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Caring for Cancer

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SENIOR THESIS APPROVAL

This Honors thesis entitled

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written by

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requirements for completion of the Carl
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meets the criteria for acceptance
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Introduction

My life has been full of challenging and motivating situations that have contributed greatly to my desire to attain a medical doctorate. The initial spark of inspiration can be attributed to my mother. She has worked in the medical field for over twenty years, and I have watched her diligently and with admiration. She has had a profound influence on my dreams, and I have, for as long as I can remember, expressed to her my desire to become a doctor.

In my adolescence, my late grandmother, who had been struggling with multiple sclerosis for many years, began a descent in her quality of life. I stayed with her most evenings during high school, by which point she required 24-hour care. While my grandfather worked, I would help her complete daily tasks, such as moving from the couch to her wheelchair, eating, and preparing for bed. My grandmother fought her disease to the best of her ability, and through the care of knowledgeable specialists, was able to be in my life until April 2012. This situation, although difficult, taught me how much of a blessing physicians are in the lives of their patients, and it inspired me to work hard for myself and for those I could potentially help by becoming a doctor.

My own health struggles have also been a source of motivation. First, I was diagnosed with Type 1 diabetes in February of 2005. I later developed stomach and heart problems. In dealing with these complications, I became acquainted with countless physicians who, through their care, taught me what a great service doctors can provide their patients. I have greatly admired all of my doctors, and they have encouraged and inspired me to pursue my dream of becoming a physician.

Perhaps nothing has had as strong an influence on my goals as working on research under Dr. Lori Hensley. Before I began my work in the laboratory, I studied and learned more about my mentor's research into Ewing's sarcoma, a detrimental pediatric bone cancer that is associated with a very low five-year survival rate. I was moved by the possible breakthroughs that could result from my assistance, and what this research meant for the lives of people affected by this cancer. Through this experience, I also gained a greater appreciation for research. When I become a physician, I should be well versed in research into new treatments for my patients, especially those that offer a more promising quality of life. I hope I can use my knowledge of current research and literature to better care for my patients.

Physician's Care

"Your child has cancer" are words that no parent wants to hear, and no physician wants to speak. However, approximately 1 in 10,000 children each year will receive this unfortunate news (Society). Another unsettling statistic is that a number of families will be hearing these unsettling words in a less than appropriate fashion, such as brief phone calls, voicemails, and unsympathetic consultations. A study performed by the National Cancer Institute surveyed patients to determine how they learned of their diagnosis. While the situations differed, 15% of the patients lost trust in their physicians as a result of their conversations (J. Leonard Lichtenfeld). This high percentage of general dissatisfaction and miscommunication is disheartening. According to Dr. J. Leonard Lichtenfeld, "If we physicians are not able to tell people life-threatening news with some shred of sensitivity and dignity, then maybe some of us are at risk of losing our human touch. That loss of humanity goes to the heart of what it means

to be a doctor. And if we lose that, my friends, it would be tragic." As a physician, empathy and sensitivity are key in all aspects of care, including the delivery of a diagnosis.

A physician's ultimate job is to care for their patients, and this care should include their families and extend past death. Also, this care should include knowledge of research and investigation into new and improved treatments. While knowledge is a highly important aspect of treating a patient, empathy and understanding should be just as present in a physician. Palliative care is an excellent theory that holistically intertwines sympathy and understanding into the care of a patient.

Palliative care aims to both alleviate the patient's symptoms and provide comfort and care when treatments directed at a cure are no longer an option (Muckaden, Dighe and Balaji). Palliative care is extremely beneficial in most situations, but especially in conditions where the treatments can fail, death is inevitable, the disease is progressive, or the disease is irreversible. In addition, palliative care also takes into consideration ethical issues surrounding care, including an assessment of "benefits vs. burdens." According to Muckaden, et. al, any treatment for a patient should be carried out only when the benefits proportionally outweigh the burdens.

The core philosophy of palliative care is that care is total. This total care includes caring for physical, psychological, social, and spiritual pain. This care is not only for the patient, but is also extended to the child's family. This support of the family further enhances the care delivered to the patient. The amount of stress and strain placed on a family of a child with a difficult illness, such as cancer, is insurmountable, and a physician should make any efforts possible to help relieve the family of burdens. Care for the family also includes bereavement

support, which includes phone calls, home visits, individual visits, cards, memorial letters, and therapy (Muckaden, Dighe and Balaji).

In children with advanced diseases that are difficult to treat, it is important to remember that the goal should to be to maximize comfort and to improve the quality of life to the best possible, while weighing possible side-effects and benefits of a given treatment. This shift to "comfort care" is a defining feature of palliative care. A physician using "comfort care" is said to be "low tech and high touch," meaning that the treatment of the disease itself is minimized and the support and personal interaction with the patient is maximized (Muckaden, Dighe and Balaji).

While total care includes both caring for different types of pain and maximizing comfort for both the patient and their family, it also includes a responsibility to research and investigate new methods of care. Research into treatments for diseases is important in developing new methods for treating diseases that increases both quantity and quality of life. This extension of the principles of palliative care into the field of research will help to improve patient care and treatments.

Ewing's Sarcoma

Palliative care could be especially beneficial in treating a pediatric patient with Ewing's sarcoma. Ewing's sarcoma is a malignant (cancerous) bone tumor that can occur any time during childhood to young adulthood and very rarely in adults over the age of 30. The tumor may start anywhere in the body, but it is usually found in the long bones of the arms and legs, the pelvis, or the chest. The tumor often metastasizes, or spreads, to the lungs and other bones (L. Baker). The current 5-year survival rate for children younger than 15 years is approximately

79%, while the 5-year survival rate is 49% in adolescents aged 15 to 19 years (Smith, Seibel and Altekruse). Metastases occur in approximately 25 % of patients with Ewing's sarcoma and greatly reduces the chance of survival (Esiashvili, Goodman and Marcus).

Patients with Ewing's sarcoma present a varied array of symptoms depending on the location of the tumor. Pain, tenderness, and swelling at the site of the tumor, however, is commonly experienced. Patients also tend to experience fever, weight loss, and fatigue. Unfortunately, these symptoms are not rare and occur in a wide variety of common diseases. The diagnosis of Ewing's sarcoma is usually not discovered until after metastasis, or after a trauma that results in an x-ray of the patient (Fayed). Since diagnosis can be delayed, treatment for Ewing's sarcoma, if treatment is possible, begins almost immediately.

Research

The treatment regimen for Ewing's sarcoma is intensive and usually begins with a multi-drug chemotherapy regimen. After a few months of chemotherapy, the patient will either undergo surgery or radiation therapy. Once this is complete, chemotherapy is continued, which results in a total treatment time of up to nine months (MD Anderson Cancer Center).

A possible alternative treatment for Ewing's sarcoma involves the use of ajulemic acid (AJA), a synthetic non-psychoactive cannabinoid. Research has shown cannabinoids such as tetrahydrocannabinol (THC), which is the active ingredient in marijuana, and AJA to be potent anti-inflammatory drugs with antitumor effects. Although THC is initially twice as potent as AJA against cancer cells, AJA's antitumor effects last longer than THC's (Figure 1) (Recht, Salmonsen and Rosetti).

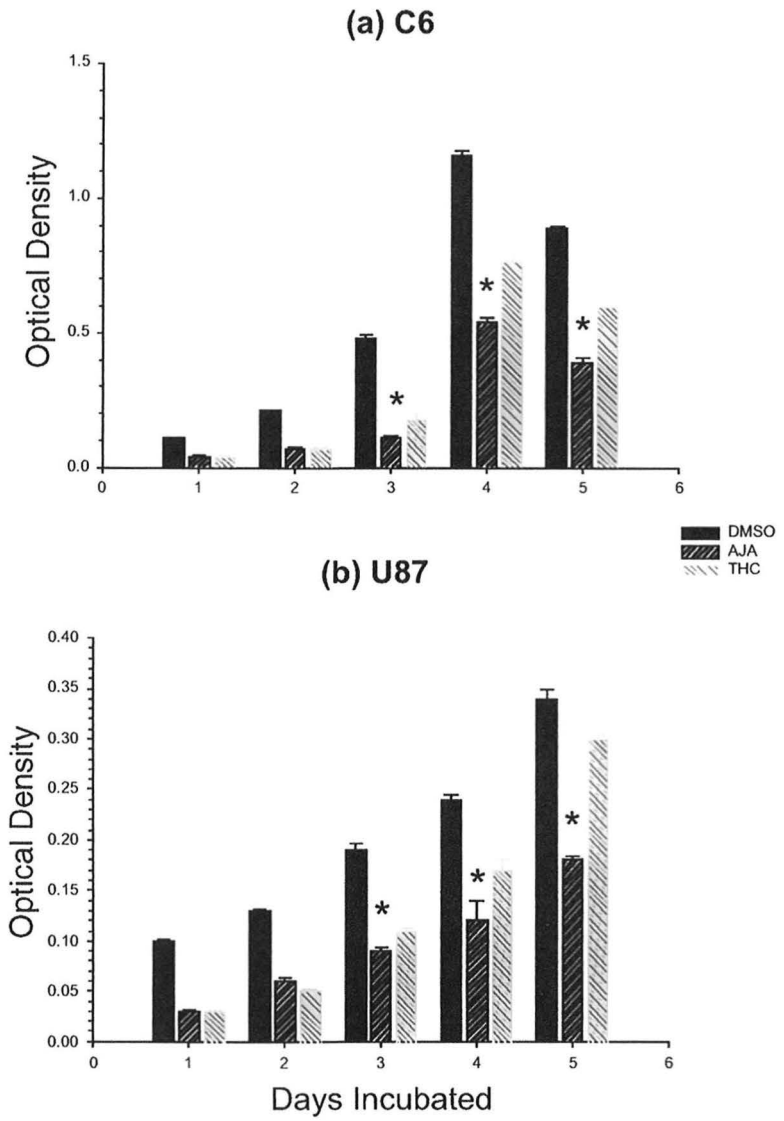

Figure 1: Assessment of duration of action of THC and AJA in two glioma cell lines, C6 and U87 (* shows differences that are statistically significant) (Recht, Salmonsen and Rosetti).

Since AJA is an effective anti-inflammatory and antitumor agent without the psychotropic effects of THC, it is a promising clinical treatment for a wide variety of cancers. Research previously conducted by Dr. Lori Hensley at Ouachita Baptist University tested the antitumor effects of AJA on Ewing's sarcoma cells. Results showed that ajulemic acid was a highly effective antitumor agent against Ewing's sarcoma. An ideal cancer drug would not only kill the cancer cells, but would also inhibit blood vessel formation and spread throughout the

body. To determine such effects of AJA on Ewing's sarcoma, further tests were performed. One such experiment was done to determine the effect of AJA on angiogenesis, the formation of blood vessels. Endothelial cell migration and aortic ring assays were used to assess *in vitro*, outside the body, effects of AJA on this process. Aortic ring assays, which provide a picture of angiogenic processes by allowing the analysis of cellular proliferation, migration, tube formation, microvessel branching, and perivascular recruitment and remodeling (Baker, Robinson and Lechertier), showed inhibition with treatment of AJA compared to controls (Figure 2).

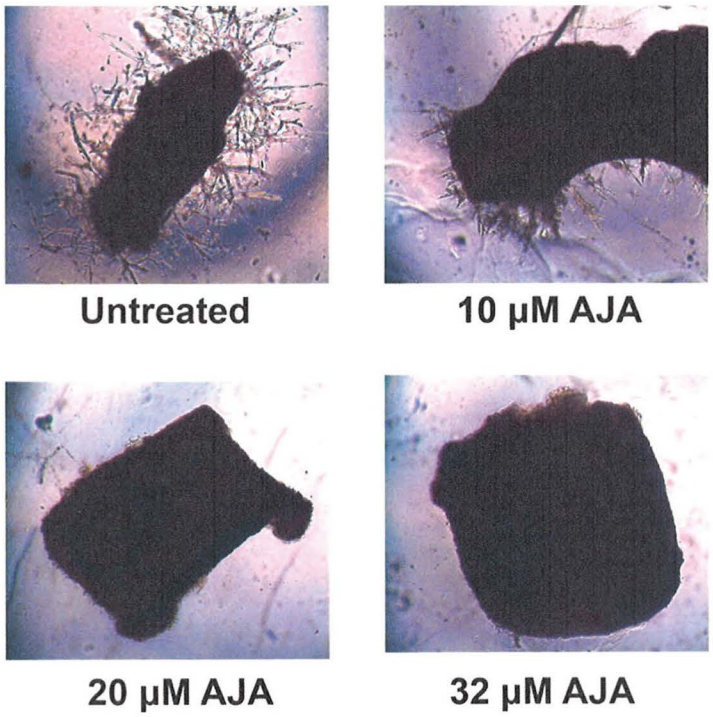

Figure 2: Aortic Ring Assay. As concentrations of AJA were increased, less blood vessel formation was observed (Hensley).

Wound scratch assays (Figure 3 and Figure 4) were also performed. Wound scratch assays measure basic cell migration parameters by growing cells to confluence and then applying a thin scratch "wound." Cells at the edge of the wound should migrate into the open

wound space unless inhibited (Cory). When compared with controls, AJA greatly inhibited the migration of endothelial cells.

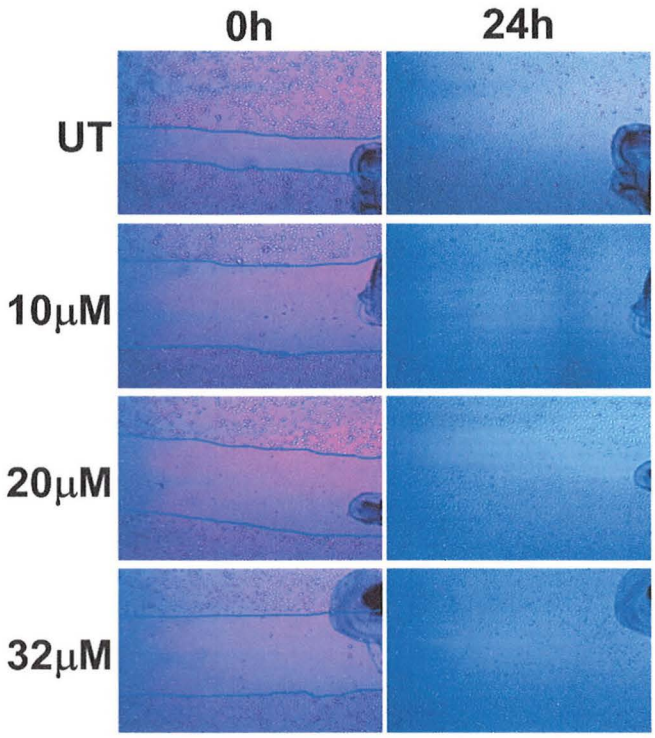

Figure 3: Wound Scratch Assay. UT is untreated with AJA. 10, 20, 32 μM, are concentrations of AJA. As concentrations of AJA were increased, less cell migration was seen after 24 hours (Hensley).

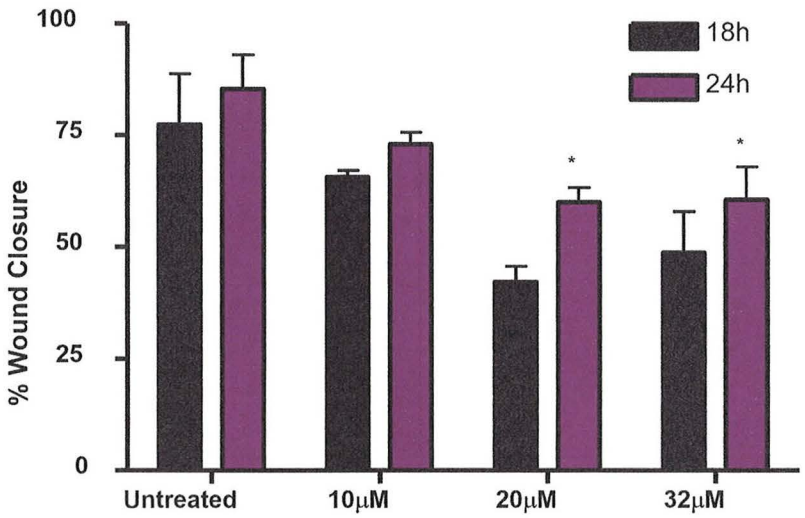

Figure 4: Wound Scratch Assay Data. Untreated is untreated with AJA. 10, 20, 32 μM, are concentrations of AJA. As concentrations of AJA were increased, less cell migration, or wound closures was seen. This data graphically represents the observations shown in Figure 3 (Hensley).

Finally, a Boyden Chamber assay was used to determine AJA's effect on cell tumor migration and invasion. A Boyden Chamber assay allows for cells that pass through a extracellular membrane-like barrier to be quantitatively analyzed. When this assay was performed, Ewing's sarcoma cells that were untreated passed through the membrane. When treated with AJA, however, the tumor cells were not able to penetrate the membrane, which means that AJA is inhibiting the cells from migrating. This finding led to the investigation of this mechanism of inhibition.

To investigate this inhibition, levels of matrix metalloproteinase 9 (MMP9) were measured. MMP9 is a protein involved in the breakdown of extracellular matrix (NCBI MMP9). If the tumor cannot breakdown the extracellular matrix in the surrounding tissues, the cells

cannot migrate and metastasize. To further analyze the effects of AJA on MMP9 levels, the use of three-dimensional spheroids (Figure 5) was implemented.

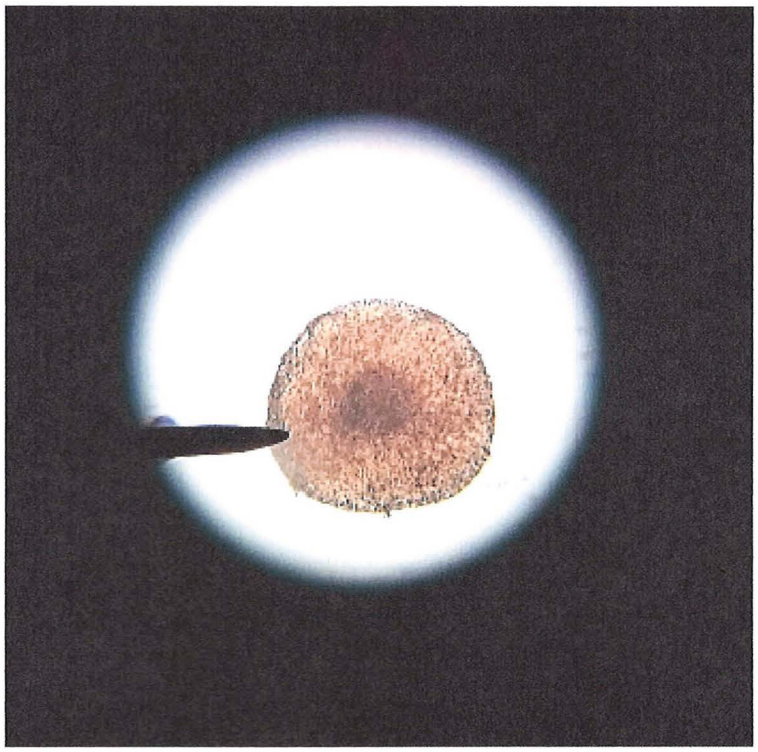

Figure 5: Tumor-Endothelial-Fibroblast three-dimensional spheroid.

Methods

Spheroids were constructed using TC71-PM4-GR cells (Ewing's sarcoma cells), EP cells (endothelial progenitor cells), and WI-38 cells (fibroblast cells). This revolutionary new approach to *in vitro* cancer research allows for a more informative and *in vivo* like setting. Previously conducted studies found spheroids to be more sensitive to anti-tumor agents and exhibit higher levels of various proteins, such as MMP9. Overall, spheroids allow provide a "model system to better understand mechanisms governing tumor initiation, growth, angiogenesis, and progression" (Upreti, Jamshidi-Parsian and Koonce).

These spheroids were constructed by first growing the three cells lines, TC71-PM4-GR, EPC, and WI-38, separately. The cells were then counted, and 100,000 of each were placed into

a single centrifuge tube. After being centrifuged for 6 min at 4°C and 1000 RPM, the supernatant was pipetted off, and the cells were suspended in 2 mL of mixed growth media, which contained 0.666 mL of each of the growth medium for the three cell lines.

After mixing the cells thoroughly in the mixed media, a 20 µL drop of the cell mixture was placed in each "circle" of a 96 well plate lid. The lid was then flipped and the spheroids were allowed to grow in the suspended media for 6 days. The spheroids were then transferred to a low binding plate, and 200 µL of Opti-Mem® was placed into each well. This method of spheroid construction and growth was not optimum, however. The transfer of spheroids to a low binding plate was difficult and inefficient, and protein levels were not as high as expected. To optimize spheroid production and growth, experiments were performed to test different methods of growing spheroids.

Experiments were performed to determine the effect of placing fresh media in wells with the spheroids on day 4. Also, the last round of spheroids were grown in an ultra low-binding plate, so the transferring of the spheroids did not occur in this instance. The spheroids grown in this plate contained 120,000 cells from each cell line.

The timeline of treatment and the concentrations of AJA used for the treatment of spheroids remained the same. After 2 additional days of growth, the spheroids were treated for 18 hours with either 0 µM, 10 µM, 20 µM, or 32 µM AJA. Samples of growth media were then obtained from each well, and MMP9 levels were measured using an enzyme-linked immunosorbent assay (ELISA). The data collected was analyzed using the GraphPad© software Prism.

Results

The concentration of MMP9 in the first set of spheroids (Figure 6) showed a decrease with increased concentrations of AJA. These spheroids were grown on the lid of a 96 well plate and not given fresh media.

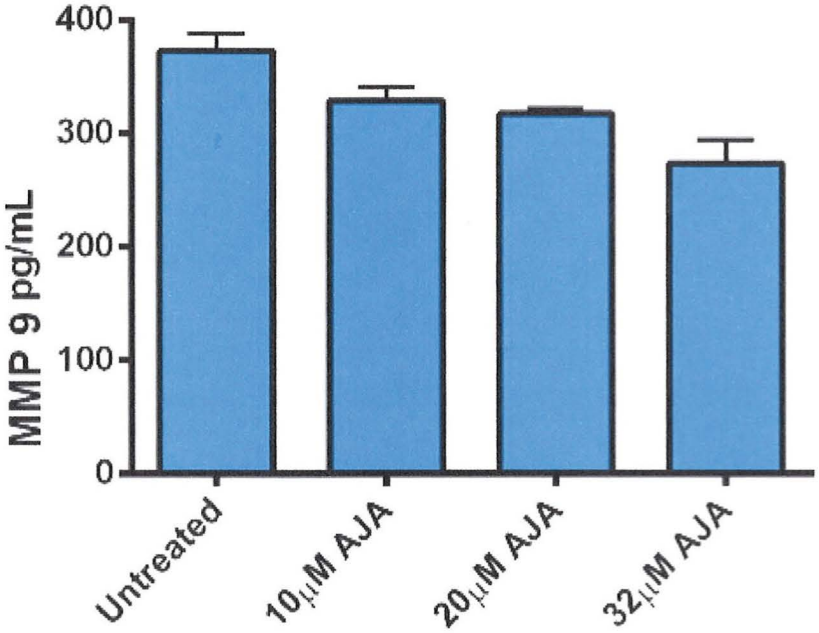

Figure 6: Concentration of MMP9 in spheroids set 1 (9-19-2014).

The second set of spheroids (Figure 7) were divided into two groups. One group was given fresh media on day 4, while the second group was not. Both groups showed a decrease in the concentration of MMP9 with increased concentrations of AJA. The spheroids given fresh media showed higher levels of MMP9, especially in the untreated wells.

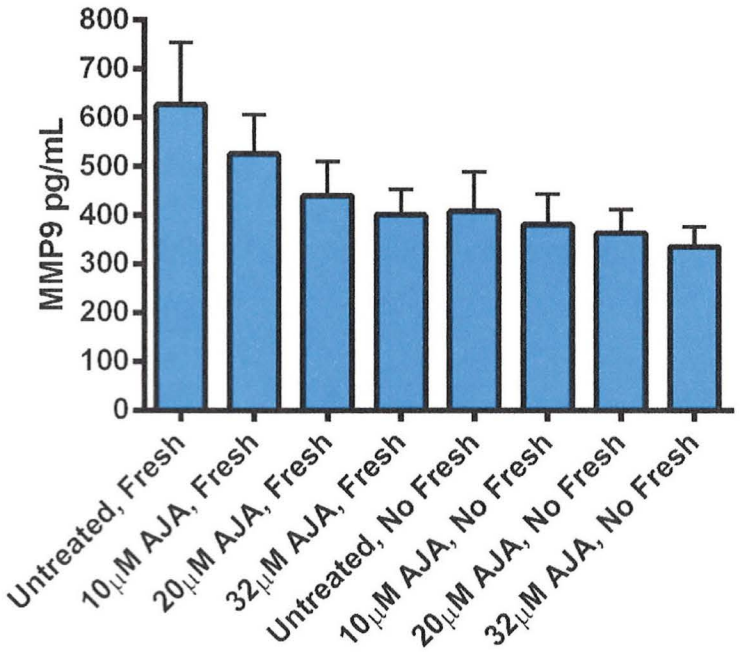

Figure 7: Concentration of MMP9 in spheroids set 2 (10-10-2014).

The third set of spheroids (Figure 8) were grown in an ultra low-binding plate. All of these spheroids were given fresh media on day 4. The decrease in MMP9 concentration is not as high as before.

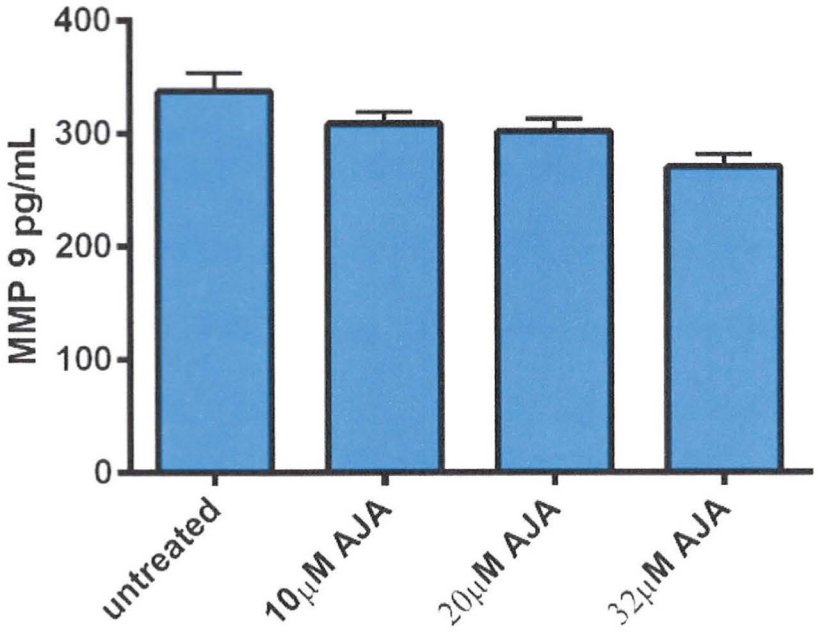

Figure 8: Concentration of MMP9 in spheroids set 3 (11-14-2014).

Finally, further sets of spheroids have been used to test the effects of AJA on MMP9 levels. These spheroids were all grown in an ultra low-binding plate and given fresh media on day 4. The data from each new set and set 3 were compiled together (Figure 9). This data shows a decrease in the concentration of MMP9 with increased concentrations of AJA.

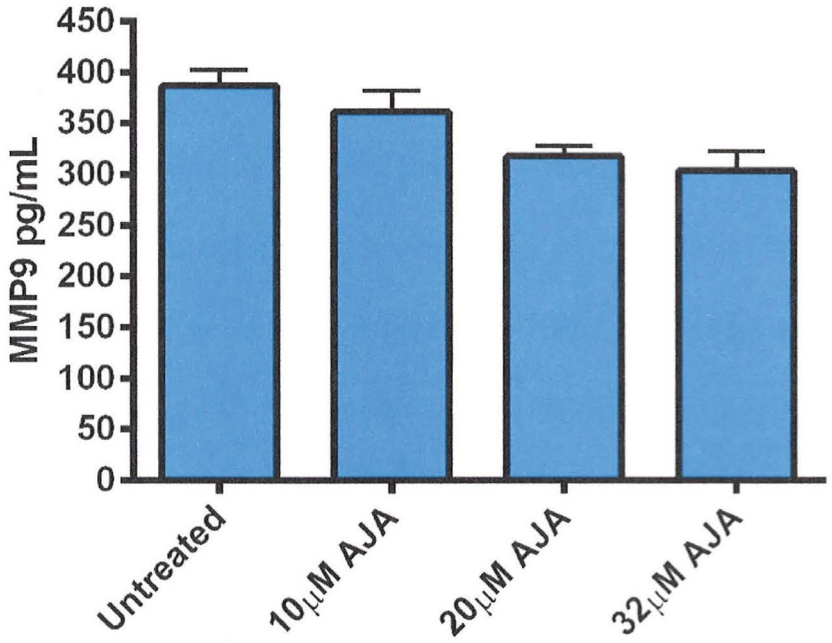

Figure 9: MMP9 concentration in combined spheroid sets.

Conclusion

AJA's suppressive effect on the levels of MMP9 may explain its ability to prevent cell migration. With decreased concentrations of MMP9, the cells cannot break down the extracellular matrix, which would inhibit invasion through the tissue. This inhibited invasive ability would decrease the tumors ability to create a vascular network and metastasize.

This novel use of spheroids to study potential cancer treatments such as AJA is a momentous step towards increasing the survival rate of patients suffering from Ewing's sarcoma. Cancer is difficult to deal with for anyone, but it is especially difficult for children and

their families. In these particularly trying situations, the approach to the care of these patients should be holistic, including tending to physical, psychological, social, and spiritual pain (Muckaden, Dighe and Balaji). In circumstances with a decreased chance of survival, such as Ewing's sarcoma, quality and comfort should be key factors in deciding the course of treatment.

As a prospective physician, I hope to be empathetic and caring to all of my patients. Also, I hope to establish trust and a lasting relationship with my patients. Most of all, I want to be successful in tending and treating my patients. In some situations, this treatment may be solely to create a comfortable atmosphere for the remainder of my patient's life. I hope that I take into consideration all aspects of my patient's well-being, and that in any situation I do what is best for my patient. Finding the best option for my patient constitutes a responsibility to be actively engaged in reading and be aware of research. Without investigation and research, medical breakthroughs are not possible. Through my newly gained knowledge of the research process, I can better understand current research and hopefully be of better assistance to my patients. If I can accomplish these tasks, I will become a successful doctor.

References

Baker, L.H. "Ewing's Sarcoma." 2011. *Medline Plus.* Document. 6 September 2013.

Baker, Marianne, et al. "Use of mouse aortic ring assay to study angiogenesis." *Nature Protocols 7* (2012): 89-104. Print.

Cory, G. "Scratch-wound assay." *Methods Mol Biol.* (2011): 25-30. Print.

Esiashvili, N, M Goodman and R B Marcus. "Changes in incidence and survival of Ewing sarcoma patients over the past 3 decades: Surveillance Epidemiology and End Results data." *J Pediatr Hematol Oncol* (2008): 425-430. Print.

Fayed, Lisa. "Symptoms of Ewing's Sarcoma." 8 August 2010. *About.com.* Web. 1 February 2014.

Hensley, Lori. "Hensley Research." *Unpublished*. n.d.

J. Leonard Lichtenfeld, MD, MACP. "Dr. Len's Cancer Blog." 8 July 2010. *American Cancer Society.* Web. 25 January 2014.

"MD Anderson Cancer Center." 2013. *Ewing's Sarcoma Treatment.* Web. 6 September 2013.

Muckaden, Maryann, et al. "Paediatric Palliative Care: Theory to Practice." *Indian J Palliat Care* (2011): S52-S60. Print.

NCBI MMP9 . 16 February 2014. Web. 18 February 2014.

Recht, Lawrence D, et al. "Antitumor effects of ajulemic acid (CT3), a synthetic non-psychoactive cannabinoid." *Biochemical Pharmacology* (2001): 755-763. Print.

Smith, M A, N L Seibel and S F Altekruse. "Outcomes for children and adolescents with cancer: challenges for the twenty-first century." *J Clin Oncol* (2010): 2625-2625. Print.

Society, American Cancer. *Cancer Facts and Figures 2007*. Atlanta: American Cancer Society, 2007. Web.

Upreti, Meenakshi, et al. "Tumor-Endothelial Cell Three-dimensional Spheroids: New Aspects to Enhance Radiation and Drug Therapeutics." *Translational Oncology* (2011): 365-376. Print.