Pew Research Proposal
Union University 2021-2022

Lunawati Bennett MS; PhD; PharmD; FACN

1. Title of Project: "Effects of Vitamin C in Triple Negative Breast Cancer (TNBC) Cells"

2. Statement of the End Product: The results from this study will be written as manuscript(s) and it will be sent for publication to journal(s) that relate to cancer or cancer pharmacology. It will also be presented as a poster or oral presentation at the American Society for Pharmacology and Experimental Therapeutics (ASPET) Annual Meeting or other conferences that are pertinent to cancer research.

3. Explanation of the Scholarly Activity

I. ***Statement of the Scholarly Activity***

Cancer is currently the second leading cause of death in the United States with estimation of more than 1.6 million new cases being diagnosed. According to the World Health Organization (WHO), cancer is the leading cause of death worldwide accounting for 15% of all death (8.2 million deaths). The most common cancers in men are lung, prostate, colorectal, stomach and liver; while among women are colorectal, lung, cervix, stomach, and breast [WHO Report]. An estimation of 276,480 new cases of invasive breast cancer and 48,530 new cases of non-invasive will be diagnosed in women in 2020 in the U.S. An estimation of 42,170 women will die due to breast cancer in the U.S [Breast Cancer Fact, 2020]. Worldwide, breast cancer effect 2.1 million women each year with death estimated at 627,000 women [WHO Report 2018]. Although rare, men get breast cancer as well. About 2620 men will be diagnosed with breast cancer and approximately 520 will die [Breast Cancer Fact, 2020].

There are many subtypes of breast cancer. One of these subtypes is known as triple-negative breast cancer (TNBC). TNBC doesn't have the receptors for the hormone estrogen, or progesterone, or doesn't express the protein HER2/neu; therefore, TNBC doesn't respond to hormonal nor targeted therapies. According to John's Hopkins Breast Center, about 20% of breast cancer diagnosis is TNBC [Silberman and Ranchod 2020; Dent *et al* 2007]. There are several characteristics that make TNBC difficult to treat. Firstly, TNBC often grows rapidly and undergoes metastasis, the process whereby cancer cells spread from their primary site to distant parts of the body. Metastasis is considered the most life-threatening aspect of cancer, responsible for approximately 90% of cancer-related death in humans [Spano *et al* 2012]. Secondly, TNBC can easily become resistant through activation of mutated pathways which make TNBC difficult to treat; therefore, oncologists often use high dosage of anti-cancer agents with multiple drug classes to prevent and treat metastasis and resistance. Thirdly, TNBC has a high recurrence rate of relapse within the first 3 years

of therapy. The 5-year survival rate is also lower (65%) for TNBC as compared to 86% for all breast cancer [Silberman and Ranchod 2020].

Because TNBC doesn't respond to hormonal or targeted therapies, treatment options become limited to using traditional anti-cancer drugs which also cause unfavorable adverse effects such as: nausea, vomiting, toxicity to bone marrow, cardiac arrest, renal failure, and infertility [Theodossiou *et al* 2019]. Therefore, several attempts have been made to find different compounds in order to alleviate this problem.

Vitamin C (ascorbic acid or ascorbate) has been known for the treatment and prevention of scurvy and common cold since 1928 [Padayatty and Levine, 2016]. In the 1970s, several studies have shown the benefit of high dose vitamin C in advanced cancer survival [Cameron and Pauling, 1976]. Due to the controversial results between Linus Pauling, the Nobel Prize recipient of chemistry, and the Mayo clinic at that time, vitamin C notation as a promising anti-cancer agent has been dismissed. In recent times, new knowledge has re-merged to investigate the mechanism of action of vitamin C as a possible anti-cancer agent [Shenoy *et al*, 2018].

II. Theoretical Framework

Because of the impact of cancer on society, efforts to prevent or to treat cancer are an ongoing research interest. In particular, recent knowledge of intravenous vitamin C pharmacokinetics and the discovery of an unexpected mechanism of vitamin C action has spawned my research interest. Although my research proposal is not using intravenous administration in animals, I am using MDA-MB-231, a cancer cell line that has characteristics of TNBC. By adding high doses of vitamin C to this line, I can mimic the pharmacologic effects of vitamin C of TNBC cells. Therefore, I can add value to research in cancer in the near future.

I attempt to study: a) the link between oxidative stress and the process of carcinogenesis (cancer formation) and cancer progression, b) the correlation between anti-cancer and anti-oxidant properties of vitamin C in relation to impairing invasion, migration, and metastasis of cancer cells, and c) understand the proteins and genes involve in the progression TNBC. I have published a few manuscripts and a book chapter regarding natural dietary compounds found in fruits, vegetables, medicinal herbs and some over the counter supplementations such as vitamin E, selenium, and vitamin C [Zhou *et al* 2016; Bennett *et al* 2012].

Bibliography

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9. Shenoy N, Creagan E, Witzig T *et al*. Ascorbic Acid in Cancer Treatment: Let the Phoenix Fly. *Cancer Cell* 2018; 34(5): 700-706.

10. Zhou Q, Bennett LL**,** Zhou S. Multifaceted Ability of Naturally Occurring Polyphenols Against Metastatic Cancer. *Clinical and Experimental Pharmacology and Physiology*. 2016; Doi:10.1111/1440-1681.12546.

11. Bennett LL, Rojas S, Seefeldt, M. 2012. Role of Antioxidants in the Prevention of Cancer. *Journal of Experimental and Clinical Medicine* 2012; 4: 215-222.

12. Bennett LL**,** Howell P. Vitamin E: Daily Requirements, Dietary Sources, Symptoms of Deficiency, and Recent Use in Clinical Studies. In: Nutrition and Diet Research Progress-Vitamin A and Vitamin E. Nova Science Publisher, Hauppauge, New York, 2013. p. 1-34.

III. Description of the activity and its goals

The overarching goal is to understand the anti-cancer effects of vitamin C on TNBC, using a cell line such as MDA-MB-231 a good model for TNBC. Specific goals of my research are:

1. To identify the concentration of vitamin C that kills 50% and 80% of the cancer cells using a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphneyltetrazolium bromide (MTT) cell viability assay.
2. To study the mechanism of action and genes involved in the pathways of cancer cell death using Western Blot techniques, in particular, genes that are involved in apoptosis (programmed cell death) such as caspase 3, 6, 7, 8 and 9. Other genes involved in cell cycle regulation, DNA damage and tumor suppression will be investigated as well.
3. To understand the mechanism of changes in morphology, DNA and mitochondrial membrane potential of cancer cells using DNA fragmentation assay and fluorescence intensification method.

IV. Scholarly Literature within Discipline

I have published a few review papers in peer reviewed journals regarding cancers (see below). I included literature to support my theoretical framework.

1. Zhou Q, Bennett LL**,** Zhou S. Multifaceted Ability of Naturally Occurring Polyphenols Against Metastatic Cancer. *Clinical and Experimental Pharmacology and Physiology*. 2016; Doi:10.1111/1440-1681.12546.

2. Bennett LL, Rojas S, Seefeldt, M. 2012. Role of Antioxidants in the Prevention of Cancer. *Journal of Experimental and Clinical Medicine* 2012; 4: 215-222.

3. Bennett LL**,** Howell P. Vitamin E: Daily Requirements, Dietary Sources, Symptoms of Deficiency, and Recent Use in Clinical Studies. In: Nutrition and Diet Research Progress-Vitamin A and Vitamin E. Nova Science Publisher, Hauppauge, New York, 2013. p. 1-34.

4. Integration of Faith and Science/Academic Discipline

I believe scholarly activity is an important part of being in academia. Scholarly activity is crucial to reflect the academic development, knowledge in the field, and contribution to the scientific community and to general public. Research and scholarly activities demand concentrated effort and tasks including extensive reading, data evaluation, collaboration with others, and hard laboratory work that require determination and perseverance. I believe we should try to achieve the highest quality research and excel in our research and endeavor in accordance with our school mission: Christ-Centered, People-Focused, Future-Directed, and Excellence-Driven.

I tried to push myself to the best of my abilities, time, and resources to do research that brings glory and honor to God. Over the past years, my research has focused on understanding the role of antioxidants on cancers and to identify natural antioxidants useful in treating this dreadful disease. As a Christian scientist, I believe I must use God's given ability, knowledge and time to be used by Him for His ultimate glory and for the betterment of humanity and service to Him and to others. I strongly believe as a healthcare provider, I can help to decrease human suffering by reducing the incidence or progression of disease such as cancer. Jesus Christ was a perfect example not only he saved us from eternal condemnation, but also he healed all sorts of illness and diseases ranging from blindness, leprosy, paralysis, to even raising the dead. The relief of being cured and healthy again is something that all of us have experienced at some point in life whether it be a serious illness or not so serious one.

The disease that I continue investigating is cancer that affects men and women equally irrespective of age, status, family background, or race. This deadly and complicated disease requires extensive research endeavors from different people in the field ranging from biomedical, biochemistry, biology, medicinal chemistry, pharmacy, medical, and pharmacology experts, to name a few. Although much research has

been done and continues to progress in the field of breast cancer, there is much research that needs to be done regarding TNBC due to the severity of the disease and the limitation of compounds or drugs available to treat or cure this advanced breast cancer. The new interest in exploring vitamin C as an option for the treatment of TNBC is very crucial. I have done preliminary research regarding the role of vitamin C in different cancer cells lines. I hope by God's grace and direction through His Holy Spirit, I can contribute to find some "cure" for cancer.

As I progress in my career, I have grown to believe that without research, my job in academia is not complete. I want to contribute to Union University with my research and writing contributions to publish in reputable journals and make the university be known as an institution of excellence with high caliber researchers. I thank God for Union University in providing research fund, so I can do research along with faculties from pharmacy and other department. Even though research is challenging and frustrating sometimes, I pray that God gives me strength and motivation to do the best for His utmost glory.

5. Time Frame for Completion and dissemination of the Project

Budget

Drug, Chemicals, …….....	$ 2000.00
Supplies, Pipet, microplate, etc……	$ 500.00
Western Blot supplies……	$ 1500.00
Attending a conference……	$ 500.00
Total	$ 4500.00

Project TimeLine

Determine vitamin C concentration that kill cells (MTT assay) ……..…….. February 2021

Determine protein of interest (Western Blot technique) …….. …….. August 2021

Presentation Results Pew Luncheon ……..... April 2022

Poster or Oral presentation at ASPET meeting .. ……... April 2022

Writing manuscript …….. May 2022

Letters of Recommendation

Forthcoming by email from:

1. Dr. Blake Watkins, Chair, Department of Pharmaceutical Sciences, Union University College Of Pharmacy, Jackson, TN
2. Dr. Qingyu Zhou, College of Pharmacy, University of South Florida, Tampa, Florida