# The Real Deal: Using Cytochalasin B in Sonodynamic Therapy to Preferentially Damage Leukemia Cells

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**Abstract.** *Background/Aim: Sonodynamic therapy (SDT) is a form of ultrasound therapy in which chemotherapeutic agents known as sonosensitizers are administered to increase the efficacy of ultrasound's preferential damage to neoplastic cells. Perhaps one of the most intriguing capabilities of ultrasound is its ability to preferentially lyse cells based on size. Cytochalasin B is a cytokinesis inhibitor that preferentially enlarges and multinucleates malignant cells, making them much more sensitive to ultrasonic irradiation. Materials and Methods: The present study investigated the extent of preferential damage inflicted by cytochalasin B on U937 leukemia/human blood cell populations. Cell mixtures were treated with cytochalasin B and then sonicated under a relatively low intensity (3W/cm$^2$). Results: Cytochalasin B preferentially damages U937 cells both before and after sonication. This agent also reduces rapid proliferation as the clonogenicity of U937 cells was considerably reduced following treatment. Conclusion: Cytochalasin B may have profound therapeutic applications when combined with SDT.*

Sonodynamic therapy (SDT) is a promising novel treatment modality that has yielded impressive anticancer effects in both *in vitro* and *in vivo* studies. Experimental evidence has indicated that ultrasound preferentially damages malignant cells based on the size differential between such cells and those of normal histology (1-3). SDT is a form of ultrasound therapy in which chemotherapeutic agents known as sonosensitizers are administered to increase the efficacy of preferential damage to neoplastic cells by ultrasound. One of the most cited shortcomings of chemotherapy in clinical practice is drug resistance acquired by tumors. SDT has been shown to reverse this potent defense mechanism (1). Studies have also indicated that the mechanisms by which ultrasound destroys malignant tissue are amplified when appropriate sonosensitizers are administered. Such drugs often attack cells through multiple mechanisms, creating a potential synergistic effect when sonosensitizers of different classes are used in collaborative efforts (2, 3). Being able to develop treatment regimens in which the synergistic effects of different sonosensitizers are applied can have monumental importance in clinical applications. Such treatments could substantially amplify the capability of ultrasound to preferentially damage malignant cells in order to reduce the rate at which drug resistance occurs.

Perhaps one of the most intriguing capabilities of ultrasound is its ability to preferentially lyse cells based on size (4). This known fact invariably gives rise to the idea of grossly enlarging tumor cells to increase their already noticeable size difference from normal cells. Cytochalasin B is a known pharmacological agent that disrupts the actin cytoskeleton and inhibits cytokinesis by interfering with formation of the contractile ring, as well as the development of the cleavage furrow (1). Consequently, the cell does not divide and an immature actin cytoskeleton remains. However, the cell continues to form nuclei and eventually becomes grossly enlarged and multinucleated. Such cells invariably have more DNA targets, increasing the likelihood of apoptosis. Furthermore, these multinucleated cells have a large cell volume, making them more susceptible to direct cell destruction. Preferential damage of malignant cells is actually easily attainable as normal cells exposed to cytochalasin B exit the cell cycle and enter a resting state until sufficient actin levels are restored. Therefore, only malignant cells that have lost the ability to enter the rest phase will become grossly enlarged and multinucleated, providing an ideal target for ultrasonic irradiation.

Work from our laboratory has indicated that cytochalasin B does indeed only damage leukemia cells, leaving normal blood cells, unaffected. Promyleocytic leukemia U937 cells are a frequent choice for *in vitro* studies. U937 cells



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routinely become grossly enlarged and multinucleated, providing an ideal target based on size. The typical erythrocyte is 6-8 μm, while leukocytes are slightly larger at a range of 10-15 μm and an average of 12 μm. By contrast, work from our laboratory has shown that cytochalasin B-treated leukemia cells easily grow in excess of 20 μm, with some reaching 40 μm in diameter after adequate exposure (1). Such cells have reduced cytoskeletal integrity and are easy targets for ultrasonic irradiation. Furthermore, cytochalasin B-treated leukemia cells are substantially multinucleated as cytokinesis is inhibited.

To demonstrate the utility of SDT in clinical applications, the effects of cytochalasin B were examined in detail. It is hoped that this comprehensive experimental evidence will convince readers of the promise of SDT as a viable treatment modality for patients with leukemia. The experiments involved preferentially lysing U937 promyeleocytic leukemia cells in the presence of normal blood cells. Only cytochalasin B was used as a sonosensitizer in order to indicate how effective this pharmacological agent is with the use of ultrasound.

## Materials and Methods

*U937 Cell and normal blood cell preparation*. U937 human promyelocytic leukemia cells were placed at $5.2\times10^4$ viable cells/ml in 20% Fetal Bovine Serum (FBS) in Isocove's medium with the following: 2% by volume of 10,000 units/ml penicillin, 10 mg/ml streptomycin, 0.5% gentamicin sulfate and 2 mM glutamine. Human blood cells acquired from SUNY Upstate Medical University (Syracuse, NY) mixed with human hematopoietic stem cells (hHSCs) (10% concentration of hHSCs) from the same patient were cultured under equivalent conditions. To ensure that cytochalasin B would alter U937 cells in the predicted manner, U937 cells were first treated individually with a 1.5 μM concentration for 48 h, corresponding to two cell cycles. Cells were seeded at $1\times10^5$ cells/ml before being examined. Cells were subsequently Wright-Giemsa and DAPI (4',6-diamidino-2-phenylindole) stained to examine nuclear structure. DAPI is a fluorescent stain that binds strongly to A-T rich regions in DNA; Wright-Giesma is a histological stain that is used primarily to stain peripheral blood smears and bone marrow aspirates. It is commonly used to stain chromosomes to facilitate diagnosis of syndromes and diseases due to its ability to readily visualize cell nuclei (5).

It has been well-cited that leukemia cells have exceedingly high mitochondrial activity due to increased metabolic rates (6). Therefore, testing whether cytochalasin B further amplifies mitochondrial activity of malignant cells has tremendous utility as this would open the door for mitochondrial-based agents. Mitochondrial activity of U937 cells was assessed with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, a colorimetric assay that relies on NADPH-dependent cellular oxidoreductase enzymes found within the organelle (7). Under appropriate conditions, the enzymes reduce MTT to an insoluble product that has a purple color. The amount of mitochondrial activity is then readily-assessed based on the deepness of purple that each sample provides.

Once it was confirmed that cytochalasin B had the suspected effect on U937 cells (*i.e.* cells were grossly enlarged and multinucleated) more U937 cells were prepared under the same conditions to ensure healthy U937 cells would be introduced to the normal blood cell populations. Before each experiment, U937 cells were mixed at a 20% concentration with normal blood cells and incubated for 24 h prior to treatment, ensuring stabilization of the heterogeneous cell population. After U937 cells were mixed with healthy blood cells, 1.5 μM cytochalasin B was administered for 48 hours

*Sonication of cell populations*. The U937–normal blood cell mixtures were put into 2.4 cm diameter vials with Mylar bottoms for sonication. To be sure cytochalasin B was truly impacting the extent of preferential damage, controls of normal blood cells alone, U937 cells alone and U937–normal blood cell mixtures (no cytochalasin B) were prepared. The cells were seeded in 1.0 ml of 20% FBS medium with 1% Gibco® Fungizone (Life Technologies, Grand Island, NY, USA). Each vial contained 1,000 μl of cells. Cells were sonicated using a Fisher Scientific® Sonic Dismembrator (23.5 kHz, 6.0 cm diameter cup) system (Fisher Scientific International Inc., Hampton, NH, USA) along with a Bellco® Orbital Shaker (Fisher Scientific International Inc., Hampton, NH, USA). Mylar vials were placed in 7.0 cm deionized, distilled and degassed water and located 6.0 cm from the sonic horn before sonication. Cells were sonicated at a constant $3W/cm^2$ for 1-4min. Trypan Blue staining was used to identify non-viable cells after sonications were performed: 50 μl of cell suspension and 50 μl of 0.4% Trypan Blue stain in isotonic saline were mixed and transferred to a hemocytometer counter chamber after sonication experiments. A Z2 Beckman-Coulter® Particle Count and Size Analyzer (Beckman Coulter Inc., Brea, CA, USA) along with a Bio-Rad® TC10 Automated Cell Counter (Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used for size determination, as well as for identifying the number of enlarged, multinucleated cells post-sonication.

*Longitudinal effects of cytochalasin B*. In order to examine the longitudinal effects of cytochalasin B on U937 cells, clonogenicity was assessed for both untreated and treated cells. A healthy U937 cell population was divided into two groups with one receiving 1.5 μM cytochalasin B, and the other acting as a control. One and a half Corning® 384 well immunoassay plates (Sigma-Aldrich Corp., St. Louis, MO, USA) were used for each cell population. Each well was loaded with 0, 1, or 2 cells that ranged from 13-19 μm in diameter for untreated cells and 14-24 μm for treated cells (48 h after cytochalasin B administration). After loading, the cells were incubated for 12 days in 5% $CO_2$ at 37˚C. Individual wells were then assessed for the presence and number of clones.

## Results

*Effects of cytochalasin B on U937 cells*. Administering cytochalasin B to U937 cells resulted in profound alterations to cytological structure and physiology. Wright-Giesma staining revealed that malignant cells became remarkably multinucleated 48 h after 1.5 μM cytochalasin B was introduced to the cell population (Figure 1). When compared to normal U937 cells, it became readily apparent that cytochalasin B had a profound effect on cytoskeletal structure as is expected from a cytokinesis inhibitor. Aberrant cytoskeletons are often a hallmark of perturbed cellular integrity, suggesting that such cells would be highly sensitive

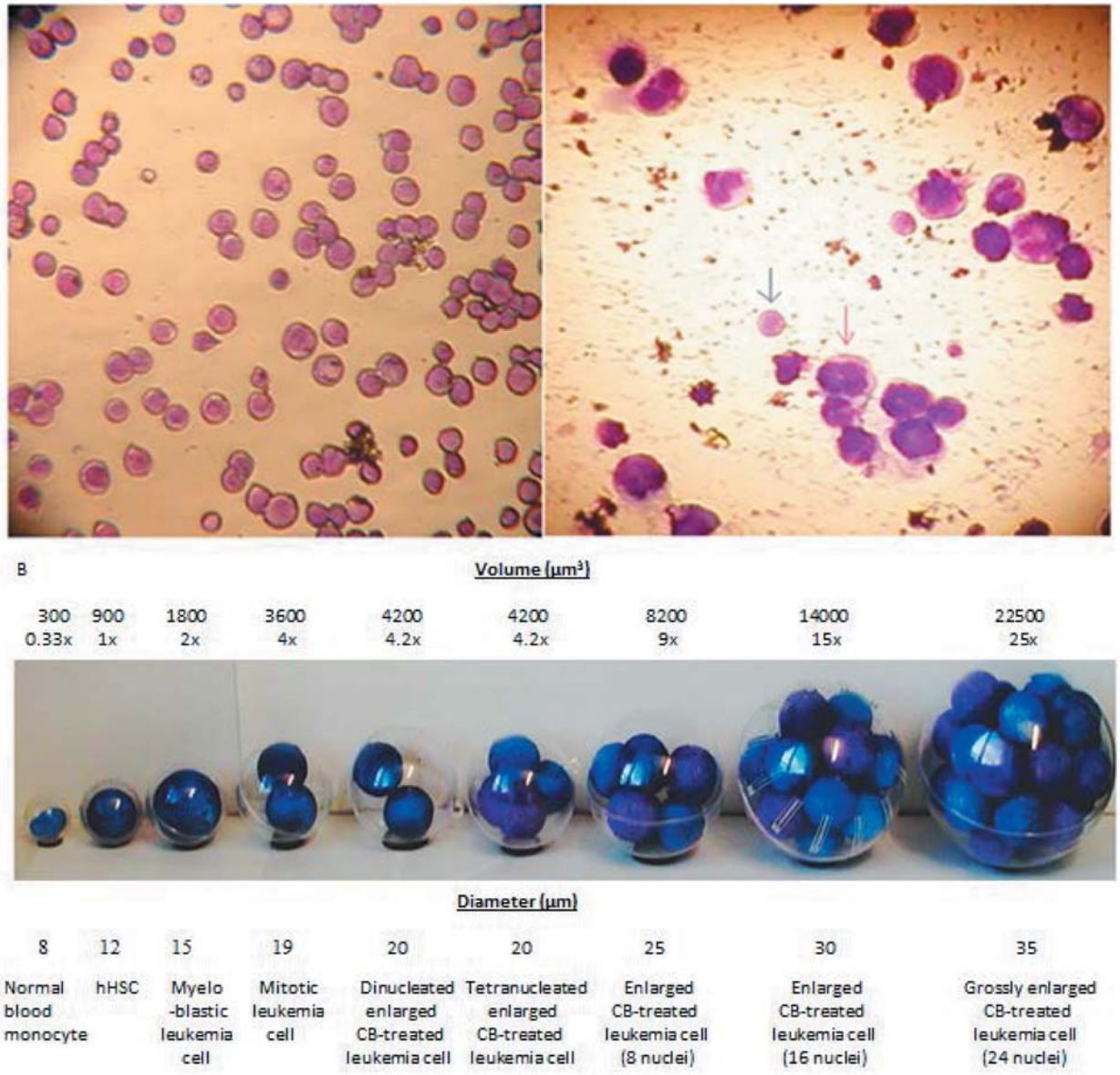


Figure 1. *Comparison of U937 cells after treatment with cytochalasin B. A: Typical U937 cells not exposed to any agent (13-18 μm in diameter) (left) and cells treated with cytochalasin B at 1.5 μM (right). The cells become grossly enlarged and multinucleated (19-40 μm in diameter) upon treatment. B: A model of the size differential between blood cells and leukemia cells treated with cytochalasin B. While normal leukemia cells are approximately 15 μm, leukemia cells treated with cytochalasin B can grow to 35 μm or larger. Such cells are substantially more sensitive to ultrasound than normal leukemia cells. The additional nuclei suggest that nucleic acid agents could be used with cytochalasin B to further increase the efficacy of ultrasound treatment in the clinical setting. Nuclei were visualized with Wright-Giesma stain at 100× magnification.*

to physical disruption (ultrasonic irradiation). The extent of multinucleation was further confirmed by DAPI staining as aggregates of DNA were readily detected (Figure 2). Results from flow cytometry and both cell counters revealed a profound shift in U937 cell size, indicated by the apparent increase in peak height. Both normal and cytochalasin B-treated U937 cells were then compared with normal blood cells for a further analysis of size differential. Subsequent results revealed a profound difference in average cell size (Figure 3). The extent of physiological disturbance induced by cytochalasin B was further demonstrated by MTT assays of normal and cytochalasin B-treated U937 cells (Figure 4). Cytochalasin B-treated cells had an approximate four-fold increase of absorbance at 590 nm, indicating enhanced

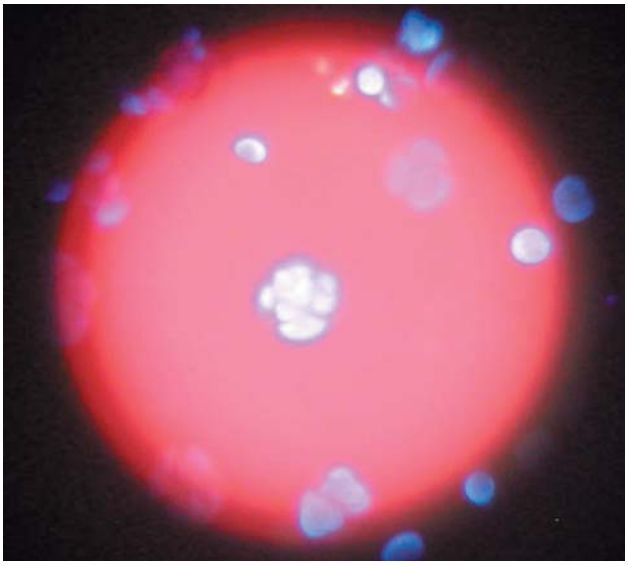

Figure 2. *DAPI staining of cytochalasin B-treated U937 cells 48 h after administration. DAPI was chosen for nuclear analysis as it passes through intact cell membranes. Therefore, it can be used to stain both live and fixed cells, necessary for visualizing the nuclei of cytochalasin B-treated cells as they do not readily undergo apoptosis in the absence of ultrasonic irradiation. DAPI staining confirmed the extent of multinucleation in treated cells.*

number or activity of mitochondria. The increased mitochondrial activity coincides with the four-fold higher DNA content on average which was previously indicated by Wright-Giemsa and DAPI staining.

*Effects of sonication on cytochalasin B-treated U937 cells.* While the effects of cytochalasin B on U937 cells were readily apparent, the malignant cells were still viable at 48 hours post-treatment. Therefore, a physical catalyst is needed to promote cell death (either by apoptosis or necrosis) of the U937 cells. Results from sonication at 3 W/cm$^2$ suggest that ultrasonic irradiation is a viable catalyst. The extent of preferential damage was readily detected post-sonication with Trypan Blue staining (Figure 5). While normal blood cells (including hHSCs) did have slight sensitivity to sonication at 3W/cm$^2$, it was minor compared to the profound sensitivity U937 cells exhibited. This sensitivity was dramatically amplified when cytochalasin B was administered, as evidenced by the significant difference in cell viability at 4 minutes.

*Longitudinal impact of cytochalasin B.* The effects of cytochalasin B on U937 cell clonogenicity were readily apparent. After the 12-day incubation period, cytochalasin B-

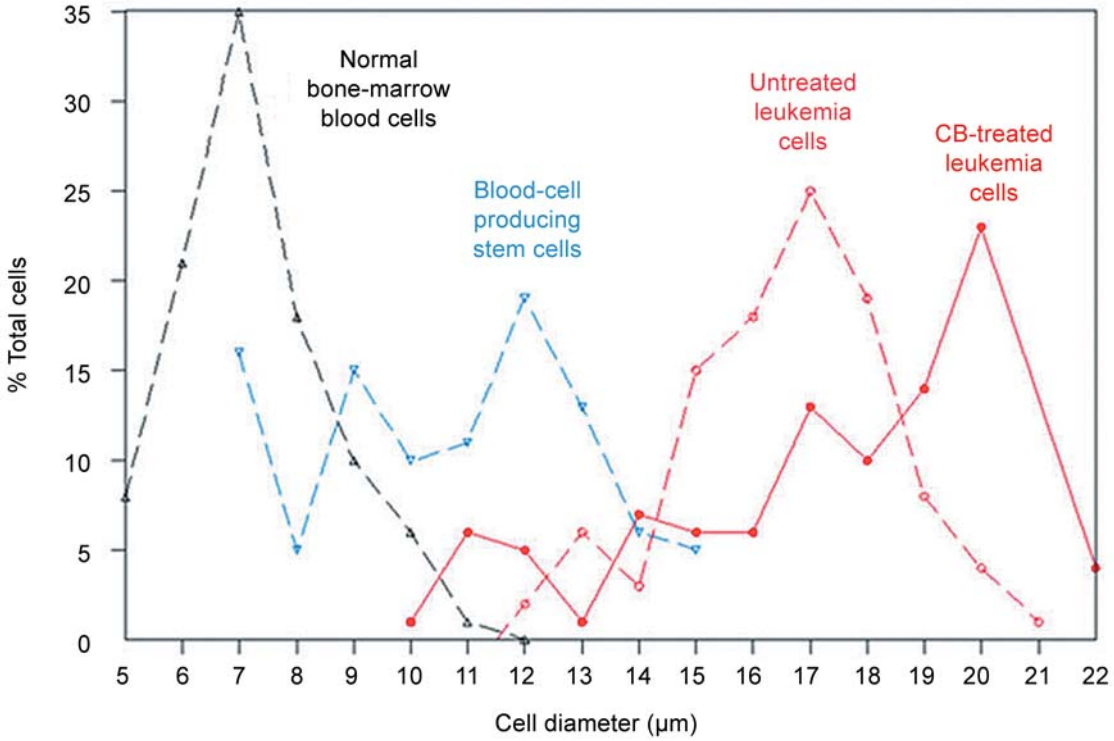


Figure 3. *Size distribution of blood cells. Flow cytometry and cell counters confirmed a significant shift in U937 cell size at 48 h post-cytochalasin B (CB) administration. Therefore, the already significant difference in size between leukemia cells and normal blood cells becomes exceedingly amplified. Note: Cytochalasin B-treated cells were still undergoing mitosis after 48 h, suggesting the size differential could be further increased if the cells were incubated further before sonication. Further incubation periods produced U937 cells in excess of 40 μm.*

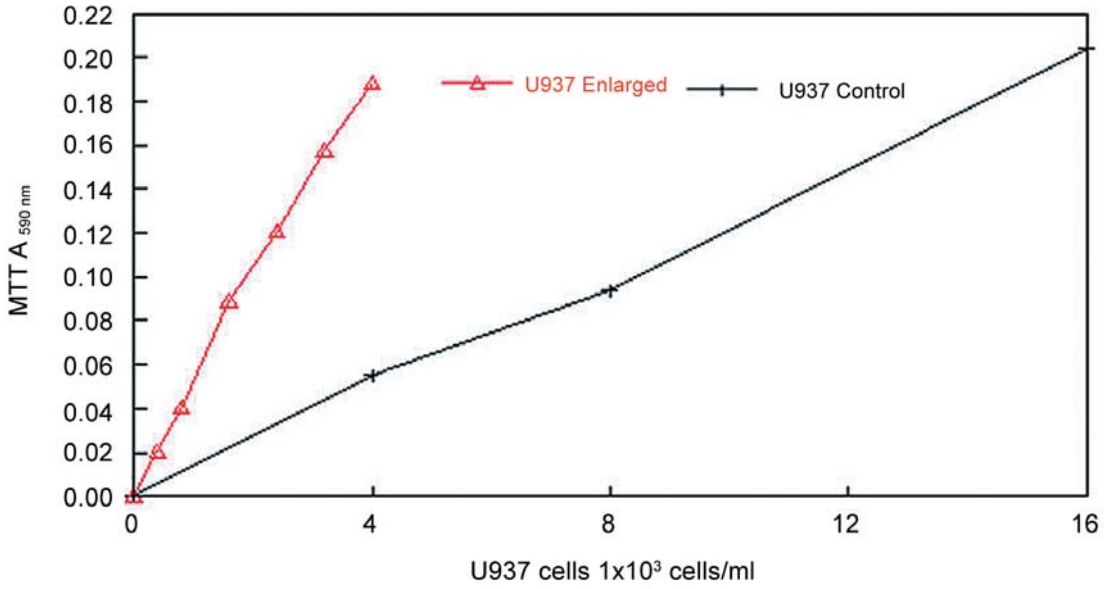


Figure 4. *MTT assay of control and cytochalasin B-treated U937 cells. U937 controls (no cytochalasin B administered) and enlarged cells were assessed for mitochondrial activity using the MTT assay. Cytochalasin B-treated cells had about a four-fold absorbance increase at 590 nm, indicating enhanced number or activity of mitochondria. The increased mitochondrial activity coincides with the four-fold DNA content on average. U937 cells were seeded at 1×10³ cells/ml for accurate MTT readings.*

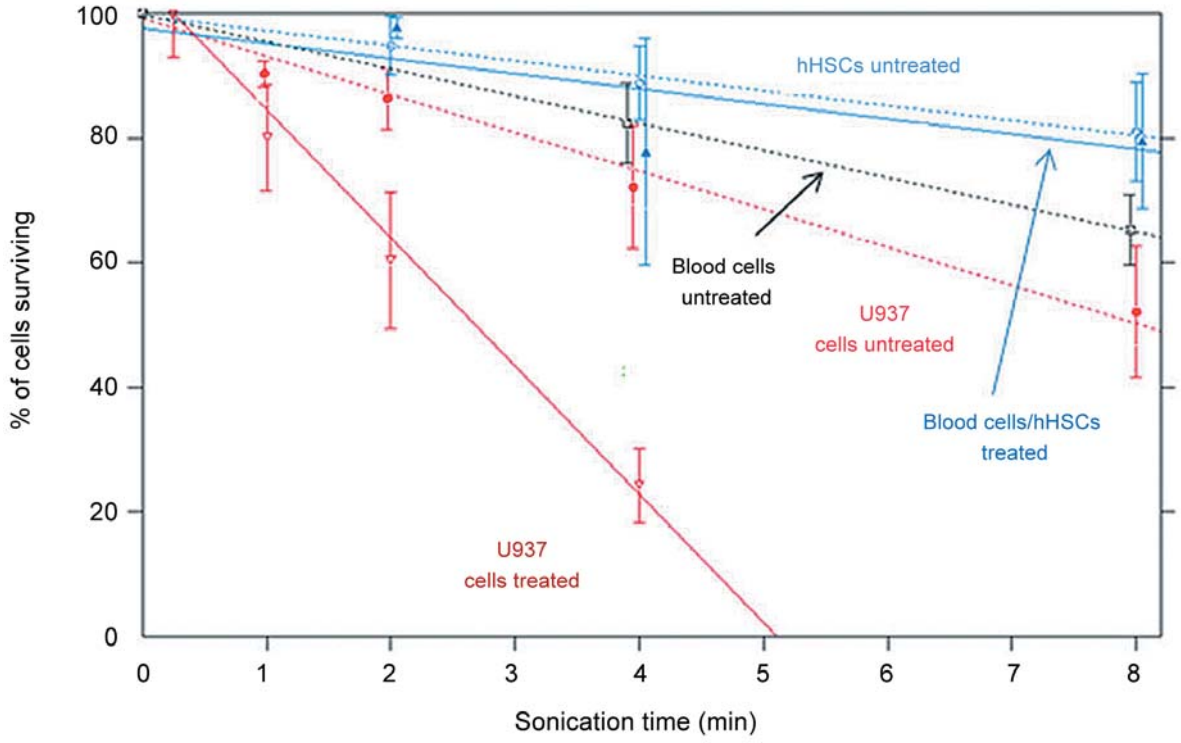


Figure 5. *Sonic sensitivity of cytochalasin B-treated U937 cells. Although normal blood cells are sensitive to 3 W/cm² of ultrasound, the effect was minor compared to that due to the profound sensitivity of U937 cells. It is important to note that cytochalasin B drastically increased the efficacy of sonication as most U937 cells were deemed non-viable by Trypan Blue staining after 4 min of sonication.*

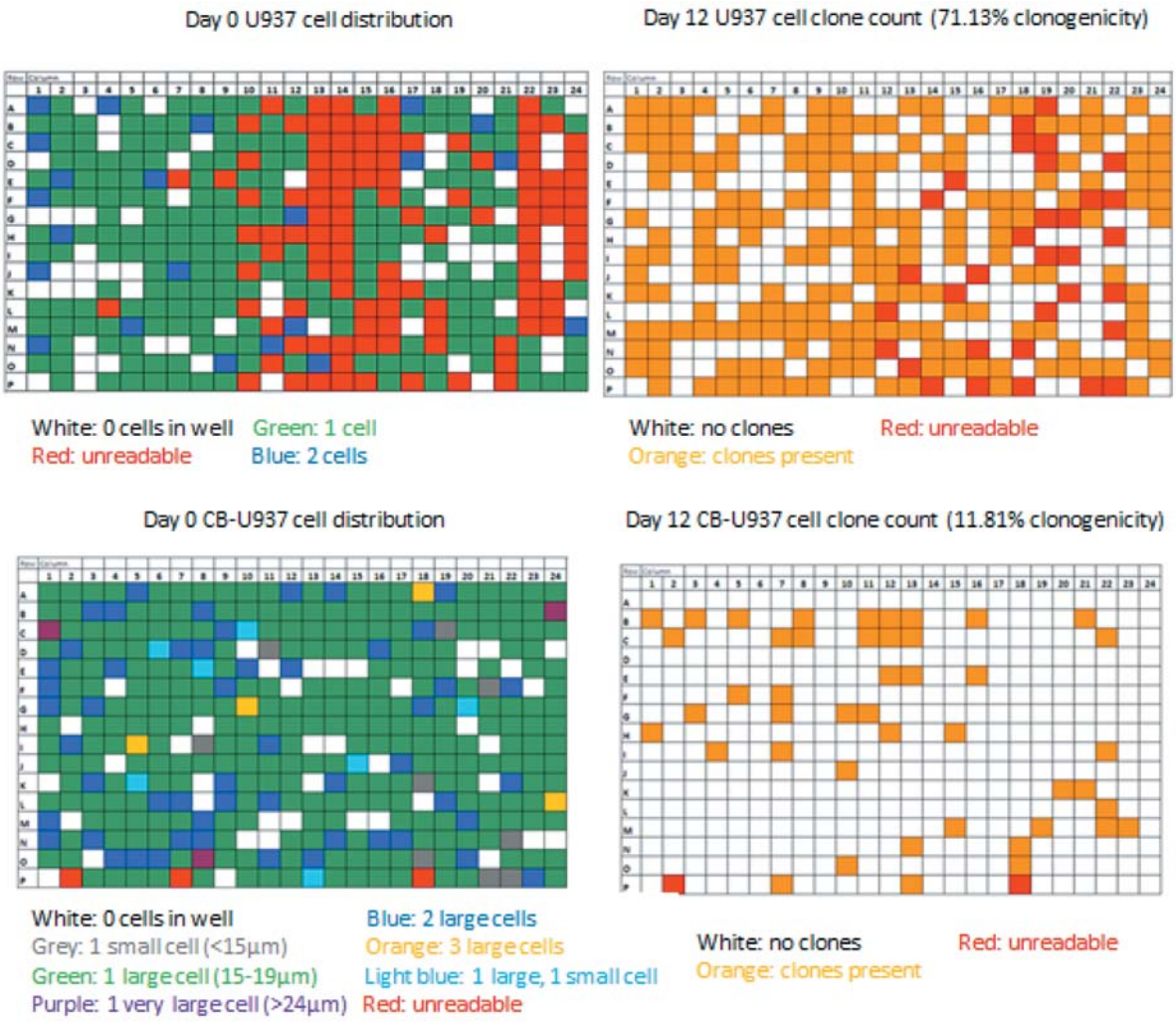


Figure 6. *The effects of cytochalasin B on U937 cell clonogenicity. Top row represents cells of the control, while the bottom row represents cells treated with cytochalasin B. U937 cells treated with cytochalasin B exhibited a markedly reduced ability to proliferate when compared to untreated cells after the 12-day incubation period.*

treated cells had a cloning efficiency of approximately 12% and exhibited the same hallmarks of increased size and multinucleation, with some being in excess of 40 μm. By contrast, the control, untreated U937 cells had a cloning efficiency of 71% (Figure 6). With such a low cloning efficiency, it is apparent that most cytochalasin B-treated U937 cells lose the ability to readily proliferate. It is important to note that the very large cytochalasin B-treated U937 cells (24 μm) had lost their capability to proliferate after the 12-day incubation period. This suggests that further along the multinucleation process (indicated by their increased size and nuclei, U937 cells continue to lose their proliferative capability. This could indicate that the most rapidly proliferating U937 cells ultimately lose their clonogenicity at the fastest rate.

## Discussion

Cytochalasin B appears to be a versatile chemotherapeutic agent that amplifies the damage ultrasonic irradiation preferentially inflicts on malignant cells. Exposed U937 cells consistently became grossly enlarged and multinucleated after being administered a relatively small dosage of 1.5 μM cytochalasin B. By contrast, normal blood cells exhibited no change in cell morphology and remained stable in size throughout the 48-h incubation period. When exposed U937 cells were assessed for mitochondrial activity using the MTT assay, the cells exhibited a fourfold increase in activity in comparison to U937 cells of typical histology. Such a dramatic increase in metabolic rate inherently suggests using mitochondrial

agents in tandem with cytochalasin B during ultrasound treatments. Indeed, Reactive Oxygen Species (ROS) agents often target the mitochondrial induced apoptotic pathway of leukemia cells, providing a viable method for developing synergistic treatments. This approach could be further supported by nucleic acid agents as cytochalasin B-treated U937 cells are considerably multinucleated. It is very likely that only a single nucleus will have to undergo apoptosis in order for the malignant cell to be destroyed; having so many nuclei present greatly increases the likelihood of this event.

By itself, a cytochalasin B, mitochondrial- and nucleic acid-directed drug cocktail appears to be a viable method for generating preferential damage to malignant cells in patients with leukemia. However, this combinatorial therapy appears to have the most promise when it is used to amplify the effects of ultrasonic irradiation. Cytochalasin B-treated U937 cells are remarkably sensitive to relatively low sound intensities (3 W/cm$^2$). Although U937 cells are much more sensitive to ultrasound than normal blood cells, the damage caused by ultrasound pales in comparison to the preferential damage inflicted on cytochalasin B-treated cells. This is why sonosensitizers are so important in SDT. Using chemotherapeutic agents make susceptible cancer cells much more sensitive to ultrasound, indicating that less sound intensities will be needed to generate substantial preferential damage. Ultrasound-only therapies would necessitate much higher intensities in order to inflict the same amount of damage to the malignant cell population. As observed with blood cells, normal cells are not immune to the effects of ultrasound. Increasing the sound intensity needed to lyse malignant cells would dramatically reduce the specificity of damage. Therefore, sonosensitizers hold the key to the efficacy of SDT, which is why more research should be invested in determining which drug combinations produce the greatest synergistic effects.

One of the most profound results of this study is the considerable loss of clonogenicity of cytochalasin B-treated U937 cells. A fundamental feature of any cancer is that it is capable of uncontrolled and often accelerated cell proliferation. This phenotypic effect is what allows such quantities of aberrant, de-differentiated cells to spread throughout the body and cause eventual death if not controlled. Cytochalasin B has the capability of mitigating this phenotype, as demonstrated by the dramatic reduction in U937 cell clonogenicity. It is likely that the first few SDT treatments will not destroy every leukemia cell found in a patient as some cells may persist in the bone marrow or spleen. However, if cytochalasin B can effectively remove the cell's ability to proliferate, it will be effectively neutralized. It is also very unlikely that an enlarged, multinucleated cell would be able to survive for extended periods of time due to its increased metabolic needs. Taking all of the evidence together, it appears that ultrasound administered with cytochalasin B is an effective method for generating preferential damage of leukemia cells in the presence of human blood cells.

While treatments with cytochalasin B-alone could yield substantial results for patients with leukemia when combined with ultrasound, the fact that affected cells become profoundly multinucleated, as well as grossly enlarged, provides the opportunity for synergistic effects with a nucleic acid agent. Although ultrasound has been shown to increase the efficacy of multiple nucleic acid agents, one agent of particular note is doxorubicin as it has been shown to attack malignant cells through a novel mechanism when applied in SDT, enabling the chemotherapeutic agent to damage doxorubicin-resistant cell lines. The human leukemia multidrug-resistant cell line K562/A02 has been shown to be damaged by ROS when doxorubicin is applied with ultrasound, a mechanism that is not typically seen for a DNA intercalating agent (8). Such effects were derived from a cell line shown to be resistant to both ultrasound and doxorubicin-alone control treatments, further substantiating the amplifying effect sonosensitizers have in SDT. There is a similar effect when ultrasound/ doxorubicin treatments are applied to U937 cells, suggesting the agent can be effective against multiple leukemia cell lines when used in tandem with ultrasonic irradiation (1, 9).

Cytochalasin B has also been shown to increase the metabolic activity of U937 cells, indicating that it could have a profound synergistic effect with mitochondrial agents. HMME (Hematoporphyrin Monomethyl Ether) has been used for multiple cancer cell lines and has shown commendable efficacy, particularly in a study that involved U937 cells (10). Immediately after administration, intracellular HMME concentrations rapidly increased within the U937 cells, reflecting its high affinity for malignant tissue. The synergistic effect of ultrasound with HMME showed significant cell destruction, indicating the necessity of sonosensitizers in ultrasound-mediated therapy. Flow cytometry with DCFH-DA (2'-7'-Dichlorodihydrofluorescein diacetate) staining confirmed that HMME-ultrasound treated cells had markedly increased ROS levels compared to control, HMME and ultrasound-alone groups. Further analysis of damaged cell populations revealed that oxidative stress was present and that cells had indeed undergone apoptosis. These results not only confirm the linkage between ROS and apoptosis within U937 cells, but ultimately suggest a novel approach to treating patients with leukemia. This damage can be further enhanced with the use of doxorubicin in collaboration with ultrasound/HMME, as a study with QBC939 leukemia cells has indicated (11). The study

demonstrated that the ultrasound/doxorubicin/ HMME group had a higher reduction in cell viability than both the ultrasound/doxorubicin and ultrasound/HMME groups, suggesting the need to investigate the cumulative effect of multiple sonosensitizers. Such results reflect a synergistic effect as doxorubicin has the ability to increase ROS content in malignant cells. Since HMME and doxorubicin can increase production of singlet oxygen when activated by ultrasound, the drugs act in tandem to create an environment that malignant cells find particularly cytotoxic due to their decreased levels of endogenous thiol buffers.

Combining ultrasound with cytochalasin B/nucleic acid/mitochondrial agent drug combinations may yield high efficacy rates when applied in the clinical setting as the sonosensitizers can preferentially damage malignant cells with remarkable precision. However, the impressive results of such treatments obtained in *in vitro* and *in vivo* studies might be grossly attenuated when actually applied in a clinical setting. The efficacy of SDT on real patients is simply unknown. Unless clinicians are willing to take a chance on this novel method, the required data necessary to accurately determine whether SDT is a viable approach will not be acquired. If clinical studies determine that ultrasound combined with cytochalasins/nucleic acid/mitochondrial agents is not effective, there are a tremendous variety of other sonosensitizers that are currently available for such rigorous testing. However, if initial trails are successful, further refinements could be made to determine conditions optimal for leukemia cell destruction. As with any novel treatment, the only way to determine actual efficacy is to give the therapeutic approach real-world experience.

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## References

1 Trendowski M: The promise of sonodynamic therapy. Cancer Metast Rev 2013 Dec 18. [Epub ahead of print].

2 Rosenthal I, Sostaric JZ and Riesz P: Sonodynamic therapy – a review of the synergistic effects of drugs and ultrasound. Ultrason Sonochem *11(6)*: 349-363, 2004.

3 Bai W, Shen E and Hu B: Induction of the apoptosis of cancer cell by sonodynamic therapy: A review. Chin J Cancer Res *24(4)*: 368-373, 2012.

4 Miller MW, Luque AE, Battaglia LF, Mazza S and Everbach EC: Biological and environmental factors affecting ultrasound-induced hemolysis *in vitro*: 1. HIV macrocytosis. Ultrasound Med Biol *29(1)*: 77-91, 2003.

5 Woronzoff-Dashkoff KK: The Wright-Giemsa stain. Secrets revealed. Clin Lab Med *221*: 15-23, 2002.

6 Kuroki M1, Hachimine K, Abe H, Shibaguchi H, Kuroki M, Maekawa S, Yanagisawa J, Kinugasa T, Tanaka T and Yamashita Y: Sonodynamic therapy of cancer using novel sonosensitizers. Anticancer Res *27(6A)*: 3673-3677, 2007.

7 Berridge MV, Herst PM and Tan AS: Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. Biotechnol Annu Rev *11*: 127-152, 2005.

8 Meng Q, Chen B, Wu W, Shao Z, Gao F and Zhao H: Enhanced antitumor effects of low-frequency ultrasound combined with adriamycin on human leukemia multidrug resistance cell line K562/A02. Chin J Cancer 27(11): 436-439, 2008.

9 Yoshida T, Kondo T, Ogawa R, Feril LB Jr., Zhao QL, Watanabe A and Tsukada K: Combination of doxorubicin and low-intensity ultrasound causes a synergistic enhancement in cell lilling and an additive enhancement in apoptosis induction in human lymphoma U937 cells. Cancer Chemother Pharmacol *614*: 559-567, 2008, 2008.

10 Su X, Wang P, Wang X, Cao B, Li L and Liu Q: Apoptosis of U937 cells induced by hematoporphyrin monomethyl ether-mediated sonodynamic action. Cancer Biother Radiopharm *28*: 207-217, 2013.

11 Liang L, Xie S, Jiang L, Jin H, Li S and Liu J: The combined effects of hematoporphyrin monomethyl Ether-SDT and doxorubicin on the proliferation of QBC939 cell lines. Ultrasound Med Biol *39(1)*: 146-160, 2013.

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