

Scurvy to stage IV cancer: the efficacy of intravenous vitamin C therapy in combination with cisplatin and paclitaxel treatment on non-small cell lung cancer strain A549

By Jasmine Goldsmith

Vitamin C has been recognized to be beneficial to many areas of human health, but the extent of its uses have yet to be fully explored, specifically in cancer research. Professional opinions on the effectiveness of vitamin C therapy for cancer patients vary across the board. The goal of this study is to work toward a deeper understanding of the effects of vitamin C therapy on the non-small cell lung cancer strain A549. Initially, nine different vitamin C concentrations ranging from 5 µg/mL to 960 µg/mL were tested. Vitamin C was then tested with two standardized drug treatments, cisplatin and paclitaxel, at concentrations ranging from 5 µM to 100 µM. Results indicate that vitamin C, whether combined with an additional treatment or not, actually increased lung cancer cell viability and proliferation. This study will ultimately assist in determining vitamin C's role in treatment plans for lung cancer patients.

Keywords: *vitamin C, cisplatin, paclitaxel, cell viability*

The challenge remains to find a dependable treatment for lung cancer. While lung cancer accounts for only 12.2% of new cancer cases, it accounts for 20.8% of all cancer deaths (National Cancer Institute, 2020). Despite many discoveries in the past couple of decades, the 5-year relative survival rate remains to be only 25.4% (National Cancer Institute, 2019). This is due to late-stage patient diagnosis, where the cancer has already spread and is difficult to treat. A treatment plan for lung cancer usually involves some combination of surgery, radiation, immunotherapy, and or chemotherapy (Mayo Clinic, 2022). One of the standard FDA-approved chemotherapeutic drugs used to treat lung cancer is cisplatin (Dasari, 2014). However, desperate patients are often willing to turn to other non-FDA-approved treatment options, such as vitamin C infusion therapy.

The benefits of vitamin C have been known for centuries; from curing scurvy to aiding in tissue repair and regulating the immune system (Purwaningsih, 2021). Unfortunately,

humans are unable to synthesize vitamin C on their own, so the only way to prevent a deficiency is through diet and or supplements; the recommended daily vitamin C intake is 90 mg for a male over nineteen and 75 mg for a female over nineteen (National Institute of Health, 2021). While the average human is fairly susceptible to a vitamin C deficiency, a specific link has been found between low vitamin C levels and cancer patients (Mussa, 2022). This suggests that vitamin C could be an even more crucial therapy to implement into cancer patient's treatment plans.

There are a few proposed mechanisms for how vitamin C interacts within the body and interferes with the proliferation of cancer cells. Vitamin C can function as an antioxidant or pro-oxidant agent, meaning it can either reduce or increase reactive oxygen species (ROS) levels within the cell. ROS, also known as free radicals, are formed when molecular oxygen interacts with escaped electrons (Mussa, 2022). The pathway that forms ROS begins when Fe³⁺ enters the cell through transferrin

receptor 1 where it is then reduced to Fe^{2+} and transported to the labile iron pool. Fe^{2+} can then interact with hydrogen peroxide and produce high levels of ROS. Vitamin C aids this process in its oxidized form Asc^- which reduces Fe^{2+} back into Fe^{3+} , helping recycle the process and generate increasing amounts of ROS. Extreme levels of ROS are toxic to cancer cells since they affect lipid, protein, and DNA function leading to an energy crisis and ultimately cell death. The reason for the selective death of cancer cells by vitamin C is due to the fact that they are more susceptible to oxidative stress as a result of their defective mitochondrias and increased metabolic reliance (Mussa, 2022). Overall, cancer cells struggle to deal and survive with increased ROS levels more than a normal cell.

The effectiveness of vitamin C for cancer patients is intensely debated due to the results of many studies being contradictory. While some studies claim that vitamin C shows extreme promise, others claim that it has no advantageous effects. For instance, a double-blind study was conducted that gave their patients either 10 g of vitamin C or a placebo pill. The results demonstrated no statistical difference between patients who received the treatment and those who did not (Mortel, 1985). On the other hand, a clinical study came to the opposite conclusion that vitamin C therapy increased the survival rate for terminally ill patients by 4.2 times (Pauling, 1976). Due to a wide range of trial outcomes, more research is needed to determine if vitamin C infusions could benefit cancer patients. Specifically, the optimal dosage, treatment time, administration method, and different combination therapies have yet to be determined.

This research project will combine methods and procedures from past pre-clinical studies and apply them to the lung cancer non-small cell lung cancer (NSCLC) strain A549. The goal is to demonstrate the effectiveness of vitamin C treatment. The proven success of

this therapy would help open a pathway to provide patients with an affordable combination treatment option with no detrimental side effects. Ultimately, if vitamin C proves to be a beneficial therapy, it could save the lives of thousands of cancer patients.

Methods

General

All trials were conducted in a biosafety cabinet. Safety precautions for FBS, F-12K medium, cisplatin, paclitaxel, A549, and CellTiter 96 AQueous One Solution Assay were followed from their respective safety data sheets from Sigma-Aldrich, LGC, Pfizer, Cayman Chemical, InvivoGen, and Promega respectively.

Culturing A549

The general cell culture and cell passage procedures from the ATCC were followed. The complete media consisted of F-12K Medium, 10% FBS, and 1% of PenStrep in a T75 flask.

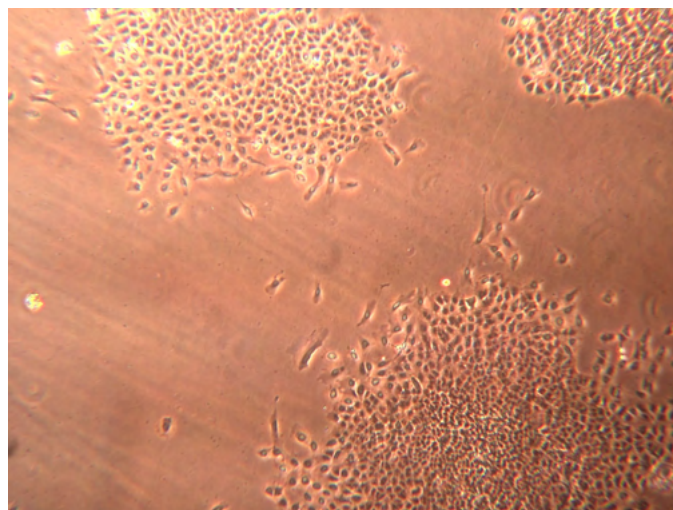


Figure 1. A549 cells adhered to the bottom of T75 flask

Control Trials

For every trial, two controls were employed: 50 μL of culture media with 50 μL of 0.9% sodium chloride and 50 μL of culture media and cells with 50 μL of 0.9% sodium chloride.

Each control trial was replicated in three wells in a 96-well plate. The well plates were incubated in a 37°C and 5% CO₂ environment. An MTS assay was then performed after 24-hours.

Vitamin C Trials

The concentrations of vitamin C that were tested were 5 µg/mL, 15 µg/mL, 25 µg/mL, 55 µg/mL, 110 µg/mL, 320 µg/mL, 640 µg/mL, 800 µg/mL, and 960 µg/mL. 0.9% sodium chloride solution was used to make the respective vitamin C concentrations above. 50 µL of each vitamin C concentration was added to 50 µL of culture medium and cells. Each vitamin C trial was replicated in three wells in a 96-well plate (Appendix Fig. 1A). The well plate was incubated in a 37°C and 5% CO₂ environment. An MTS assay was then performed after 24-hours.

Vitamin C and Cisplatin Trials

The concentrations of cisplatin tested were 5 µM, 10 µM, 50 µM, and 100 µM. 0.9% sodium chloride solution was used to make a 1 mg/mL stock and each following cisplatin concentration. For additional control trials, 25 µL of each drug solution was added to 50 µL of culture media and cells and 25 µL of 0.9% sodium chloride. Each cisplatin control trial was replicated in three wells in a 96-well plate. The four concentrations of cisplatin selected were used in conjunction with the nine vitamin C concentrations. One well consisted of 50 µL of culture media and cells, 25 µL of vitamin C solution, and 25 µL of cisplatin solution. Each vitamin C and cisplatin trial was replicated in three wells in a 96-well plate (Appendix Fig. 1B). The well plates were incubated in a 37°C and 5% CO₂ environment. An MTS assay was performed after 24-hours.

Vitamin C and Paclitaxel Trials

The concentrations of paclitaxel that were tested were 5 µM, 10 µM, 50 µM, and 100 µM. 0.9% sodium chloride solution was used to

make a 1 mg/mL stock and each following paclitaxel concentration. For additional control trials, 25 µL of each drug solution was added to 50 µL of culture media and cells and 25 µL of 0.9% sodium chloride. Each paclitaxel control trial was replicated in three wells in a 96-well plate. The four concentrations of paclitaxel selected were used in conjunction with the nine vitamin C concentrations. One well consisted of 50 µL of culture media and cells, 25 µL of vitamin C solution, and 25 µL of paclitaxel solution. Each vitamin C and paclitaxel trial was replicated in three wells in a 96-well plate (Appendix Fig. 1C). The well plates were incubated in a 37°C and 5% CO₂ environment. An MTS assay was then performed after 24-hours.

Combination Vitamin C and Drug Trials

The concentrations for cisplatin and paclitaxel were used from above. For additional control trials, one well consisted of 50 µL of culture media and cells, 25 µL of 0.9% sodium chloride, 12.5 µL of cisplatin solution, and 12.5 µL of paclitaxel solution. Each combination drug control trial was replicated in three wells in a 96-well plate. The four cisplatin and paclitaxel concentrations selected were used in conjunction with the nine vitamin C concentrations. One well consisted of 50 µL of culture media and cells, 25 µL of vitamin C solution, 12.5 µL of cisplatin solution, and 12.5 µL of paclitaxel solution. Each vitamin C, cisplatin, and paclitaxel trial was replicated in three wells in a 96-well plate (Appendix Fig. 1D). The well plates were incubated in a 37°C and 5% CO₂ environment. An MTS assay was then performed after 24-hours.

MTS Assay

After a 24-hour treatment exposure, 20 µL of CellTiter96Aqueous was added to each well already containing 100 µL total of culture medium, cells, and treatments. The well plate was incubated in a 37°C and 5% CO₂ environment for one hour. The well plate was

transferred to a microplate reader and the absorbance was recorded with the primary filter set at 490 nm and the differential filter set at 630 nm (Promega, 2012). The cell viability was measured and results were analyzed.

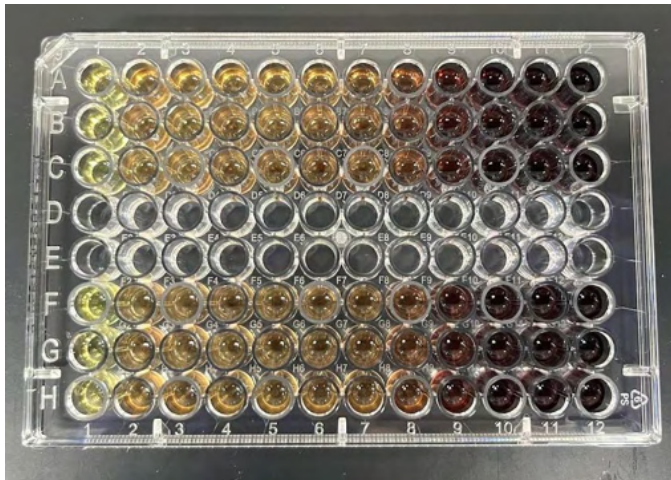


Figure 2. Vertical view of 96-well plate immediately after the addition of MTS assay to wells containing cells, media, and or exposure treatments

Results

An increase in vitamin C concentrations led to an increase in absorbance levels. Higher absorbance values correspond to less light from the spectrophotometer being able to pass through a well. Therefore, increased absorbance levels correlate to increased cell viability. Effects of vitamin C on cell viability and proliferation appear to flatten out at around 800 µg/mL.

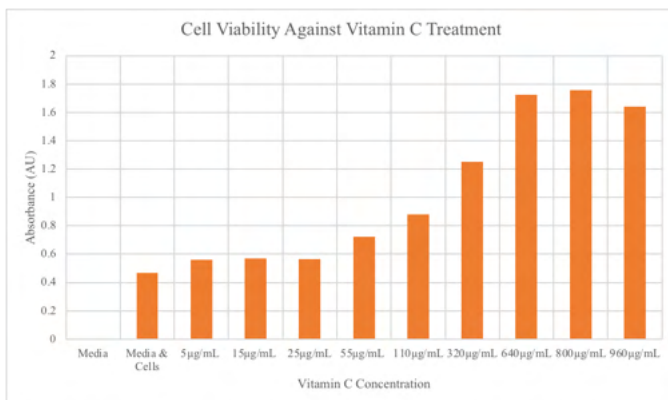


Figure 3. Comparison of increasing vitamin C

concentrations to absorbance level as a product of spectrophotometry

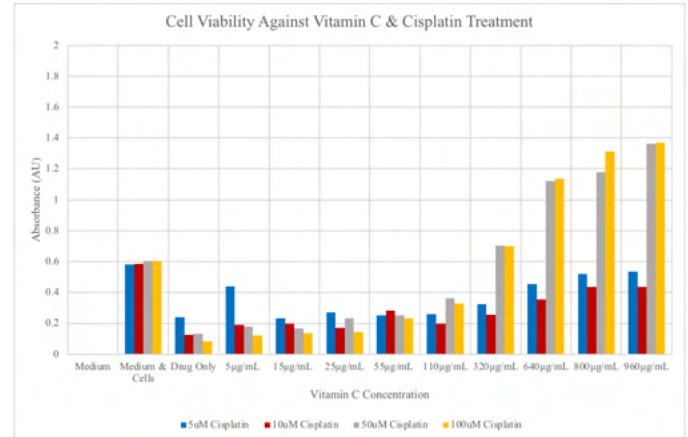


Figure 4. Comparison of increasing vitamin C concentrations to absorbance level at four different cisplatin concentrations as a product of spectrophotometry

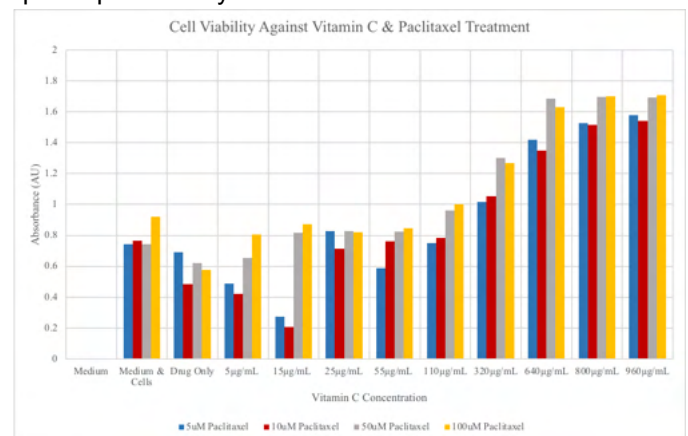


Figure 5. Comparison of increasing vitamin C concentrations to absorbance level at four different paclitaxel concentrations as a product of spectrophotometry

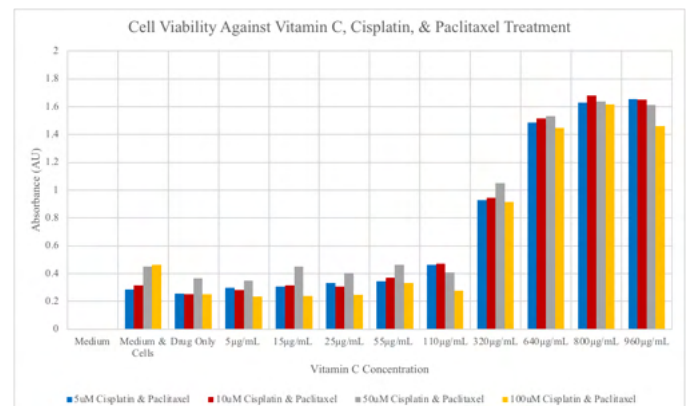


Figure 6. Comparison of increasing vitamin C

concentrations to absorbance level at four different cisplatin and paclitaxel concentrations as a product of spectrophotometry

Discussion

A multiple regression statistical analysis was performed and a R-squared value of 0.73 was calculated. Therefore, roughly 73% of the variability in absorbance can be explained by the entire set of independent variables (vitamin C, cisplatin, and paclitaxel). A significance F value of 0 was also calculated. As a result, the null hypothesis is rejected at the significance level of 0.01. Therefore, there is 99% confidence that an increase in vitamin C concentrations leads to a significant increase in absorbance levels. Furthermore, the P-values for each independent variable of vitamin C, cisplatin, and paclitaxel were calculated to be 5.45E-35, 4.42E-06, and 5.96E-08 respectively.

Based on the data and statistical analysis, vitamin C is not a beneficial therapy for lung cancer as either an individual or combination therapy. The trend of increased cell viability was seen in all exposure trails with different drugs at different concentrations. Therefore, patients with lung cancer would be ill advised to add vitamin C therapy to their treatment plan.

Methods Improvement

The first recommendation would be to use an automatic micropipette during the plating of the 96- well plates. In this project, a micropipette was used to transfer all solutions into each individual well. Instead, using an automatic micropipette could reduce potential variability in the volume of each well. The second recommendation would be to perform dilutions from the original cell culture. In this project, a hemocytometer was used to acquire a cell count for recording purposes prior to the transfer of cells from a T75 flask to the 96-well plate. Cell counts were generally similar, but not exact. Performing a cell dilution would ensure consistency in the number of cells per milliliter and reduce potential variability in

results between trials run on different days at different passage points.

Future Research

More research could be conducted in order to test the efficacy of vitamin C therapy on other cancer strains. While vitamin C proved to be beneficial for cell viability in cell strain A549, the results in this project provide no inference for its potential effectiveness in other cancers. Additionally, it is possible that at some vitamin C concentration higher than 960 $\mu\text{g/mL}$, recorded absorbance begins to decrease, demonstrating the desired detrimental effect. Therefore, a wider range of vitamin C concentrations should be tested in order to completely rule out the possibility of this therapy option for patients.

References

- A549 - CCL-185 | ATCC. ATCC. (n.d.). <https://www.atcc.org/products/ccl-185>
- Cancer of the lung and bronchus - cancer stat facts. SEER. (n.d.). <https://seer.cancer.gov/statfacts/html/lungb.html>
- Creagan ET;Moertel CG;O'Fallon JR;Schutt AJ;O'Connell MJ;Rubin J;Frytak S; (1979a, September 27). Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. A controlled trial. The New England journal of medicine. <https://pubmed.ncbi.nlm.nih.gov/384241/>
- Dasari, S., & Tchounwou, P. B. (2014, October 5). Cisplatin in cancer therapy: Molecular mechanisms of action. European journal of pharmacology. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4146684/>
- L.;, C. E. (1976, October). Supplemental ascorbate in the supportive treatment of cancer: Prolongation of survival times in terminal human cancer. Proceedings of the National Academy of Sciences of the United States of America. <https://pubmed.ncbi.nlm.nih.gov/1068480>
- Mayo Foundation for Medical Education and Research. (2022, March 22). Lung cancer. Mayo Clinic. <https://www.mayoclinic.org/diseases-conditions/lung-cancer/diagnosis-treatment/drc-20374627>
- Home. CellTiter 96® AQueous One Solution Cell Proliferation Assay System Protocol. (n.d.). <https://www.promega.com/resources/protocols/technical-bulletins/0/celltiter-96-aqueous-one-solution-cell>



- proliferation-assay-system-protocol/
Mussa, A., Mohd Idris, R. A., Ahmed, N., Ahmad, S., Murtadha, A. H., Tengku Din, T. A. D. A. A., Yean, C. Y., Wan Abdul Rahman, W. F., Mat Lazim, N., Uskoković, V., Hajissa, K., Mokhtar, N. F., Mohamud, R., & Hassan, R. (2022, June 3). High-dose vitamin C for cancer therapy. Pharmaceuticals (Basel, Switzerland). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9231292/>
- Purwaningsih, E., Mustofa, S., & Pendrianto. (2021, September 6). Cytotoxicity effects of vitamin C on cancer cells MCF-7. Nusantara Science and Technology Proceedings. <https://nstproceeding.com/index.php/nusciencetech/article/view/446>
- U.S. Department of Health and Human Services. (n.d.). Office of dietary supplements - vitamin C. NIH Office of Dietary Supplements. <https://ods.od.nih.gov/factsheets/VitaminC-HealthProfessional/>

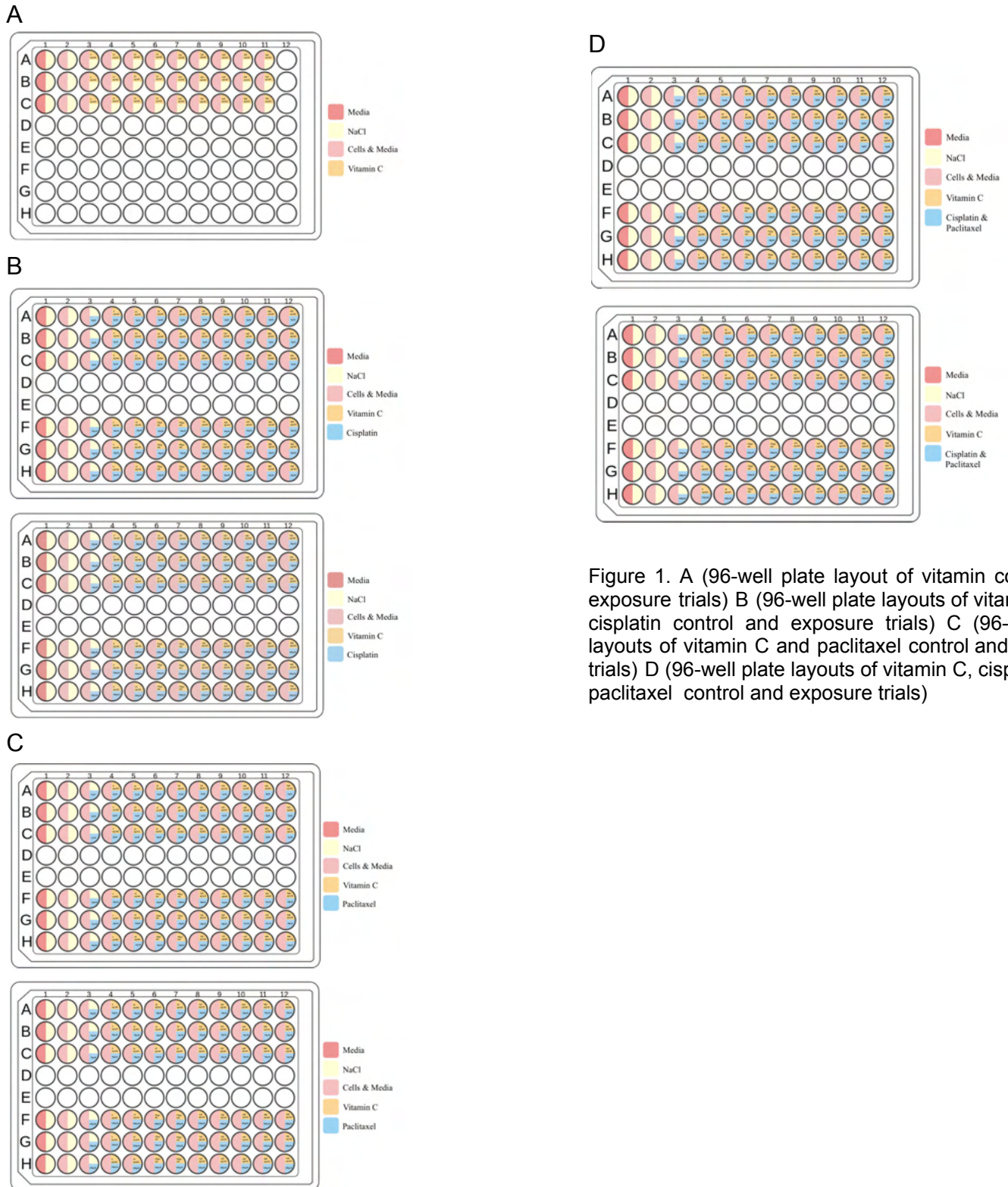


Figure 1. A (96-well plate layout of vitamin control and exposure trials) B (96-well plate layouts of vitamin C and cisplatin control and exposure trials) C (96-well plate layouts of vitamin C and paclitaxel control and exposure trials) D (96-well plate layouts of vitamin C, cisplatin, and paclitaxel control and exposure trials)