectiveness is uncertain or limited.

A recent laboratory finding may provide a new strategy to counter taxane cytotoxicity, that a brief exposure to low density ultrasound waves was sufficient to eliminate paclitaxel cytotoxicity cells in culture by transiently breaking microtubule filaments, which were then relocated to lysosomes for disposal. Thus, ultrasonic force to break rigid microtubules is an effective solution to counter taxane cytotoxicity. The discovery and concept of low intensity ultrasound as an antidote may have the potential to provide a practical strategy to counter paclitaxel-induced peripheral neuropathy and alopecia that resulted from chemotherapy.

Taxanes are a class of important drugs used in chemotherapy to treat several major cancers. This article reviews a new laboratory discovery that ultrasound can be used as an antidote for the peripheral cytotoxicity of taxane drugs and discusses the potential development and application of low intensity ultrasound to prevent side effects in chemotherapeutic treatment of cancer patients.

Key words: Ultrasound, shock wave, microtubules, Taxol/paclitaxel, cytotoxicity, peripheral neuropathy, alopecia, neutropenia, cancer chemotherapy, side effects

1. Cancer chemotherapy: taxanes/paclitaxel

Paclitaxel (brand name Taxol) is a key drug in the current treatment of several major solid tumors, including ovarian cancer [1,2]. A paclitaxel dose-dense protocol is also effective to treat recurrent ovarian cancer [3-5]. Paclitaxel targets tubulin, and alters the dynamics and stabilizes microtubule filaments [6-8], leading to cell death [9-11]. The major side effects (neutropenia, peripheral neuropathy, and

alopecia) are thought to be caused by the impact of paclitaxel on the dynamics of the microtubules and killing of mitotic cells [12], including rapidly dividing matrix keratinocytes in the hair follicles [13,14] and the replenishment of neutrophils [15]. The interference of paclitaxel on neuronal microtubule dynamic remodeling leads to peripheral neuropathy.

Although generally paclitaxel is highly effective with tolerable side effects, several key side effects include peripheral neuropathy, neutropenia, and alopecia [15]. Neutropenia and alopecia are putatively caused by the high proliferative activity of the hematopoietic stem cells and matrix cells of the hair follicles, respectively. The presentation of peripheral neuropathy is assumed because of the critical roles of microtubules in the function and maintenance of neuronal axons.

2. Taxane/paclitaxel mechanism of action

Paclitaxel, the first Taxane class of compounds, is a surprisingly successful anti-cancer drug [2,5,16]. Paclitaxel was first identified to have cytotoxicity to cancer cells in a search for anti-cancer activity from compounds derived from plants, in paclitaxel's case the Pacific yew tree (*Taxus brevifolia*) [10,12,17]. The discovery of its activity to bind and stabilize microtubules [6-8] and consequently inhibit mitosis [9-11] prompted its clinical development.

The traditional view, largely based on studies of paclitaxel on cancer cells in culture, is that paclitaxel binds and stabilizes microtubules, and the key consequences relevant to its efficacy is mitotic inhibition and ultimately apoptosis [8,9,18]. Additional studies indicate that slippage in mitotic inhibition and the aberrant mitosis and mitotic catastrophe that result are an important mechanism in the efficacy of paclitaxel treatment [10,19].

However, alternative opinions of a minority contend that paclitaxel also kills cancer cells by a non-mitotic mechanism [20-24], which may be even more important than targeting the proliferative characteristic of cancer cells. Remarkably, paclitaxel killing is p53-independent as high grade ovarian cancer generally has inactivated p53 [16], and the cancer cells are commonly insensitive to apoptotic stimulation [19]. Several studies also suggest that paclitaxel appears not to directly stimulate the activation of caspase-3, and its efficacy is independent of caspase-3 activation or through a classical apoptosis pathway [19].

Nevertheless, the observations are consistent that microtubules are the specific and relevant drug targets [16]. Another observation is that paclitaxel causes the formation of multiple micronuclei without chromatin condensation in cancer cells, a phenomenon coined as "micronucleation", to distinguish from "nuclear fragmentation" that describes apoptosis and involves chromatin condensation [19]. This formation of multiple micronuclei is proposed to be important for cancer killing through the activation of the innate immunity and inflammatory pathway [19].

Generally, in the presence of paclitaxel and its interference on microtubule function, the formation of multiple micronuclei likely results from aberrant, multipolar mitosis [10,19]. A new study proposes a non-mitotic mechanism for the paclitaxel-stimulated formation of micronuclei, as the rigid microtubule bundles associating with the nuclear envelope physically pull and distort the structure [25]. The proposal of a physical force exerted by paclitaxel-induced rigid microtubule filaments in breaking malleable cancer nuclei provides a non-mitotic mechanism to generate multiple micronuclei [24,25] (Fig. 1). This proposed mechanism offers a possible alternative explanation for the well-established dogma that paclitaxel targets mitosis in cancer therapy; rather, paclitaxel likely aims at the weakened nuclear envelope of malignant cells. The study provides a new realization that paclitaxel can induce the generation of micronuclei in cells at S-phase by a non-mitotic mechanism [25]. In addition, for paclitaxel to target proliferative, mitotic cells, the nuclear envelope malleability appears to be another characteristic that favors cancer versus benign cells. The loss or reduction of nuclear lamina proteins, especially Lamin A/C, in cancer cells has been previously noted [26-29]. Thus, malleability of cancer nuclear envelope provides another specificity for paclitaxel, in addition to cell proliferation [24].

Another observation is that a brief treatment of ovarian cancer cells with paclitaxel induced the formation of rare cells with enlarged and deformed nuclei, likely a result of suppression of mitosis but continuous genomic replication [30]. These cells, termed "polyploid giant cancer cells" (PGCCs) have been found also to be present in cancer tissues and to harbor characteristics of stem cells found in early embryos [31]. An emerging concept, established by a series of publications in the last decade [30-33], proposes that the PGCCs are ovarian cancer stem cells, and they may account for the ability of cancer to gain resistance to chemotherapy [32,33]. In a previous study giving similar idea, the paclitaxel-induced multinucleated cells were suggested to associate with paclitaxel resistance [34]. Thus, paclitaxel-induced nuclear structural changes have interesting implications in ovarian cancer progenitor cells, mechanisms in paclitaxel cell killing, and drug resistance.

3. Retention of paclitaxel within affected cells

The pharmacokinetic properties of paclitaxel in human patients have been documented [35]. Paclitaxel is commonly administered by infusion over several hours. Although a high plasma

concentration is reached, the level in blood declines rapidly following infusion, with a half time of just hours [35]. Very little paclitaxel or its metabolites are secreted through urine, and sequestration of paclitaxel in tissues/cells is likely a key mechanism in the clearance of the drug [36].

Tumor cells and normal tissues take up and sequester abundant drugs into cells during the infusion, at several hundreds of times the concentration found in the extracellular space [8,18]. Paclitaxel binds with high affinity to alpha-tubulin within microtubules at nearly a 1:1 stoichiometry [37], and the concentration of tubulin in cells is calculated to be in the range of 10-20 μM [8,18,38,39]. The ability of cells to uptake and concentrate paclitaxel results in part from paclitaxel sequestration by binding to abundant microtubules and tubulins [8,18,38,39]. Intracellular paclitaxel is not washed out, but rather retained over several days after exposure, during which time the rigid microtubules persist [8,18,40,41].

In laboratory studies, paclitaxel was found to be concentrated several hundred-fold into cultured cells [8,37]. In animal and patient studies, paclitaxel was

found present from several days to a week within cells and tumor tissues, though the drug level had been well cleared in plasma [41,42]. Thus, following drug administration, paclitaxel is sequestered and retained within cells by binding to microtubules for a prolonged period of time. Paclitaxel activity persists, resulting in the death of the cancer cells over a few days. However, the persisting activity also causes undesirable side effects (Fig. 2). Although it seems to have not been emphasized, this unique property of intracellular paclitaxel retention is likely an important factor for the success of paclitaxel as an anti-cancer drug.

Microtubules are polymers of alpha- and beta-tubulin heterodimers, and play multiple roles in cellular functions [43,44]. Microtubules are dynamic: the filaments are constantly extending and shortening, with a balance between the cellular pool of alpha- and beta-tubulin dimers and microtubule polymers, which are about half and half under normal conditions [39,45]. Paclitaxel promotes 90-100% of tubulins into polymerized forms [39,45,46].

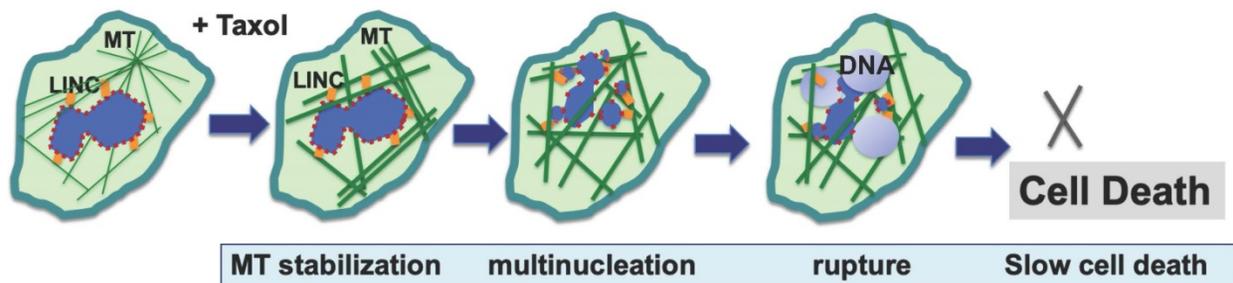


Figure 1. Proposed mechanisms for paclitaxel-induced breaking of the nuclear envelope and multiple micronucleation in cancer killing. Based on new studies, a mechanism has been suggested for the anti-cancer activity of paclitaxel: paclitaxel induces disorganized and rigid microtubule (MT) bundles, which apply physical forces to the nuclear envelope through LINC (linker of nuclear and cytoskeleton) bridges, resulting in the breaking of associated malleable nuclei of neoplastic cells and the formation of multiple micronuclei. Paclitaxel induces breaking of nuclear envelope in both mitotic and non-mitotic cancer cells. The micronuclei are defective in membrane structure (illustrated by dotted outline) and have high propensity for rupture and release of chromatin material, resulting in compromised nuclear structure and cell death.

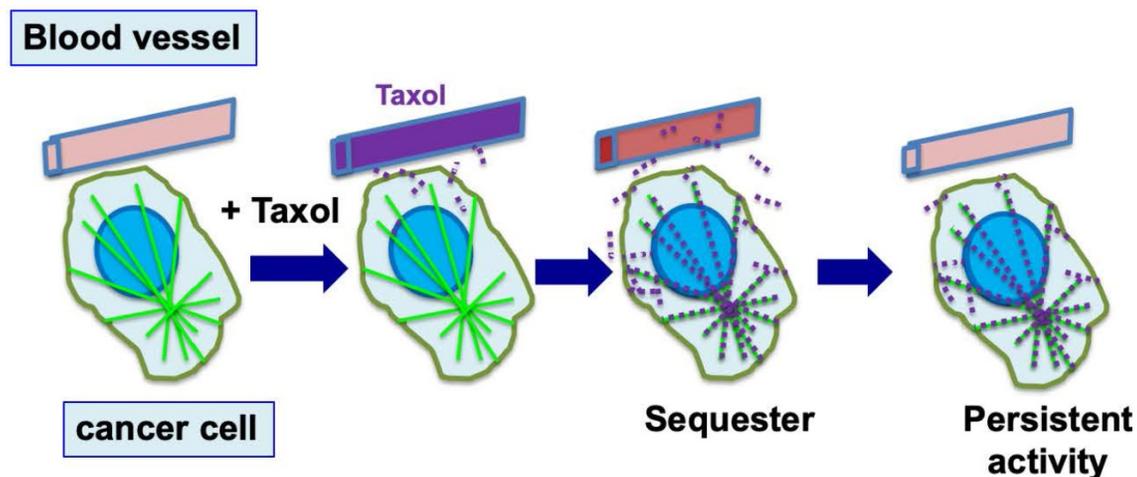


Figure 2. Cellular sequestration and retention of paclitaxel enables efficient killing of tumor cells. During chemotherapy, paclitaxel (Taxol) is administered to patients over 3-6 hours, and reaches a peak concentration in plasma by the end of drug infusion. Over the next 6 to 10 hours, paclitaxel level declines rapidly, and the drug is concentrated in cells (largely by binding to microtubules) several hundred times over the blood level (illustrated by red dots). By binding to microtubules, paclitaxel persists at high levels inside cells for the next 3 to 7 days, where the drug triggers nuclear envelope breakage and the death of cancer cells.

Tubulins are relatively stable, and the tubulin protein is removed by proteasome- (but not lysosome-) mediated degradation [47] and via degradation by cathepsin D [48]. Because of the importance of microtubules in cellular function, the homeostasis of tubulins is tightly regulated [49,50]. Tubulins control their synthesis by autoregulation at the mRNA stability [51]. Thus, addition of paclitaxel to eliminate alpha- and beta-tubulin dimers (into polymers) stimulates production of new tubulins. Newly synthesized tubulins will further sequester paclitaxel until all available drugs are depleted.

4. Taxane side effects: pathology and mechanisms

The common side effects of taxane chemotherapy are neutropenia, peripheral neuropathy, and alopecia [15] (Fig. 3). Both neutropenia and peripheral neuropathy are often the dose limiting factors in cancer treatment using taxanes [15], and effective interventional procedures would be highly valuable, but are not available currently. Alopecia, though it may not limit treatment protocol, is nevertheless a distressful quality-of-life issue for many cancer patients and is the top concern voiced by patients during consultations of chemotherapy treatment [52]. These side effects are explained by the targeting of microtubules in the host cells, either with roles in mitosis for hematopoietic stem cells or for matrix cells in hair follicles, resulting in neutropenia or alopecia, respectively, or a role in axonal maintenance and function resulting in peripheral neuropathy.

Neutropenia is the principal dose-limiting toxicity of paclitaxel with an early onset (around day 8), when neutrophil counts drop by day 8 to day 11, followed by rapid recovery on days 15 to 21 [15]. Neutropenia is not cumulative, suggesting that the drug does not permanently damage the hematopoietic stem cells, which seem to be able to recover fully. Obviously, for hematopoietic stem cells, which are one of the most proliferative cell types, inhibition of mitosis by paclitaxel impedes renewal of the immune cells [15]. However, the suppression of white blood cells often is transient, and the neutrophil level often can recover in a few days [15]. Additionally, the issue when presented is routinely managed by giving granulocyte colony stimulating factor (G-CSF) to enhance the expansion of hematopoietic stem cells [15].

Another highly proliferative cell type is the matrix cells of the hair follicles. Hair follicles undergo cycles of growth (anagen), regression (catagen), and relative quiescence (telogen) throughout life [53]. In humans, normally 85~90% of scalp hair follicles are in anagen at any given time, when hair follicle matrix

cells undergo rapid proliferation, making them extremely susceptible to mitotic inhibitors such as paclitaxel. Paclitaxel induces massive mitotic defects and apoptosis in transit amplifying hair matrix keratinocytes and also severely damages epithelial stem/progenitor cells in the bulge and outer root sheath of human scalp hair follicles [14]. This results in hair shedding that begins as early as 1-3 weeks after initiation of chemotherapy [13,54]. More than 80% of patients receiving paclitaxel develop alopecia, and beard, eyebrows, and eyelashes can also be affected, in addition to scalp hair. Hair regrowth usually takes 3~6 months after cessation of chemotherapy, but in a substantial number of cases, hair loss is irreversible [52,55]. While killing of mitotic hair matrix keratinocytes accounts for the drastic hair loss, direct epithelial stem cell damage likely causes irreversible hair loss [14].

Sensory neuropathy, presenting as numbness and pain of feet and hands, is often the dose-limiting toxicity of the taxane agents [12,15,56,57]. In this case, mitosis is not the target in this terminally differentiated cell type. However, microtubules play critical functions in neuronal axons, and paclitaxel targeting would cause a pathological situation. This major side effect (peripheral neuropathy) is thought to be caused by the impact of paclitaxel on the dynamics of the axonal microtubule structure, presumably altering the distribution and structure of the peripheral neuron network and the transmission of nerve stimuli [12,58]. Clinical features of peripheral neuropathy often present with a reduced density of peripheral neuron fibers, visualized using diagnostic ultrasound [56,59,60] (Fig. 3). Extensive laboratory research using cell systems, nonmammalian animal models, and rodent models has been attempted and is ongoing to understand the complex mechanisms of paclitaxel in causing peripheral neuronal damage [61-63]. Paclitaxel causes neurite retraction in these cultured neuronal cells, and it is considered that the phenomenon models the neuronal damage in paclitaxel-induced peripheral neuropathy [12]. Although the binding and stabilization of neuronal microtubule bundles by paclitaxel is thought to be the initial cause, the subsequent pathways and mechanisms leading to neuronal damage are complex and remain unresolved.

5. Research efforts to counter paclitaxel/taxane side effects

Oncologists have invested substantial research effort to find strategies to counter the side effects and fully realize the power of taxanes in the treatment of a wide range of cancer types [15]. Developing a practical strategy to prevent the side effects of

paclitaxel is indeed a difficult problem to solve, and various models have been used to investigate [63]. Potential drugs to block paclitaxel cytotoxicity will also inhibit the cancer killing activity, making the potential drugs unusable. Many studies tested agents with alternative mechanisms, though so far none have been found to reduce the symptoms [63,64].

The underlying pathobiology of paclitaxel chemotherapy-induced alopecia remains poorly understood. Recently it was shown that paclitaxel induces massive mitotic defects and apoptosis in transit amplifying hair matrix keratinocytes and also severely damages epithelial stem/progenitor cells in the bulge and outer root sheath of human scalp hair follicles [14]. This newly identified damage directly to stem/progenitor cells likely explains the severity and permanence of paclitaxel-induced alopecia. Since paclitaxel, as a mitotic inhibitor, targets cells at mitosis, or the M phase of the cell cycle [10,11,65], blocking of hair matrix cells from entering into mitosis is a potential strategy to prevent hair follicle damage and possibly alopecia. Indeed, using the CDK4/6 inhibitor palbociclib to keep cells of human hair follicles in organoid culture in G1, paclitaxel-induced apoptosis (shown by caspase-3 activation) is prevented [14]. This concept has been applied to test CDK4/6 inhibition for the protection against chemotherapy-induced acute kidney injury [66,67] and chemotherapy-induced hematopoietic stem cell exhaustion [68]. However, systematic blocking of mitosis likely will also reduce efficacy of paclitaxel activity in chemotherapy. Additionally, the CDK4/6 inhibitor itself already presents alopecia as a side effect [69-71]. Thus, the possibility of using the CDK4/6 inhibitor to prevent paclitaxel-induced

alopecia may require fine calibration of drug dosage and drug administration sequence and schedule.

In addition to neutropenia, which is often transient and readily recoverable, severe peripheral neuropathy is accumulative and is often the dosage limiting factor in treatment using taxane drugs [15,36]. In patient biopsies and animal models, paclitaxel treatment was observed to induce reduction of sensory neuron ends [63]. *In vitro* studies indicate that paclitaxel causes neurite retraction and neuron cell degeneration, leading to the idea that paclitaxel targeting of axon microtubules is the cause of neuronal retraction and peripheral neuropathy [58,72]. However, some studies indicate peripheral neuropathy may precede axon retraction [73,74]. Although the initial step appears to be the paclitaxel binding to axonal microtubules and their stabilization, downstream pathways leading to the pathology are more complex [63].

Many studies and various proposed mechanisms have been reported [63]. As an example, studies propose that the damage to epidermal mitochondria and generation of H_2O_2 , and subsequent activation of MMP-13, are involved in neurite degeneration [62,75]. Upregulation of MMP-13 by paclitaxel has also been confirmed in mammals to be a potential mechanism for paclitaxel-induced peripheral neuropathy [62,75]. MMP-13 is a member of the matrix-metalloproteinase family of matrix-degrading enzymes, and its suppression likely will not affect the activity of paclitaxel in killing cancer cells. Thus, pharmacologic inhibition of MMP-13 may be a potential strategy to prevent paclitaxel-induced peripheral neuropathy [62,75].

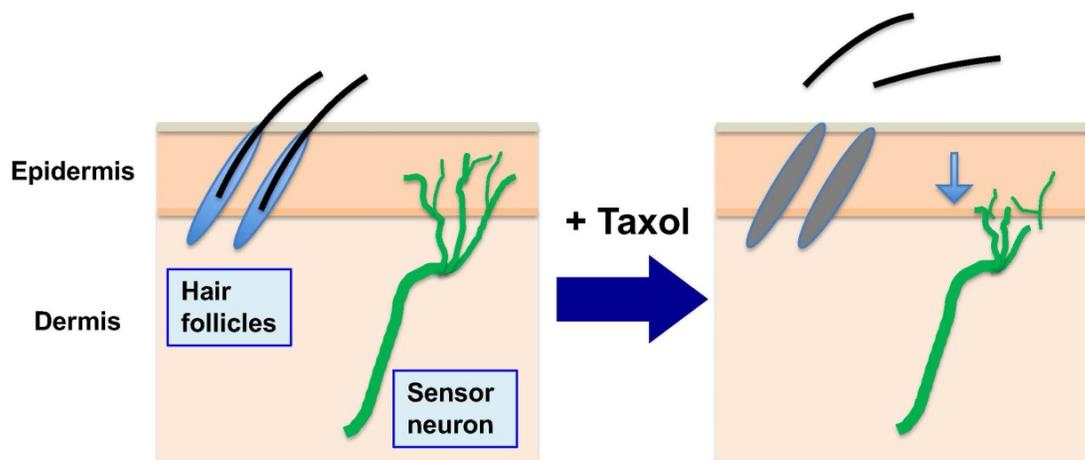


Figure 3. Alopecia and peripheral neuropathy (in addition to neutropenia) are the main side effects of paclitaxel in chemotherapy. During chemotherapy to kill cancer cells, paclitaxel (Taxol) is administered to patients over several hours (3 to 6 hours), and the drug is sequestered and concentrated in both tumor and normal cells (by binding to microtubules). While paclitaxel presented in high levels inside cells triggers death of cancer cells over the next 2-3 days, it also causes death of the proliferative hair matrix keratinocytes, leading to shedding of the hair shaft and alopecia. The retained paclitaxel also damages and causes retraction of sensory neuron terminals, causing peripheral neuropathy. (Noted that neutropenia is another major side effect, not illustrated here).

Despite their recognized importance and the extensive efforts devoted to the issues related to paclitaxel side effects, to date, few approaches are practical and available to counter taxane side effects in chemotherapy [63]. The mechanisms of paclitaxel induced neuronal damage and development of peripheral neuropathy have been extensively explored, and many drugs and agents have been suggested to have neuroprotective effects in basic laboratory studies. Some of these drugs have been tested in clinical studies for their protective effects. In the pre-clinical research, these neuropathy inhibitors are proposed to have mechanisms targeting oxidative stress, inflammatory response, ion channels, transient receptor potential channels, cannabinoid receptors, and the monoamine nervous system. However, very few drugs have demonstrated any efficacy in protecting paclitaxel-induced peripheral neuropathy in clinical trials [64], and none has reached clinical utility.

6. Prevention of taxane chemotherapy side effects using cryo-treatment

Currently, no satisfactory methods are available to reverse the side effects of paclitaxel, though cooling of hands and feet to limit drug exposure has been studied as a possible strategy to limit peripheral neuropathy of hands and feet [76,77]. For protection from hair loss, only scalp cooling has been established as a method to limit drug exposure to the scalp and to prevent or reduce alopecia [78], but its success is limited and unpredictable [79,80], and scalp cooling caps cannot protect eyebrows, eyelashes and facial hair. Therefore, novel approaches to prevent paclitaxel-induced alopecia are urgently needed to improve the quality of life of cancer patients.

The cooling needs to be maintained during the entire paclitaxel infusion period, generally 3 to 6 hours [35]. Cooling hands and feet with cooling gloves and socks, or the scalp with a cold hat/cap, works to limit the blood flow and thus the exposure to drugs. However, possible mechanisms such as the impact of temperature on microtubules polymerization, and slowing the rate and number of mitotic hair follicle matrix keratinocytes that enter mitosis, may also contribute to the outcome.

7. Discovery that low intensity ultrasound to be an antidote for paclitaxel cytotoxicity

The new discovery that low intensity ultrasound is an antidote of paclitaxel cytotoxicity [81] may offer a unique and effective strategy to eliminate the side effects of paclitaxel in cancer chemotherapy.

Ultrasound technologies have extensive applications in medicine, either for diagnosis (sonogram) or therapy [82-84]. Typically, ultrasound with extremely low intensity (1-50 mW/cm²) and high frequency (such as 50 MHz) is used for diagnostic (imaging) purposes. High intensity (> 8 W/cm², 20-60 kHz) ultrasound that can deliver strong energy is used for surgery and disruption through heating and acoustic cavitation. The medical application of ultrasound with an intensity that is low yet sufficiently high to produce biological activity is known as ultrasound physiotherapy [83,84], which uses sufficiently strong but non-disruptive ultrasound shock waves (0.5-3.0 W/cm²). The most commonly used devices produce ultrasound waves with frequencies either around 1-3 MHz or 20-150 kHz (known as long wavelength ultrasound). Similar effects by either 1-3 MHz or 45 kHz ultrasound waves on cells and tissues were reported in several studies [85,86]. The majority of ultrasound for physiotherapy uses frequencies in the range of 1-3 MHz, which traditionally is thought to produce less cavitation and thus less tissue damage. However, more recent laboratory findings indicate that the low frequency (20 to 100 KHz) ultrasound seems to produce a stronger biological impact [82,83,87,88], and at the same time seems to produce no cell or tissue damage [87,89,90]. With the availability of efficient low frequency ultrasound devices [91], low frequency ultrasound has found suitable application in several medical procedures [82-84].

A serendipitous discovery is that ultrasound can eliminate paclitaxel cytotoxicity in cells. Although exposure to low intensity ultrasound (1 W/cm²) alone, for up to 10 minutes, had no noticeable impact on cultured cells, it actually reversed the cytotoxicity of paclitaxel in cancer cells [81]. Ultrasound at low intensity can disrupt microtubule cytoskeleton transiently without significant impact on cell survival [92,93]. For cells in culture, paclitaxel/Taxol treatment resulted in the appearance of strong staining of microtubule filaments, which was abolished by low intensity ultrasound (**Fig. 4**) [81]. After treatment with ultrasound and recovery, the microtubule cytoskeleton appeared to have the same morphology in paclitaxel-treated cells as those without paclitaxel treatment (**Fig. 4A**), but the ultrasound exposure completely eliminated paclitaxel cytotoxicity (**Fig. 4**) [81]. The finding was repeated and confirmed in various cell types, and it was concluded that low intensity ultrasound is capable of eliminating paclitaxel induced cytotoxicity in all cell types tested, by transiently breaking the rigid microtubule filaments [81] (**Fig. 4**).

Based on these results, a concept was developed

that ultrasound reverses cytotoxicity by disrupting rigid microtubule filaments induced by paclitaxel treatment of proliferative cells (Fig 4) [81]. The physical breakage of paclitaxel-bound microtubules by ultrasound shock waves will result in the relocation of paclitaxel-bound microtubule fragments or tubulin heterodimers to lysosomes for degradation, and new microtubule networks will form rapidly from tubulins not bound to paclitaxel [81]. Tubulin levels in cells are auto-regulated, and newly synthesized tubulins quickly replace degraded paclitaxel-bound tubulins to form the microtubule cytoskeleton [49,51,94]. Thus, a brief pulse exposure to ultrasound efficiently removes the negative impact of paclitaxel on microtubule dynamics and cell cytotoxicity (Fig. 4).

A potential strategy is to reverse the early step of paclitaxel cytotoxicity by disrupting the rigid microtubule filaments induced by paclitaxel with low intensity ultrasound. Thus, the strategy may be successful to counter paclitaxel-induced peripheral neuropathy at an early step, regardless of the complex down-stream mechanisms by which paclitaxel induces peripheral neuropathy.

In chemotherapy, paclitaxel also causes stabilization and bundling of microtubules in peripheral neuronal cells, and consequently leads to retraction (Fig. 5A). This may reflect the underlying issue in peripheral neuropathy, which is thought to be caused by the impact of paclitaxel on the dynamics of the axonal long microtubule structure, presumably altering the distribution and structure of the peripheral neuron network and the transmission of nerve stimuli [63,95]. Low intensity ultrasound

transiently disrupts the paclitaxel-induced rigid microtubule bundles (Fig. 5B). The paclitaxel bound microtubule fragments undergo lysosomal degradation. Additionally, paclitaxel is rapidly (8-24 hours) eliminated in the environment by binding to and being sequestered by cellular microtubules (about 10-20 μM in cells) [38,39,42], or bound by other cellular components. Increased synthesis of tubulins likely also contributes to paclitaxel elimination.

8. Potential prevention of taxane chemotherapy side effects using low intensity ultrasound

Low density ultrasound has been explored to influence peripheral neurons [96] or treat pain and neuropathy in clinical trials [97]; however, the current concept is based on a new discovery that ultrasound can eliminate acute paclitaxel cytotoxicity by breaking the rigid microtubules. While low intensity ultrasound has been extensively used for various medical applications [83,84], and laboratory studies show that ultrasound waves impact cells and may have biological activity even on hair follicles [98], the evidence to support medical applications of ultrasound is largely anecdotal. Most medical applications of ultrasound physiotherapies were determined to lack true merit in large and rigorous clinical studies [83,84]. Instead, the surprising findings [81] and unique hypothesis discussed here may be possible to introduce a rational, evidence-based use of ultrasound therapy for overcoming paclitaxel cytotoxicity into medical practice.

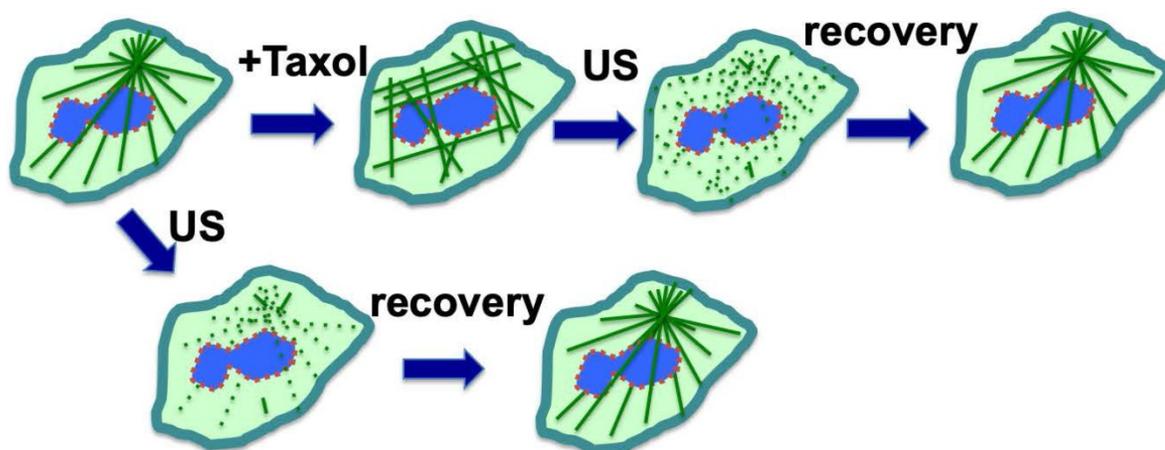


Figure 4. Ultrasound exposure reverses cytotoxicity by disrupting rigid microtubule filaments induced by paclitaxel (Taxol) treatment of cells. Microtubule bundles radiate out from the microtubule organizing center. Ultrasound (US) is known to transiently disrupt microtubule networks, which reform within 1-2 hours. Paclitaxel (Taxol) induces rigid microtubule filaments that lead to growth arrest and subsequent cell death in proliferative cells such as cancer cells or matrix keratinocytes of the hair follicles. We suggest a mechanism through which ultrasound reverses cytotoxicity by disrupting rigid microtubule filaments induced by paclitaxel. The paclitaxel-bound microtubule fragments and tubulins are relocated to lysosomes for degradation, and newly synthesized tubulins form a new network of microtubule cytoskeleton without bound paclitaxel. Thus, a brief exposure to low intensity ultrasound removes cellular paclitaxel activity/cytotoxicity.

Since ultrasound treatment is considered a safe procedure, potential clinical development is eminently feasible. Ultrasound devices suitable to apply low intensity shock waves to the skin surface and hair follicles may be designed, and water may be suitable to be used as an ultrasound-transmitting medium (Fig. 6A). The human scalp is a strong barrier for the penetration of low frequency ultrasound energy [84], which actually eases the potential concern that the ultrasound waves may affect human brain.

The application of ultrasound to hands, feet, and scalp a few hours after drug administration may be able to eliminate the persistent activity of paclitaxel already bound to the microtubules of the cells. Both the water bath and probe types of ultrasound devices can be considered for clinical application (Fig. 6B). The bath device produces 45-150 kHz, 1-3 W/cm² ultrasound waves with adjustable frequency and energy levels. The probe device produces adjustable 1-3 W/cm² and 1-3 MHz ultrasound waves.

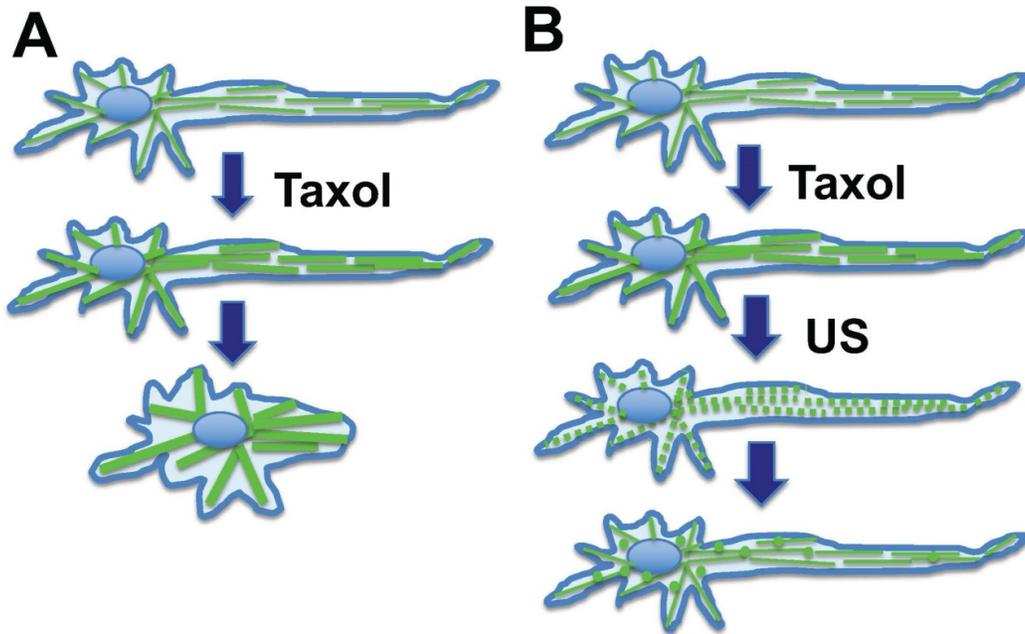


Figure 5. Hypothesis: Ultrasound reverses neurite retraction by disrupting rigid microtubule filaments induced by paclitaxel. Axon maintenance in peripheral neuronal cells depends on microtubule dynamics. (A) Taxol/paclitaxel stabilizes microtubules and leads to neurite retraction. This may model the underlying issue of paclitaxel-induced peripheral neuropathy. (B) Ultrasound (US) is known to disrupt the microtubule network, which in turn reforms rapidly. A mechanism is suggested that ultrasound disrupts paclitaxel-induced rigid microtubule bundles, leading to prevention of neurite retraction, and this concept may be explored to prevent paclitaxel-induced peripheral neuropathy.

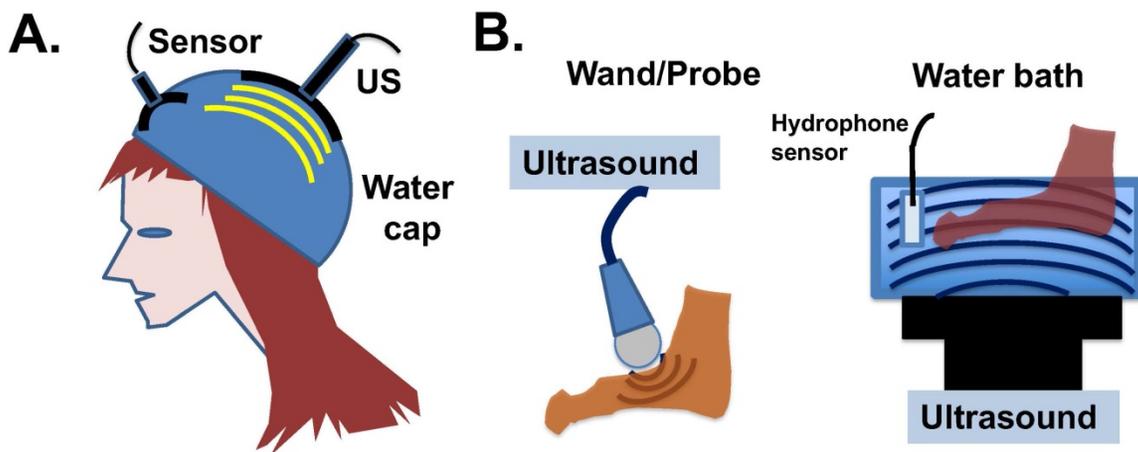


Figure 6. Potential application of ultrasound devices for physiotherapy to prevent paclitaxel side effects in chemotherapy. (A) Illustration of ultrasound design to counter cytotoxicity in hair follicles of cancer patients undergoing paclitaxel (Taxol) treatment. A water-filled bag/cap is used to couple the transfer of ultrasound energy to epidermal and hair follicles under the wetted hair. Ultrasound intensity will be monitored (and potentially feedback regulated) by a built-in hydrophone sensor. (B) Illustration of two available devices for the transmission of ultrasound waves through water to peripheral tissues. The water bath ultrasound device gives out 45 kHz ultrasound shock wave with 1 to 3 W/cm² energy, and the probe ultrasound device produces 1 to 3 MHz ultrasound in 1 to 3 W/cm² energy waves.

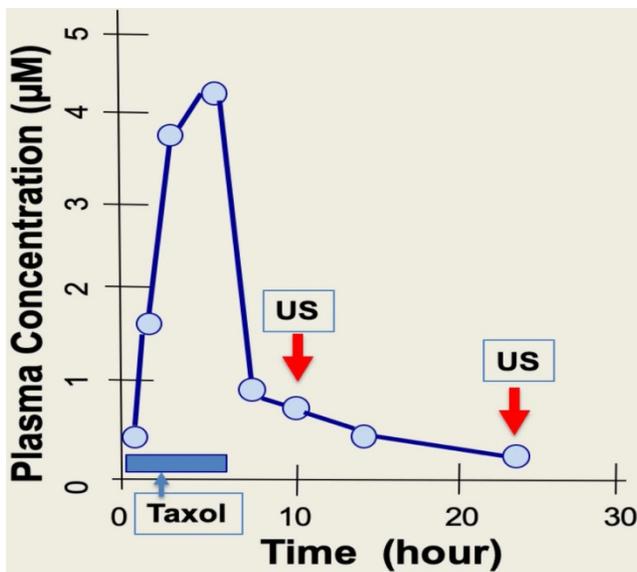


Figure 7. Timing of ultrasound application for physiotherapy to prevent paclitaxel side effects in chemotherapy. During chemotherapy, paclitaxel (Taxol) is administered to patients over 3-6 hours, and taxane concentration reaches a peak level in plasma by the end of drug infusion. Paclitaxel plasma levels fall rapidly following infusion over next 6 to 10 hours. It is reasoned that intermittent ultrasound (US) pulse treatment (5 min exposure) over a period of 4-10 hours may be suitable, when plasma drug levels are much lower. A second ultrasound treatment at around 24 hours may also help to further eliminate paclitaxel cytotoxicity locally at hands, feet, and scalp.

Paclitaxel is administered through IV over a 4- to 6-hour time course. Upon completion of drug infusion, blood paclitaxel rapidly declines, and the drug enters and accumulates within cells [8,35,42]. The cellular level of paclitaxel is concentrated several hundred-fold higher than in blood, partly due to binding to cellular microtubules [8,35,37]. A short exposure of cells to paclitaxel leads to persistent cytotoxicity over several days even when extracellular sources of paclitaxel is absent, as the paclitaxel bound rigid microtubule bundles are present several days after [41]. During the 2-7 days after paclitaxel administration, the drug triggers death of cancer cells, but also causes damage to peripheral neurons that presents as peripheral neuropathy, and also to hair matrix keratinocytes that results in alopecia [14]. Thus, for a 3- to 6-hour infusion of paclitaxel, it may be suitable to treat patients with intermittent, low intensity ultrasound pulse therapy (5 min, about 1 W/cm²) over just a few hours (e.g. 4 to 10 hours) following chemotherapy (Fig. 7). Possibly, a second ultrasound treatment may be applied 24 hours after chemotherapy to ensure complete localized elimination of paclitaxel cytotoxicity (e.g., in scalp skin but not in cancer cells) (Fig. 7). The optimal timing of ultrasound exposure to reverse paclitaxel activity may be the window between the time when free paclitaxel is depleted, and the time required for causing damage and harm by the paclitaxel-bound rigid microtubules (Fig. 7). Thus, ultrasound

treatment appears to disrupt the persistent paclitaxel-bound rigid microtubules and thereby eliminate the prolonged cytotoxicity of paclitaxel.

Newer microtubule stabilizing drugs and delivering platforms, such as Abraxanes, cabazitaxel, epothilones (ixabepilone), laulimalide, and discodermolide, etc., are under development and coming into clinical application [99-104]. These new agents act with a mechanism same as paclitaxel, the first generation of microtubule stabilizing drugs, with similar side effects. Similarly, low intensity ultrasound likely will disrupt microtubules affected by these new drugs in the same way the ultrasound acts on paclitaxel-induced rigid microtubule bundles. Thus, ultrasound treatment may also relieve side effects from the new microtubule stabilizing drugs.

The application of ultrasound to counter paclitaxel-induced peripheral neuropathy seems to be very feasible and practical. In fact, probe ultrasound has been used in clinical trials to treat paclitaxel-induced peripheral neuropathy [97]. However, the current research findings suggest that ultrasound may prevent, but not reverse paclitaxel-induced peripheral neuropathy and alopecia, and this will be a new rationale based on a solid scientific basis.

9. Summary

Paclitaxel (and other taxane drugs) binds and stabilizes microtubules, while ultrasound breaks microtubules, thus acting as an antidote. The mechanism may be quite self-explanatory. Based on our recent discovery that low intensity ultrasound treatment can effectively and almost completely neutralize the cytotoxic effects of paclitaxel in various cell types, a strategy may be developed to counter the side effects of paclitaxel-based chemotherapy for cancer patients. The concept that a brief exposure of paclitaxel-treated cells to ultrasound is sufficient to break paclitaxel-induced rigid microtubules opens up the possibility to use ultrasound locally to eliminate cytotoxicity at only the desirable anatomic sites, without affecting paclitaxel activity towards neoplastic cells. In essence, ultrasound is able to remove the persistent activity of paclitaxel at intended areas after systematic drug infusion. The possibilities to use this paclitaxel antidote can be contemplated to prevent chemotherapy-induced alopecia, and peripheral neuropathy, which will ensure the full use of paclitaxel dosage in treatment and improve the quality of life for cancer patients.

Acknowledgments

We thank our lab members and alumni for the research work supporting this review/opinion paper.

We acknowledge that many additional excellent works and publications are relevant to the discussed topic, but are not cited due to the limited scope of this review/opinion manuscript.

Funding

This work from our lab discussed and cited was supported by funds from grant NICHD R03HD071244 (E.R.S.), concept awards BC097189 and BC076832 from Department of Defense (USA). Grants R01 CA230916, R01 CA095071, R01 CA099471, and CA79716 to X-X Xu from NCI, NIH also contributed to the studies. Internal research funding from the Sylvester Comprehensive Cancer Center and University of Miami also supported this work.

Competing Interests

The authors declared no competing interests.

References

- Gallego-Jara J, Lozano-Terol G, Sola-Martínez RA, Cánovas-Díaz M, de Diego Puente T. A compressive review about taxol: History and future challenges. *Molecules*. 2020; 25(24):5986.
- Rowinsky EK, Donehower RC. Paclitaxel (taxol). *N Engl J Med*. 1995 Apr 13; 332(15):1004-1014.
- Baird RD, Tan DS, Kaye SB. Weekly paclitaxel in the treatment of recurrent ovarian cancer. *Nat Rev Clin Oncol*. 2010 Oct; 7(10):575-582.
- Baker KG, Robertson VJ, Duck FA. A Review of Therapeutic Ultrasound: Biophysical Effects. *Physical Therapy*. 2001; 81(7):1351-1358.
- Jain A, Dubashi B, Reddy KS, Jain P. Weekly paclitaxel in ovarian cancer-the latest success story. *Curr Oncol*. 2011 Jan; 18(1):16-17.
- Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly *in vitro* by taxol. *Nature*. 1979; 277(5698):665-667.
- Schiff PB, Horwitz SB. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc Natl Acad Sci USA*. 1980 Mar; 77(3):1561-1565.
- Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer*. 2004; 4:253-265.
- Horwitz SB. Taxol (paclitaxel): mechanisms of action. *Ann Oncol*. 1994; 5 Suppl 6:S3-6.
- Weaver BA. How Taxol/paclitaxel kills cancer cells. *Mol Biol Cell*. 2014; 25(18):2677-2681.
- Morse DL, Gray H, Payne CM, Gillies RJ. Docetaxel induces cell death through mitotic catastrophe in human breast cancer cells. *Mol Cancer Ther*. 2005; 4:1495-1504.
- Canta A, Chiorazzi A, Cavaletti G. Tubulin: a target for antineoplastic drugs into the cancer cells but also in the peripheral nervous system. *Curr Med Chem*. 2009; 16:1315-1324.
- Paus R, Haslam IS, Sharov AA, Botchkarev VA. Pathobiology of chemotherapy-induced hair loss. *Lancet Oncol*. 2013 Feb; 14(2):e50-59.
- Purba TS, Ng'andu K, Brunken L, Smart E, Mitchell E, Hassan N, O'Brien A, Mellor C, Jackson J, Shahmalak A, Paus R. CDK4/6 inhibition mitigates stem cell damage in a novel model for taxane-induced alopecia. *EMBO Mol Med*. 2019 Oct; 11(10):e11031.
- Rowinsky EK, Eisenhauer EA, Chaudhry V, Arbusk SG, Donehower RC. Clinical toxicities encountered with paclitaxel (Taxol). *Semin Oncol*. 1993; 20(4 Suppl 3):1-15.
- Blagosklonny MV, Fojo T. Molecular effects of paclitaxel: myths and reality (a critical review). *Int J Cancer*. 1999 Oct 8; 83(2):151-156.
- Wani MC, Horwitz SB. Nature as a remarkable chemist: a personal story of the discovery and development of Taxol. *Anticancer Drugs*. 2014; 25(5):482-487.
- Jordan MA. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. *Curr Med Chem Anticancer Agents*. 2002; 2(1):1-17.
- Mitchison TJ, Pineda J, Shi J, Florian S. Is inflammatory micronucleation the key to a successful anti-mitotic cancer drug? *Open Biol*. 2017; 7(11):170182.
- Komlodi-Pasztor E, Sackett D, Wilkerson J, Fojo T. Mitosis is not a key target of microtubule agents in patient tumors. *Nat Rev Clin Oncol*. 2011; 8(4):244-250.
- Komlodi-Pasztor E, Sackett DL, Fojo AT. Inhibitors targeting mitosis: tales of how great drugs against a promising target were brought down by a flawed rationale. *Clin Cancer Res*. 2012; 18(1):51-63.
- Mitchison TJ. The proliferation rate paradox in antimetabolic chemotherapy. *Mol Biol Cell*. 2012; 23:1-6.
- Fürst R, Vollmar AM. A new perspective on old drugs: non-mitotic actions of tubulin-binding drugs play a major role in cancer treatment. *Pharmazie*. 2013; 68:478-483.
- Smith ER, Xu XX. Breaking malignant nuclei as a non-mitotic mechanism of taxol/paclitaxel. *J Cancer Biol*. 2021; 2(4):86-93.
- Smith ER, Leal J, Amaya C, Li B, Xu XX. Nuclear Lamin A/C Expression Is a Key Determinant of Paclitaxel Sensitivity. *Mol Cell Biol*. 2021 Jun 23; 41(7):e0064820.
- Capo-chichi CD, Cai KQ, Simpkins F, Ganjei-Azar P, Godwin AK, Xu XX. Nuclear envelope structural defects cause chromosomal numerical instability and aneuploidy in ovarian cancer. *BMC Med*. 2011; 9:28.
- Capo-Chichi CD, Yeasky TM, Smith ER, Xu XX. Nuclear envelope structural defect underlies the main cause of aneuploidy in ovarian carcinogenesis. *BMC Cell Biol*. 2016; 17:37.
- Smith ER, Capo-Chichi CD, Xu XX. Defective Nuclear Lamina in Aneuploidy and Carcinogenesis. *Front Oncol*. 2018; 8:529.
- Smith ER, George SH, Kobetz E, Xu XX. New biological research and understanding of Papanicolaou's test. *Diagn Cytopathol*. 2018; 46:507-515.
- Niu N, Zhang J, Zhang N, Mercado-Urbe I, Tao F, Han Z, Pathak S, Multani AS, Kuang J, Yao J, Bast RC, Sood AK, Hung MC, Liu J. Linking genomic reorganization to tumor initiation via the giant cell cycle. *Oncogenesis*. 2016 Dec 19; 5(12):e281.
- Niu N, Mercado-Urbe I, Liu J. Dedifferentiation into blastomere-like cancer stem cells via formation of polyploid giant cancer cells. *Oncogene*. 2017 Aug 24; 36(34):4887-4900.
- Niu N, Yao J, Bast RC, Sood AK, Liu J. IL-6 promotes drug resistance through formation of polyploid giant cancer cells and stromal fibroblast reprogramming. *Oncogenesis*. 2021 Sep 29; 10(9):65.
- Liu J, Niu N, Li X, Zhang X, Sood AK. The life cycle of polyploid giant cancer cells and dormancy in cancer: Opportunities for novel therapeutic interventions. *Semin Cancer Biol*. 2021 Oct 17; S1044-579X(21)00255-8.
- Merlin JL, Bour-Dill C, Marchal S, Bastien L, Gramain MP. Resistance to paclitaxel induces time-delayed multinucleation and DNA fragmentation into large fragments in MCF-7 human breast adenocarcinoma cells. *Anticancer Drugs*. 2000 Apr; 11(4):295-302.
- Wiernik PH, Schwartz EL, Strauman JJ, Dutcher JP, Lipton RB, Paietta E. Phase I clinical and pharmacokinetic study of taxol. *Cancer Res*. 1987 May 1; 47(9):2486-2493.
- Rowinsky EK, Donehower RC. The clinical pharmacology of paclitaxel (Taxol). *Semin Oncol*. 1993 Aug; 20(4 Suppl 3):16-25.
- Manfredi JJ, Parness J, Horwitz SB. Taxol binds to cellular microtubules. *J Cell Biol*. 1982 Sep; 94(3):688-696.
- Cassimeris L, Silva VC, Miller E, Ton Q, Molnar C, Fong J. Fueled by Microtubules: Does Tubulin Dimer/Polymer Partitioning Regulate Intracellular Metabolism? Cytoskeleton. 2012; 69:133-143.
- Zhai Y, Borisy GG. Quantitative determination of the proportion of microtubule polymer present during the mitosis-interphase transition. *J Cell Sci*. 1994; 107:881-890.
- Michalakakis J, Georgatos SD, de Bree E, Polioudaki H, Romanos J, Georgoulas V, Tsiftsis DD, Theodoropoulos PA. Short-term exposure of cancer cells to micromolar doses of paclitaxel, with or without hyperthermia, induces long-term inhibition of cell proliferation and cell death *in vitro*. *Ann Surg Oncol*. 2007 Mar; 14(3):1220-1228.
- Mori T, Kinoshita Y, Watanabe A, Yamaguchi T, Hosokawa K, Honjo H. Retention of paclitaxel in cancer cells for 1 week *in vivo* and *in vitro*. *Cancer Chemother Pharmacol*. 2006 Nov; 58(5):665-672.
- Koshiha H, Hosokawa K, Mori T, Kubo A, Watanabe A, Honjo H. Intravenous paclitaxel is specifically retained in human gynecologic carcinoma tissues *in vivo*. *Int J Gynecol Cancer*. 2009 May; 19(4):484-488.
- Desai A, Mitchison TJ. MICROTUBULE POLYMERIZATION DYNAMICS. *Annu Rev Cell Dev Biol*. 1997; 13:83-117.
- Baas PW. Microtubules and axonal growth. *Curr Opin Cell Biol*. 1997; 9:29-36.
- Diaz JF, Andreu JM. Assembly of purified GDP-tubulin into microtubules induced by taxol and taxotere: reversibility, ligand stoichiometry, and competition. *Biochemistry*. 1993; 32:2747-2755.
- Holmfeldt P, Sellin ME, Gullberg M. Predominant regulators of tubulin monomer-polymer partitioning and their implication for cell polarization. *Cell Mol Life Sci*. 2009; 66:3263-3276.
- Huff LM, Sackett DL, Poruchynsky MS, Fojo T. Microtubule-disrupting chemotherapeutics result in enhanced proteasome-mediated degradation and disappearance of tubulin in neural cells. *Cancer Res*. 2010 Jul 15; 70(14):5870-5879.

48. Johnson GV, Litersky JM, Whitaker JN. Proteolysis of microtubule-associated protein 2 and tubulin by cathepsin D. *J Neurochem*. 1991 Nov; 57(5):1577-1583.
49. Caron JM, Jones AL, Kirschner MW. Autoregulation of tubulin synthesis in hepatocytes and fibroblasts. *J Cell Biol*. 1985; 101:1763-1772.
50. Laferriere NB, MacRae TH, Brown DL. Tubulin synthesis and assembly in differentiating neurons. *Biochem Cell Biol*. 1997; 75:103-117.
51. Lin Z, Gasic I, Chandrasekaran V, Peters N, Shao S, Mitchison TJ, Hegde RS. TTC5 mediates autoregulation of tubulin via mRNA degradation. *Science*. 2020 Jan 3; 367(6473):100-104.
52. Rossi A, Fortuna MC, Caro G, Pranteda G, Garelli V, Pompili U, Carlesimo M. Chemotherapy-induced alopecia management: Clinical experience and practical advice. *J Cosmet Dermatol*. 2017; 16:537-541.
53. Paus R, Cotsarelis G. The biology of hair follicles. *N Engl J Med*. 1999; 341:491-497.
54. Botchkarev VA, Paus R. Molecular biology of hair morphogenesis: development and cycling. *J Exp Zool B Mol Dev Evol*. 2003 Aug 15; 298(1):164-180.
55. Freitas-Martinez A, Chan D, Sibaud V, Shapiro J, Fabbrocini G, Tosti A, Cho J, Goldfarb S, Modi S, Gajria D, Norton L, Paus R, Cigler T, Lacouture ME. Assessment of Quality of Life and Treatment Outcomes of Patients With Persistent Postchemotherapy Alopecia. *JAMA Dermatol*. 2019 Jun 1; 155(6):724-728.
56. Kudlowitz D, Muggia F. Clinical features of taxane neuropathy. *Anticancer Drugs*. 2014; 25:495-501.
57. Mielke S, Sparreboom A, Mross K. Peripheral neuropathy: a persisting challenge in paclitaxel-based regimens. *Eur J Cancer*. 2006 Jan; 42(1):24-30.
58. Malacrida A, Meregalli C, Rodriguez-Menendez V, Nicolini G. Chemotherapy-Induced Peripheral Neuropathy and Changes in Cytoskeleton. *Int J Mol Sci*. 2019 May 9; 20(9):2287.
59. Boyette-Davis JA, Cata JP, Driver LC, Novy DM, Bruel BM, Mooring DL, Wendelschafer-Crabb G, Kennedy WR, Dougherty PM. Persistent chemoneuropathy in patients receiving the plant alkaloids paclitaxel and vincristine. *Cancer Chemother Pharmacol*. 2013 Mar; 71(3):619-626.
60. Velasco R, Bruna J. Taxane-Induced Peripheral Neurotoxicity. *Toxics*. 2015; 3(2):152-169.
61. Cirrincione AM, Rieger S. Analyzing chemotherapy-induced peripheral neuropathy *in vivo* using non-mammalian animal models. *Exp Neurol*. 2020 Jan; 323:113090.
62. Cirrincione AM, Pellegrini AD, Dominy JR, Benjamin ME, Utkina-Sosunova I, Lotti F, Jergova S, Sagen J, Rieger S. Paclitaxel-induced peripheral neuropathy is caused by epidermal ROS and mitochondrial damage through conserved MMP-13 activation. *Sci Rep*. 2020 Mar 4; 10(1):3970.
63. Staff NP, Fehrenbacher JC, Caillaud M, Damaj MI, Segal RA, Rieger S. Pathogenesis of paclitaxel-induced peripheral neuropathy: A current review of *in vitro* and *in vivo* findings using rodent and human model systems. *Exp Neurol*. 2020 Feb; 324:113121.
64. Kawashiri T, Inoue M, Mori K, Kobayashi D, Mine K, Ushio S, Kudamatsu H, Uchida M, Egashira N, Shimazoe T. Preclinical and Clinical Evidence of Therapeutic Agents for Paclitaxel-Induced Peripheral Neuropathy. *Int J Mol Sci*. 2021; 22:8733.
65. Abal M, Andreu JM, Barasoain I. Taxanes: microtubule and centrosome targets, and cell cycle dependent mechanisms of action. *Curr Cancer Drug Targets*. 2003; 3(3):193-203.
66. DiRocco DP, Bisi J, Roberts P, Strum J, Wong KK, Sharpless N, Humphreys BD. CDK4/6 inhibition induces epithelial cell cycle arrest and ameliorates acute kidney injury. *Am J Physiol Renal Physiol*. 2014 Feb 15; 306(4):F379-388.
67. Pabla N, Gibson AA, Buege M, Ong SS, Li L, Hu S, Du G, Sprowl JA, Vasilyeva A, Janke LJ, Schlatter E, Chen T, Ciarimboli G, Sparreboom A. Mitigation of acute kidney injury by cell-cycle inhibitors that suppress both CDK4/6 and OCT2 functions. *Proc Natl Acad Sci U S A*. 2015 Apr 21; 112(16):5231-5236.
68. He S, Roberts PJ, Sorrentino JA, Bisi JE, Storrle-White H, Tiessen RG, Makhuli KM, Wargin WA, Tadema H, van Hoogdalem EJ, Strum JC, Malik R, Sharpless NE. Transient CDK4/6 inhibition protects hematopoietic stem cells from chemotherapy-induced exhaustion. *Sci Transl Med*. 2017 Apr 26; 9(387):eaal3986.
69. Lashen S, Shohdy KS, Kassem L, Abdel-Rahman O. Fatigue, alopecia and stomatitis among patients with breast cancer receiving cyclin-dependent kinase 4 and 6 inhibitors: a systematic review and meta-analysis. *Expert Rev Anticancer Ther*. 2017 Sep; 17(9):851-856.
70. Yang L, Xue J, Yang Z, Wang M, Yang P, Dong Y, He X, Bao G, Peng S. Side effects of CDK4/6 inhibitors in the treatment of HR+/HER2-advanced breast cancer: a systematic review and meta-analysis of randomized controlled trials. *Ann Palliat Med*. 2021 May; 10(5):5590-5599.
71. Zhang M, Zhang L, Hei R, Li X, Cai H, Wu X, Zheng Q, Cai C. CDK inhibitors in cancer therapy, an overview of recent development. *Am J Cancer Res*. 2021 May 15; 11(5):1913-1935.
72. Fukuda Y, Li Y, Segal RA. A mechanistic understanding of axon degeneration in chemotherapy-induced peripheral neuropathy. *Frontiers in Neuroscience*. 2017; 11:481.
73. Polomano RC, Mannes AJ, Clark US, Bennett GJ. A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug, paclitaxel. *Pain*. 2001 Dec; 94(3):293-304.
74. Flatters SJL, Bennett GJ. Studies of peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy: evidence for mitochondrial dysfunction. *Pain*. 2006 Jun; 122(3):245-257.
75. Lisse TS, Middleton LJ, Pellegrini AD, Martin PB, Spaulding EL, Lopes O, Brochu EA, Carter EV, Waldron A, Rieger S. Paclitaxel-induced epithelial damage and ectopic MMP-13 expression promotes neurotoxicity in zebrafish. *Proc Natl Acad Sci USA*. 2016 Apr 12; 113(15):E2189-198.
76. Griffiths C, Kwon N, Beaumont JL, Paice JA. Cold therapy to prevent paclitaxel-induced peripheral neuropathy. *Support Care Cancer*. 2018 Oct; 26(10):3461-3469.
77. Hanai A, Ishiguro H, Sozu T, Tsuda M, Yano I, Nakagawa T, Imai S, Hamabe Y, Toi M, Arai H, Tsuboyama T. Effects of Cryotherapy on Objective and Subjective Symptoms of Paclitaxel-Induced Neuropathy: Prospective Self-Controlled Trial. *J Natl Cancer Inst*. 2018 Feb 1; 110(2):141-148.
78. Rice BA, Ver Hoeve ES, DeLuca AN, Esserman LJ, Rugo HS, Melisko ME. Registry study to assess hair loss prevention with the Penguin Cold Cap in breast cancer patients receiving chemotherapy. *Breast Cancer Res Treat*. 2018 Jan; 167(1):117-122.
79. van den Hurk CJ, Peerbooms M, van de Poll-Franse LV, Nortier JW, Coebergh JW, Breed WP. Scalp cooling for hair preservation and associated characteristics in 1411 chemotherapy patients - results of the Dutch Scalp Cooling Registry. *Acta Oncol*. 2012 Apr; 51(4):497-504.
80. Wang S, Yang T, Shen A, Qiang W, Zhao Z, Zhang F. The scalp cooling therapy for hair loss in breast cancer patients undergoing chemotherapy: a systematic review and meta-analysis. *Support Care Cancer*. 2021 Nov; 29(11):6943-6956.
81. Amaya C, Luo S, Baigorri J, Baucells R, Smith ER, Xu XX. Exposure to low intensity ultrasound removes paclitaxel cytotoxicity in breast and ovarian cancer cells. *BMC Cancer*. 2021 Sep 1; 21(1):981.
82. Abramavičius S, Volkevičiūtė A, Tunaitytė A, Venslauskas M, Bubulis A, Bajoriūnas V, Stankevičius E. Low-Frequency (20 kHz) Ultrasonic Modulation of Drug Action. *Ultrasound Med Biol*. 2020 Nov; 46(11):3017-331.
83. Ahmadi F, McLoughlin IV, Chauhan S, ter-Haar G. Bio-effects and safety of low-intensity, low-frequency ultrasonic exposure. *Prog Biophys Mol Biol*. 2012; 108(3):119-138.
84. ter Haar G. Therapeutic applications of ultrasound. *Prog Biophys Mol Biol*. 2007; 93(1-3):111-129.
85. Reher P, Doan N, Bradnock B, Meghji S, Harris M. Therapeutic ultrasound for osteoradionecrosis: an *in vitro* comparison between 1 MHz and 45 kHz machines. *Eur J Cancer*. 1998 Nov; 34(12):1962-1968.
86. Robertson VJ, Ward AR. Subaqueous ultrasound: 45kHz and 1MHz machines compared. *Arch Phys Med Rehabil*. 1995 Jun; 76(6):569-575.
87. Iida K, Luo H, Hagsisawa K, Akima T, Shah PK, Naqvi TZ, Siegel RJ. Noninvasive low-frequency ultrasound energy causes vasodilation in humans. *J Am Coll Cardiol*. 2006; 48:532-537.
88. Samuels JA, Weingarten MS, Margolis DJ, Zubkov L, Sunny Y, Bawiec CR, Conover D, Lewin PA. Low-frequency (<100 kHz), low-intensity (<100 mW/cm²) ultrasound to treat venous ulcers: a human study and *in vitro* experiments. *J Acoust Soc Am*. 2013 Aug; 134(2):1541-1547.
89. Fischell TA, Abbas MA, Grant GW, Siegel RJ. Ultrasonic energy. Effects on vascular function and integrity. *Circulation*. 1991 Oct; 84(4):1783-1795.
90. Scarponi C, Nasorri F, Pavani F, Madonna S, Sestito R, Simonacci M, De Pità O, Cavani A, Albanesi C. Low-frequency low-intensity ultrasounds do not influence the survival and immune functions of cultured keratinocytes and dendritic cells. *J Biomed Biotechnol*. 2009; 2009:193260.
91. Sunny Y, Bawiec CR, Nguyen AT, Samuels JA, Weingarten MS, Zubkov LA, Lewin PA. Optimization of un-tethered, low voltage, 20-100kHz flexural transducers for biomedical ultrasonics applications. *Ultrasonics*. 2012 Sep; 52(7):943-948.
92. Adler J, Necas O, Hrazdira I. Dissassembly of microtubules due to low intensity ultrasound. *Folia Biol. (Praha)* 1993; 39:55-57.
93. Samandari M, Abrinia K, Mokhtari-Dizaji M, Tamayol A. Ultrasound induced strain cytoskeleton rearrangement: An experimental and simulation study. *J Biomech*. 2017; 60:39-47.

94. Gasic I, Boswell SA, Mitchison TJ. Tubulin mRNA stability is sensitive to change in microtubule dynamics caused by multiple physiological and toxic cues. *PLoS Biol.* 2019; 17(4):e3000225.
95. Gornstein E, Schwarz TL. The paradox of paclitaxel neurotoxicity: Mechanisms and unanswered questions. *Neuropharmacology.* 2014; 76 Pt A:175-183.
96. Jiang W, Wang Y, Tang J, Peng J, Wang Y, Guo Q, Guo Z, Li P, Xiao B, Zhang J: Low-intensity pulsed ultrasound treatment improved the rate of autograft peripheral nerve regeneration in rat. *Sci Rep.* 2016; 6:22773.
97. Al Onazi MM, Yurick JL, Harris C, Nishimura K, Suderman K, Pituskin E, Chua N, McNeely ML. Therapeutic Ultrasound for Chemotherapy-Related Pain and Sensory Disturbance in the Hands and Feet in Patients With Colorectal Cancer: A Pilot Randomized Controlled Trial. *J Pain Symptom Manage.* 2021 Jun; 61(6):1127-1138.
98. Liao AH, Lin KH, Chuang HC, Tsai CH, Lin YC, Wang CH, Shih CP, Liu HL. Low-frequency dual-frequency ultrasound-mediated microbubble cavitation for transdermal minoxidil delivery and hair growth enhancement. *Sci Rep.* 2020 Mar 9; 10(1):4338.
99. Altaha R, Fojo T, Reed E.. Abraham Epothilones: a novel class of non-taxane microtubule-stabilizing agents. *J Curr Pharm Des.* 2002; 8(19):1707-1712.
100. Cao YN, Zheng LL, Wang D, Liang XX, Gao F, Zhou XL. Recent advances in microtubule-stabilizing agents. *Eur J Med Chem.* 2018; 143:806-828.
101. Hunt JT. Discovery of ixabepilone. *Mol Cancer Ther.* 2009; 8(2): 275-281.
102. Sofias AM, Dunne M, Storm G, Allen C. The battle of "nano" paclitaxel. *Adv Drug Deliv Rev.* 2017; 122:20-30.
103. Zhao Y, Mu X, Du G.. Microtubule-stabilizing agents: New drug discovery and cancer therapy. *Pharmacol Ther.* 2016; 162:134-143.
104. Kundranda MN, Niu J. Albumin-bound paclitaxel in solid tumors: clinical development and future directions. *Drug Des Devel Ther.* 2015; 9:3767-3777.