Retinoblastoma and Etoposide

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**Table of Contents**

Abstract ......................................................................................................................... 3

Introduction

- Purpose....................................................................................................................... 5
- Background................................................................................................................. 5
- Tumor Growth and Diagnosis.................................................................................. 9
- Genetics..................................................................................................................... 13
- Treatments................................................................................................................. 16
- Etoposide.................................................................................................................. 20

Hypothesis ..................................................................................................................... 23

Materials and Methods ............................................................................................... 28

Expected Results ......................................................................................................... 32

Discussion .................................................................................................................... 33

Literature Cited ............................................................................................................. 35
Abstract

Retinoblastoma is a cancer that develops within the retina of the eye. Although it may be found in adults, retinoblastoma cases seen globally are mostly in young children. Therefore, it is also categorized among the childhood cancers, along with leukemia, bone cancer, and neuroblastoma.\textsuperscript{1,2} If retinoblastoma is treated at its initial stages, the cancer is curable. However, if left untreated, this malignant tumor spreads out from the eye and may only be treated with enucleation. Enucleation is the last resort to treating the cancer because it requires the complete removal of the eye. In order to avoid enucleation, various chemotherapy treatments have been developed, which utilize an array of drugs such as etoposide. Dunkel et al presented a study that showed successful management of retinoblastoma with chemotherapy.\textsuperscript{3} Among the drugs prescribed during chemotherapy, etoposide was one of them. As an inhibitor of topoisomerase, etoposide directly affects the growth of retinoblastomas at the core, the DNA, and affects the cell cycle of the retinoblastoma cells. The result is a reduction in tumor growth; the cells do not properly divide and cannot contribute to the growth of the tumor.

For this thesis, a hypothetical treatment was created to study the efficacy of etoposide with a higher dosage on tumor reduction. The dosage was increased from 75 mg/m\textsuperscript{2} to 90 mg/m\textsuperscript{2} for a single cycle length of 7 to 15 days. Simultaneously, the dosages prescribed before the start of the clinical trial will remain unchanged for the duration of each cycle. Utilizing the higher dosage, it is hypothesized that the higher dosage will cause a reduction in tumor size among the patients. The higher dosage will be delivered via a slow-releasing oral capsule. It is hypothesized that the slow-releasing capsule will allow children to take a higher dosage of the drug for a longer cycle and, therefore, result
in a significant reduction in tumor size. Although the clinical trial may not be carried out under the circumstances at the time of this thesis, it is predicted that a large majority of the patients will present a significant reduction in tumor size.
Introduction

Purpose

The purpose of this honors thesis is to utilize the information provided through a review of retinoblastoma and its various treatments available to propose a hypothetical clinical trial that may potentially be carried out. The hypothetical clinical trial will focus on etoposide, which is one of the main chemotherapeutic drugs used in treating retinoblastoma. Etoposide, prescribed with other chemotherapeutic drugs such as carboplatin and thiotepa, has been found to be effective in the management and reduction of tumor size.\textsuperscript{4,5,6} Etoposide, as well as other commonly used chemotherapeutic drugs, is restricted due to toxic effects on the normal cells and tissues of the body. Young children form the bulk of patients that receive etoposide in a mixture, or cocktail, of other chemotherapeutic drugs. Therefore, the proposed clinical trial will present a higher dosage, which will be taken orally in a time-released oral capsule. By using an extended release oral dosage, it is hypothesized that the higher dosage will present better results in terms of the management and reduction of the tumor size.

Background

Retinoblastoma is a malignant tumor of the retina. It is a hereditary disease that accounts for 4% of all childhood cancers and is most commonly found in children of ages 0-4.\textsuperscript{7,8} Retinoblastoma appears in the retina as a white mass with many calcifications, or accumulation of calcium deposits. The mass may develop in various directions. Retinoblastoma is classified as either intraocular, the tumor is present within the eye, or extraocular, the tumor has spread to areas around the eye. An intraocular retinoblastoma
may be bilateral, present in both eyes, or unilateral, present in only one eye. An extraocular retinoblastoma may be an orbital retinoblastoma or metastic retinoblastoma. Orbital retinoblastomas are tumors that have only spread to the eye socket. Metastic retinoblastomas are tumors that have spread to distant parts of the body such as the brain.

Figure 1. Anatomy of the Human Eye – cross-sectional view

The retina converts the images that enter through the eye’s lens to electrical signals, which are sent to the brain through the optic nerve. The retina is light-sensitive and contains photoreceptors, cones and rods across its surface. As seen in Figure 1, the retina is positioned posterior to the lens of the eye and receives a large supply of oxygen due to the large number of blood vessels present, which gives it its red color. The retina is relatively visible when a light is shown through the pupil as it is dilated. A color change in the retina may be one of the first signs of a disease. In the case of retinoblastomas, when the retina is observed through the dilated pupil, leucocoria, a white tumor, is seen, which marks the beginning stages of the disease.
Figure 2. Retinoblastoma Samples: (a) Retinoblastoma invasion of the choroid but not the optic nerve. (b) Retinoblastoma invasion of the optic nerve

The retinoblastoma cells that become extraocular invade other areas of the eye including the choroid, as seen in Figure 2. In a sample, the tumor cells are distinctly darker than the cells of the healthy tissue. As the retinoblastoma cells continue to invade areas around the retina, the healthy tissues are rapidly affected. Eventually, if left untreated, the retinoblastoma cells will reach the optic nerve. In Figure 2b, the retinoblastoma cells have proliferated significantly and most of the optic nerve has been affected; there is little healthy tissue present.

Although fatal if left untreated, retinoblastoma has developed into a curable disease since its first identification in the 1500s. In 1597, Peter Pawius of Amsterdam was the first person to provide a description of a tumor of what is now retinoblastoma in a three-year-old. In 1809, James Warthrop, a Scottish surgeon, of Edinburg defined retinoblastoma as a unique clinicopathologic entity. The term “retinoblastoma” did not appear until the 1920s, when Hermann Von Helmholtz, a German physicist and physician, proposed the name. Helmholtz believed that the tumor arose from embryonic retinal cells. The American Ophthalmological Society adopted the term in 1926. The exact date at which retinoblastoma became curable is not
known, but treatments were present since the 1930s. The pioneer work on possible treatments for retinoblastoma was done by Dr. Algernon Reese and Dr. Hayes Martin.\textsuperscript{12,13} Enucleation had been the primary option to treat retinoblastoma, but Dr. Reese and Dr. Martion were able to treat and save patients using radiation therapy.

Retinoblastoma was originally thought to be a fungating mass that resulted in the destruction of the internal construction of the eye. A fungating mass, or wound, forms when the growing cancer breaks through the epithelial tissue, or skin.\textsuperscript{14} However, the extensive molecular research based on the work of Kyritsis et al. concluded retinoblastoma was a cancer that results from the overproduction of immature retinal cells. There is also large evidence that genetics is a factor in the presence of retinoblastoma.\textsuperscript{15} Chromosomal abnormalities as well as spontaneous mutation have become key players in identifying and explaining retinoblastoma.

Retinoblastoma cells have shown a distinct rosette-like arrangement (Figure 3). Predominant retinoblastoma cells are described as “a round cell with basophilic nucleus of variable size and relatively little cytoplasm.”\textsuperscript{16} The nuclei present pleomorphic changes as well as mitotic figures. As seen in Figure 3, more clearly differentiated areas present the distinct arrangement seen. The rosette-like arrangement has been determined to be a distinct, or pathognomonic, characteristic of retinoblastoma. A study performed by Ts’o, Fine, and Zimmerman revealed the rosettes, formed by cuboidal tumor cells, originated from photoreceptors and the comparable lining of the lumen of the rosette to the outer membrane of the retina.\textsuperscript{17}

\textbf{Figure 3. Retinoblastoma cells that present a rosette-like arrangement.\textsuperscript{18}}
Figure 4: Retinoblastoma cells that form long cell processes.\textsuperscript{4}

Tumor Growth and Diagnosis

Retinoblastoma tumor growth may be endophytic or exophytic. Endophytic growth represents growth into the vitreous, interior of the eye, which contains a gelatinous mass, and emerges from the inner layers of the retina. On the other hand, exophytic growth emerges from the outer layers of the retina and represents growth under the retina toward the choroid. Retinoblastoma tumor growth may also be a mix of both endophytic and exophytic growth. Diffuse infiltrating tumor growth represents a grayish plaque present on the retinal surface. Retinoblastoma may also be identified as intraocular or extraocular. Intraocular retinoblastoma describes tumor growth that is still contained within the eye. Extraocular retinoblastoma describes tumor growth that has spread from the interior of the eye, such as into the optic nerve.

Extraocular retinoblastoma is a risk factor for metastatic growth due to the invasion into the optic nerve, which creates a more accessible path into the central nervous system.\textsuperscript{9} Extraocular retinoblastoma is localized to the optic nerve beyond the margin of resection or to the soft tissues surrounding the eye.\textsuperscript{19} The location of tumor development is a vital factor in determining the amount of visual loss that is experienced.
In one study, using a visual field analysis, various degrees of visual loss were examined. The patients were placed in one of four groups: no residual defect, absolute scotoma, arcuate scotoma, and pseudo-visual field defect. Scotoma refers to partial loss of vision; it may also refer to the presence of a blind spot in normal visual field. Arcuate represents the shape of the loss of visual field in which the loss of vision begins at a blind spot and curves around to the nasal field of vision. The relationship between the location of the tumor to visual loss is that if the tumor is present only at the blind spot, where the retina is attached to the other nerve, vision is not completely gone. However, once the tumor begins to move out from the blind spot, vision loss becomes more prominent and may eventually result in complete vision loss.

Aside from the retina, the optic nerve and the central nervous system are affected by retinoblastoma. Optic nerve involvement is mostly seen in retinoblastomas due to its direct connection to the retina. The central nervous system is affected when the disease has reached a metastatic level. The tumor is classified using the Reese-Ellsworth classification, which contains five groups, Group I to Group V, based on tumor size and location. Group I designates tumors as very favorable for maintenance of sight and are relatively small. Group I includes solitary tumors that are smaller than 4 disc diameters (DD), which is based on the size of the optic disc, and multiple tumors that are no larger than 4 DD. The solitary tumors are at or behind the equator. The equator of the eye is the plane that is equidistant to the anterior and posterior ends of the eye. Group II designates tumors as favorable for maintenance of sight and include solitary and multiple tumors with a size that ranges from 4 DD to 10 DD. The solitary tumors are at or behind the equator. The multiple tumors are all behind the equator. Group III designates tumors
as possible for maintenance of sight. This group includes solitary tumors that are larger than 10 DD and any lesions anterior to the equator. Group IV designates tumors as unfavorable for maintenance of sight. This group includes multiple tumors that may be larger than 10 DD and any lesions extending anteriorly to the ora serrata. The ora serrata is the jagged junction between the retina and ciliary body. Group V designates tumors as very unfavorable for maintenance of sight. This group includes significantly large tumors that reach a size of one-half of the retina and vitreous seeding. Seeding in retinoblastoma tumors is seen when small pieces of the tumor have broken off and are floating around the vitreous.

The Internal Classification of Retinoblastoma (ICRB) is also used to categorize the various tumors. Unlike the Reese-Ellsworth classification, ICRB categorizes tumors according to the extent of tumor seeding within the subretinal space and vitreous cavity. ICRB contains also five categories, A to E, and utilizes tumor size, location and absence of subretinal and vitreous seeds. As a patient moves up the classification, such as B to E, it will become more difficult to control the growth of the tumor and preserve as much useful vision as possible. Patients present in Group A display small intraretinal tumors that are 3 mm or smaller in greatest dimension and are present away from the foveola and disc, farther than 3 mm from foveola and 1.5 mm from optic disc. Group B presents all tumors that are discrete and are confined to the retina; these tumors are distinct from those present in Group A. Group C presents discrete tumor(s) with minimal subretinal or vitreous seeding. Subretinal and vitreous seeding refer to tumors that have broken apart and are present in the subretinal or vitreous portion of the eye. Patients classified into Group D present tumor(s) that may be massive or diffuse and the disease presents
significant vitreous or subretinal seeding. Group E indicates one or more signs of poor prognosis such as tumor touching the lens of the eye, opaque media from hemorrhage, and diffuse infiltrating retinoblastoma. Patients classified into Group E have limited treatments available due to the low probability of preserving any vision in the eye.

Figure 5. Slit-lamp photograph of multiple white nodular tumors present in the inferior anterior chamber.

Figure 6. Right: Unilateral Retinoblastoma – tumor seeding in the iris. Left: Vitreous seeding

In diagnosing retinoblastoma, the most common sign of the presence of retinoblastoma is leucocoria. Leucocoria is the white tumor that is reflected in the presence of light and blocks the view of the retina. The white tumor is life-threatening and is the first sign of the tumor growth, which is still contained in the interior of the eye. The presence of leucocoria in children may be observed if the pupils naturally dilate in dim light as a beam of light is shone on the eyes. Figures 5 and 6 display the presence of leucocoria, which may be confined to one area, Figure 5, or spread out, Figure 6. If the treatment is begun within 3 to 6 months of discovering leucocoria, retinoblastoma is
curable; the tumor is still intraocular. The effectiveness of treatment for retinoblastoma begins to decrease as treatment is delayed after the first sign of leucocoria is discovered. If the cancer is not responsive to chemotherapy, enucleation is the only option available to treat retinoblastoma.

**Genetics**

Genetics plays a large role in the diagnosis and treatment of retinoblastoma. Retinoblastoma is a hereditary disease when it is transmitted as an autosomal dominant trait. The autosomal dominant inheritance is present in retinoblastoma that contains a mutation within the germline, the reproductive cells in the body.\(^\text{25}\) However, there have been cases in which retinoblastoma is the result of a *de novo* mutation. The retinoblastoma-1 gene has been localized to chromosome 13q14, or more specifically 13q14.2.\(^\text{26,27}\) According to Online Mendelian Inheritance in Man (OMIM), retinoblastoma is not the only phenotype that is expressed as a result of microdeletions of DNA in this region. Microdeletions are chromosomal deletions that span across several genes but are too small to be detected under the microscope using conventional cytogenetic methods.\(^\text{28}\) Cytogenetic analysis is the basic element of diagnosing genetic disorders caused by chromosomal abnormalities.\(^\text{29}\) The basic method of cytogenetic analysis is fluorescence *in situ* hybridization (FISH). To detect microdeletions, genetic tests such as microarrays and whole exome sequencing are commonly used. Microarrays and whole exome sequencing allow a more in depth analysis of the various microdeletions that may be present on each chromosome. By running a patient’s chromosomes through a robotic machine, which is able to run hundreds to thousands of gene sequences placed on a single microscope slide, results obtained immediately present
any microdeletions or abnormalities that may have been documented to cause various
diseases.

Aside from retinoblastoma, microdeletions on this chromosome result in somatic
osteosarcoma, somatic bladder cancer, and somatic small cell cancer of the lung. The
retinoblastoma gene (RB1) was also the first tumor suppressor to be cloned. The function
of the RB1 gene contains the characteristics of a regulatory element of the cell cycle. The
function of the gene is controlled by a phosphorylation/dephosphorylation mechanism in
various stages of cell proliferation and differentiation.

The retinoblastoma protein, Rb, functions as an active transcriptional repressor
while it is attached to a promoter through binding with E2F. E2F is also known as
Transcription Factor E2F, Retinoblastoma-Binding Protein 3 (RBP3), and
Retinoblastoma-Associated Protein 1 (RBAP1). Rb may interact with transactivating
domains that surround transcription factors on the protein. By surrounding these factors,
the interaction is blocked with a basal transcription complex. Rb can also recruit histone
deaetylase while it is bound to E2F. Histone deacetylation is the removal of negatively
charged acetyl groups to histones. Histone acetylation stabilizes the nucleosomes and
prevents transcription. The nucleosomes are induced by histone acetylation to assemble.
Rb is bound to E2F during the recruitment of histone deacetylase and blocks the access of
transcription factors to the promoters. With the formation of Rb-E2F complexes, E2F
sites operate as transcriptional silencers.

When a defective gene copy of chromosome 13q14 undergoes somatic mutation,
the result is the loss of the normal remaining copy of the gene. Experiments have shown
that retinoblastoma tumor formation results in the loss of chromosome during mitotic
divisions of predisposed cells.\textsuperscript{33} According to research done by Zhang et al., retinoblastoma showed the lowest mutation rate reported in human cancer to date.\textsuperscript{34} The ploidy, a number or set of chromosomes within a cell, of retinoblastoma cells were stable \textit{in vitro} and \textit{in vivo} despite many instances in which retinoblastoma cells presented mitotic defects. These defects could lead to errors in chromosome segregation.

Heritable retinoblastoma requires two mutations, which includes a mutation in the germline and another in the developing retina. Germline mutation is present in 90\%-95\% of patients with bilateral retinoblastoma.\textsuperscript{35} In heritable retinoblastoma, an Rb gene mutation is present in most of the cells of the body, if not all. The Rb gene mutation results in the inactivation of the both alleles of the Rb gene, which categorizes retinoblastoma as an autosomal recessive cancer.\textsuperscript{4} Autosomal recessive retinoblastoma is found in patients that do not contain a mutation in the germline.\textsuperscript{20} Non-heritable retinoblastoma also requires two mutations but both mutations occur in the developing retina. Due to the presence of the mutation in non-germline cells, patients with autosomal recessive retinoblastoma usually do not pass on the Rb gene mutations. However, genetic testing is highly advised to verify the location of the Rb gene mutation.

Those with a \textit{de novo} mutation cannot be traced back to a family member for he/she contains a spontaneous mutation that occurred during fertilization. For patients with retinoblastoma family history, a pedigree may be created from genetic testing to identify the possibility of having a child with retinoblastoma. Genetic counseling plays an important role in keeping families educated on the disease as well as providing solutions to obtain treatment as soon as possible.
**Treatments**

As the understanding of retinoblastoma developed, various treatments emerged. The treatments include enucleation, external beam radiation therapy, focal therapy, laser photocoagulation, thermotherapy, and system chemotherapy. Enucleation was introduced by Warthrop after his observation in 1809. Warthrop identified this treatment as a life-saving measure for patients with retinoblastoma. Enucleation, by definition, involved the complete removal of the eye containing the tumor. This treatment was performed on patients in whom extraocular extension had yet to occur. However, during the time of Warthrop, enucleation was avoided due to high mortality rate after the procedure. The development of anesthesia, as well as the presence of antibiotics, resulted in fewer traumas after enucleation and, therefore, reduced the mortality rate. Von Graefe, a pioneer of German ophthalmology, identified that retinoblastoma often grew into and down the optic nerve into the brain. Graefe advised that a long stump of the optic nerve be removed in the enucleation. This remains the standard practice today. Today, enucleation is used when there is no presumption that any useful vision can be preserved. Enucleation involves the removal of the eye and may also include the removal of the optic nerve if it has been invaded. For highly lethal retinoblastomas and Group E tumors, enucleation is the only solution.

Chemotherapy refers to the use of a drug or chemical to treat an illness. Many times chemotherapy is automatically thought to refer only to cancer treatment. One type of chemotherapy is systemic chemotherapy. Systemic chemotherapy uses anticancer drugs in its treatment, which travels to cells throughout the body through the bloodstream. Another type of chemotherapy is intra-arterial chemotherapy. Intra-arterial
Chemotherapy is a newer treatment that includes intravascular cannulation of the ophthalmic artery. A tube is inserted in the ophthalmic artery to allow direct delivery of chemotherapy to the eye and areas surrounding the eye.

Stem cell rescue is an approach in treating metastatic retinoblastomas. Stem cell rescue is a technique that harvests the stem cells of the patient receiving chemotherapy that uses lethal doses of drugs. During the length of the chemotherapy treatment, the tissue of the bone marrow is heavily destroyed. Therefore, the harvested stem cells are placed back into the patient after high-dose chemotherapy and used to help the bone marrow recover from the treatment and make healthy cells. After healthy cells have been made and replaced in the patient, another cycle of chemotherapy can be performed after another round of harvesting stem cells, which will be replanted after the treatment. Stem cell rescue is used to allow patients to undergo many chemotherapy treatments that require lethal doses of chemotherapeutic drugs.

High-dose chemotherapy, also known as bone marrow transplant, is another treatment used to treat retinoblastoma. In an experiment by B. Kremens, et. al, high-dose chemotherapy was performed with thiotepa, an alkylating agent, etoposide, and carboplatin. The dosage for etoposide was 40 mg/kg and the dosage for carboplatin was 1.5 g/m². The transplantation involves peripheral hematopoietic stem cells and is an attempt to increase the survival rate of children with metastatic retinoblastomas. For the children used in the experiment, thiotepa and carboplatin were not prescribed; the drugs prescribed were BCNU, cyclophosphamide, and etoposide. The dosage used on the child was 1.6 g/m². Thiotepa, carboplatin, BCNU, and cyclophosphamide are all alkylating agents. Alkylating agents work by directly damaging DNA and, therefore, directly
blocking the cancer cells from reproducing. Alkylating agents target various stages of the cell phase and used to treat not only retinoblastoma but other cancers as well, such as leukemia and ovarian cancer.\textsuperscript{41} Alkylating agents are found to have long-term effects on the body, such as damage to bone marrow, due to its direct effect on DNA.

Cyclophosphamide belongs to a class of alkylating agents called nitrogen mustards. Nitrogen mustards are a derivative of nitrogen gas and were discovered to have an effect on bone marrow and white blood cells. BCNU, or carmustine, belongs to the class of alkylating agents called nitrosoureas. Nitrosoureas are found to be the most active among the anticancer drugs “both qualitatively and quantitatively.”\textsuperscript{42} Nitrosoureas interfere with the enzymes that are needed for DNA repair and are able to pass the blood-brain barrier, which represents its involvement in the treatment of retinoblastomas. Thiotepa belongs to the class of alkylating agents called ethylenimines. Similar to the other classes of alkylating agents, it interferes with DNA processes and is found to be highly reactive when protonated. Carboplatin belongs to the class of anticancer drugs called platinum drugs, which are sometimes grouped with alkylating agents. The mechanism of action of platinum drugs are similar to that of alkylating agents, but are found to have a lower chance of causing leukemia later in life. Etoposide belongs in a different group of anticancer drugs called topoisomerase inhibitors, which interfere with the enzyme topoisomerase.

Aside from chemotherapy, which utilizes drugs such as etoposide, there are other treatments available for patients with retinoblastoma. The different types of treatment include external beam radiation therapy, focal therapy, and ophthalmic artery chemosurgery. Each treatment tackles various groups of tumor growth and is
continuously studied and advancing as alternative treatments. Enucleation is a very common treatment as well, but it is the extreme choice when all other treatments do not show any signs of improvement. Enucleation is also performed if the patient wishes to remove the tumor entirely, which may include the optic nerve in certain cases.

External beam radiation therapy has been the preferred vision-sparing treatment for many years. It is used on progressive or persistent retinoblastomas after chemotherapy and focal treatment. It is also used on retinoblastoma cases in which vitreous or subretinal seeding has occurred. Abramson et al. interprets their data to suggest that external beam radiotherapy may be the only treatment that will potentially cure and spare the patients from significant long-term visual complications.20

Focal therapy refers to a group of treatments that are applied directly to the eye and are used on small tumors that are not located at the center of vision or the optic nerve. For tumors that are determined to be too large for focal therapy, chemotherapy is the first method of treatment in order to shrink the tumor size. There are three main types of focal therapy: cryotherapy, laser therapy, and brachytherapy. Cryotherapy is a type of focal therapy in which an instrument is used to repeatedly freeze the tumor and thaw it at temperatures as low as -60°C to -80°C.43 The tumor cells are targeted and killed through the process of thawing. The ice crystals penetrate the tumor cell’s membranes. Cryotherapy is used primarily on small peripheral retinoblastoma tumors. Laser therapy utilizes heat to physically destroy the tumor cells and is used on small tumors as well as residual and reoccurring tumors of retinoblastoma. Brachytherapy, also known as radioactive plaque, consists of a small disc that is surgically placed directly over the tumor. The disc contains a small amount of radioactive material that is delivered at small
doses directly to the tumor. The disc is present for two to five days before it is surgically removed. Brachytherapy is not used on tumors near the optic nerve and/or the center of vision. However, brachytherapy is used when the retinoblastoma has not responded to cryo therapy and laser therapy.

Ophthalmic artery chemosurgery is intra-arterial chemotherapy modified.\(^3\) It is delivered through the external carotid artery when the ophthalmic artery cannulation is impossible. David H. Abramson modifies the chemotherapy treatment by noting that the largest proportion of the blood flow usually goes to the supratrochlear artery and not directly to the eye.\(^{16}\) The aim of the experiment is to reduce the dose of melphalan, an alkylating agent. The results reveal that the lower doses had no hematologic toxicity. Although it was originally used as chemical warfare, its effect on bone marrow and white blood cells was discovered.\(^{44}\) Also, after treating eyes that were to be enucleated, the results show that 58% of eyes were saved. Ophthalmic artery chemosurgery makes it possible to save the most severe of retinoblastomas that would have been enucleated.

**Etoposide**

Etoposide is a derivative of podophyllotoxin, one of the compounds found in podophyllin (Figure 7).\(^{45}\) The drug was introduced in 1971 and has become one of the first-line drugs for treating various cancers. It treats cancers such as small cell lung cancer, acute nonlymphocytic leukemia, and lymphomas. The effects of etoposide, along with a large variety of other podophyllotoxin derivations, are mediated by topoisomerase II and interact with DNA. Topoisomerases are “enzymes that are involved in changing the supercoiled state of DNA.”\(^{46}\) Topoisomerase II belongs to the second class of
Topoisomerases that cut the ends of both strands of DNA, pass parts of DNA between the cut ends, and reseal the DNA. Etoposide specifically prompts the breakage of the double-stranded DNA by forming the cleavable complex. The cleavable complex is a complex formed between DNA and topoisomerase II. The cleavable complex creates a toxic effect due to the conversion of an essential enzyme, topoisomerase II, into a cellular toxin.

Topoisomerase II is an essential enzyme which alters DNA topology and is required to resolve “knots and tangles” in the genetic material. The knots and tangles are produced by normal cellular processes, which include DNA replication and recombination.

Despite the common use of etoposide in cancer treatment, it is found to have moderate potency, metabolic inactivation, toxic effects, poor water solubility, and may result in the development of resistance. According to the BC Cancer Manual, the distribution of the drug depends on a patient’s weight. On average, the prescribed dosage is 32% of a patient’s body weight, which ranges from 7 to 17 L/m².

![Figure 7. Etoposide Structure](image)

Within the five-ring system of podophyllotoxin, the rings labeled A and E in Figure 8 indicate the pharmacophores. A pharmacophore is portion of the compound that is responsible for biological activity. Ring A contains a methylenedioxy ring that is crucial for optimal antitumoral activity. The low potency is due to the hydroxy groups on
carbon 3 and 5 on ring E. However, despite the low potency, the hydroxyl groups play a role in the stabilization of topoisomerase II DNA intermediates.\textsuperscript{18} By modifying the groups attached to the fourth carbon in ring C, such as bulky groups, the anticancer and topoisomerase activities are amplified. As can be seen in etoposide, unlike the hydroxyl group (-OH) attached to carbon 4 in podophyllotoxin, etoposide contains a rather bulky group at carbon 4 of ring C. Any changes to ring B will lead to a loss of activity.

![Figure 8. Structure of Podophyllotoxin\textsuperscript{43}](image)

According to an experiment performed by Debra L. Friedman, et al., etoposide, prescribed with carboplatin and vincristine, presented positive results for those with tumors classified as Reese-Ellsworth groups 1-3. By prescribing these drugs during systemic chemotherapy, treatments became available to treat retinoblastoma and eliminated enucleation as only option of managing the tumor.\textsuperscript{51} Etoposide was found to be effective in treating retinoblastomas and avoiding as the only treatment to control tumor growth. Etoposide has also been used in the management of metastatic tumor growth after enucleation.\textsuperscript{52} Due to the fact that etoposide has shown many positive results in treating retinoblastoma, a clinical trial can be performed to focus on the effects of
etoposide. By emphasizing the dosage of etoposide, its effect on treating retinoblastoma can be more prevalent and, therefore, lead to an array of treatments that may present positive outcomes.

Hypothesis

Etoposide is commonly taken to treat retinoblastoma. It is specifically chosen for this hypothetical clinical trial to treat retinoblastoma because, aside from it being a commonly prescribed drug, it belongs to a class of drugs that directly affect the core of tumor growth. Tumors are the result of irregular cell growth, which is directly affected by DNA replication. Etoposide works at the core of DNA replication by damaging the DNA to prevent any further cells from reproducing. This clinical trial focuses on reducing tumor growth and, therefore, etoposide is the best drug to prescribe. By targeting the effects of increasing its dosage, it may provide the treatment needed to reduce tumor growth. Due to the fact that other drugs are simultaneously taken, the dosage varies depending on the chemotherapy and the group of tumor growth. In a potential clinical trial, all patients participating will receive etoposide as well as all other drugs prescribed. However, the patients that are placed in the experimental group will receive an elevated dosage of etoposide while the dosages of all other drugs will remain the same. The patients not in the experimental group will receive the dosage before it was elevated. By increasing only the dosage of etoposide, it can be assumed that any reduction of tumor size that occurs will be due to the higher etoposide dosage. The dosage of all other drugs will not be reduced and remain consistent before, during, and after the trial to focus on the effect of etoposide. If the experimental group presents reduced tumor size, it can be
concluded that it is due to the higher etoposide dosage. In other words, if the patients who do not receive the higher dosage do not show signs of tumor reduction, the relationship between the higher dosage of etoposide and tumor reduction becomes clearer. The clinical trial will focus solely on the effect of etoposide as the main drug with the highest efficacy in the treatment of retinoblastoma tumor growth. As the tumor growth categorization goes down, such as from B to D, the effect of dosage increase may not have substantial effects. However, retinoblastomas of group C to E will be included for this clinical trial to provide random and normal sampling.

The hypothesis for this clinical trial is that when the dosage of etoposide increases from an average of 75 mg/m²/day to 90 mg/m²/day and taken for single cycle length of 7-15 days, there will be signs of tumor reduction among the patients. According to the British Columbia Cancer Agency, BC Cancer Agency, the usual dose of etoposide given orally is 50 mg – 100 mg PO (orally) once daily for 3-10 days. The 3-10 days refer to a single cycle length, which is a single period of chemotherapy given. In a study performed Dunkel et al, the etoposide dosage was 250 mg/m²/day for 3 days. This dosage is higher than what is proposed for this hypothetical clinical trial, but given in a shorter period. Therefore, the dosage that has not been elevated will be 75 mg/m²/day but given for a longer period. The dosage was also determined based on the draft guidance of etoposide provided by the U.S. Food and Drug Administration. The draft guidance recommends an oral dosage that is estimated to be twice the dosage given intravenously. It is hypothesized that the dosage for IV injections is approximately 37 mg/m²/day to 38 mg/m²/day.
The higher dosage of etoposide has been chosen as the center of this hypothetical clinical trial because in high-dose chemotherapy, etoposide has shown positive results in treating the tumor by reducing tumor size.\textsuperscript{40} By increasing the number of days in a cycle from 3-20 days to 7-15 days, it is hypothesized that the higher dosage will present a reduction in tumor size among the patients.

What is unique to this hypothetical trial is evaluating the effect of a higher dosage of etoposide. Like all other chemotherapeutic drugs, to advance in the number of treatments available, different dosages of etoposide may be tested. However, in other clinical trials, etoposide is present in a cocktail and the dosages of all drugs vary but are elevated somewhat equally.\textsuperscript{54-56} By elevating only etoposide within the cocktail and maintaining the concentrations of the other drugs, the effects of etoposide on tumor reduction may be observed and analyzed.

Although intravenous (IV) injections are the standard procedure in the distribution of etoposide and other chemotherapy drugs, for this clinical trial, the focus will be on the oral distribution, or capsule, of etoposide given each cycle length. There are many FDA-approved dosages for intravenous distribution of etoposide\textsuperscript{57} On the other hand, there is only one FDA-approved dosage for the capsule distribution, which is 50 mg distributed by the drug company Mylan.\textsuperscript{58} Many clinical trials also focus on various dosages distributed through IV. Therefore, utilizing the clinical trials that discuss the oral distribution of etoposide, a hypothetical clinical trial will be created in search of a dosage that may potentially be FDA-approved and distributed. Despite the focus of oral distribution for this trial, it is important to know that many patients receive etoposide intravenously only, orally only, or intravenously and orally. Patients may receive IV
injections of etoposide during hospitalized stays, but during this trial, the patients will only receive etoposide orally. All other drugs will continue to be distributed as normal, which may be IV injections.

A slow-releasing capsule will be used to distribute the higher dosage of etoposide, which may result in lowering toxicity and alleviate toxic shock. Toxic shock occurs when the body reacts to healthy cells that are destroyed by drugs used in chemotherapy. This occurs because drugs prescribed during chemotherapy are poisonous. Therefore, the drugs are not used for prolonged periods, but in high dosages for short periods of time. For this trial, the slow-releasing capsule will allow a higher dosage to be prescribed for a longer single cycle of chemotherapy.

The body weight of the patients selected for this trial will be highly focused due to its large contribution in the process of determining a safe dosage of etoposide. Although the dosage is not significantly increased, it is assumed it is high enough to show results among the younger age group. The higher dosage is assumed to be safe for the clinical trial, which will be determined through blood work and supervision throughout the clinical trial.

For this trial, etoposide will be given in a slow-releasing capsule. The effect of a slow release will potentially reduce the level of toxicity present at the body at one moment. By controlling the rate at which etoposide will be released into the body, the level of toxicity may also be controlled. It is hypothesized that a higher dosage of etoposide could cause the body to react through toxic shock. To mediate, or counteract to a certain extent, the slow-releasing capsule will provide the parameters to prevent a severe response to the higher dosage of etoposide. The slow-releasing capsule is also
used for this clinical trial to study its efficacy of a higher dosage of etoposide for children. It is hypothesized that the slow-releasing capsule will allow allowing a higher dosage of etoposide to be prescribed for a prolonged period of time, 7-15 days. The body will not go into toxic shock as the dosage of etoposide is released in this hypothetical treatment. There are many drugs currently on the market that utilizes slow-releasing capsules such as Tylenol and Advil.

James R. Lawter and Michael G. Lanzilotii patented the composition of the microencapsulation for drugs that are slow-releasing in October 1990. The company that originally distributed the microencapsulation was the American Cyanamid Company. The composition of the capsules included the volatile silicone fluids that were used in the hardening of the capsule. These silicone fluids included octamethylcyclotetrasiloxane and decamethylcyclopentasiloxane; both molecules are volatile, can be easily evaporated at room temperature. Many slow-releasing capsules are now made with a cellulose derivative polymer called hydroxypropylmethylcellulose; it is also called methocel. As a cellulose derivative polymer, hydroxypropylmethylcellulose forms a consistent drug diffusion through the gel that forms when it is hydrated. The release of the drug is controlled by the diffusion of the drug across the gel and through the dissolution of the soluble drug into the water, which penetrates into the drug core after it dissolves the capsule.

In an experiment by A. Solano, et. al, the effects of a sustained release of etoposide in a poly(ε-caprolactone), PCL, implants were studied. Polycaprolactone, a non-toxic, biodegradable polymer, is used commonly in controlled drug delivery due to its high permeability to several drugs. It also has the possibility of a long, sustained drug
release rate and can be fully excreted from the body. Using the Melt Method, etoposide was thoroughly dispersed into the PCL that had melted. The results of this experiment showed promising results with approximately 12% of etoposide being released during the first 15 days in analysis performed on the implant, in vitro. The first phase presented a small burst of fast release. However, results showed a gradual decrease in release rate with approximately 66% of etoposide released in the second phase. Results performed in vivo also presented similar results with approximately 19% of the drug being released from the implant during the period between the 6th and 25th day of the trial.

Using the results from Solano’s experiment, it can be predicted that the higher etoposide dosage will be released slowly, which will allow the body to take on the higher dosage. If the PCL implants were to be utilized for the hypothetical trial, the higher dosage will have approximately 12%-19% of etoposide released from the implants. There also would not be safety concerns regarding the long-term effects of using a slow-releasing capsule due to PCL’s ability to be fully excreted from the body. Although the body may retain levels of etoposide, the effects of the drug will be not be elevated due to the use of a slow-releasing capsule.

**Materials and Methods**

In a potential clinical trial, a group of 240 children varying from the ages of 3 months to 10 years will be studied. Hypothetically, 240 children will be chosen to accommodate for any patients that will have to drop out in the middle of the trial. It is highly unlikely that the results will be obtained from all 240 children. The reasons behind why some of the children will be removed from the trial will be discussed in the results
section. 120 children will be placed in the experimental group and receive the capsule with the elevated dosage of etoposide. The other 120 children will be placed in the control group and receive the dosage before it was increased, which is 75 mg/m$^2$/day. The control group will be used to directly observe the effect of the increased dosage of etoposide. For both the experimental and control group, all patients will continue to receive the dosages of all other drugs prescribed prior to the clinical trial. The children will be recruited from hospitals such as Mount Sinai, New York Presbyterian, and North Shore. All participants will be chosen randomly among the children that have been diagnosed with retinoblastoma at any of these hospitals. In order to provide the most accurate information, ophthalmologists will perform a variety of thorough examinations, which includes a comprehensive dilated eye exam$^{62}$ and magnetic resonance imaging (MRI), on each patient. Neurologists will also study the MRI photos taken to truly understand the extent of the growth of tumor for each patient. The identification of the tumor growths of each patient will vary from Group A to Group E, with Group E being the most severe. Any patients with tumors that have reached the stage at which enucleation is the only treatment available have been dismissed.

The comprehensive dilated eye exam will be performed to allow an examination of the interior of the eye seen through the pupil. By widening the pupil, the ophthalmologists will be able to visualize the extent of the tumor growth to a certain extent. If the tumor growth has reached Group C in its classification, it should be visible during this test. The tumor may be immediately visible on the lower region of the iris or may be visible when the ophthalmologists examine the interior region of the eye, including the vitreous. The MRI will be done to obtain a more extensive image of the
tumor growth within the eye. As seen in Figure 9, the eye that contains the tumor is substantially larger than the other. However, the size of the eye on the images will not be the defining characteristic for the presence of a tumor. The MRI images of the patients will be compared to those of normal subjects, subjects that do not present any chromosomal abnormalities or presence of tumor growth in the eye. With the results of the MRI image comparisons, the eligibility of all 300 patients will be verified in order to continue the clinical trial.

![Figure 9. MRI of extraocular extension of retinoblastoma](image)

Once the patients have been verified, a full physical examination will be performed. Along with the physical examination, blood work will be completed to record the blood pressure, iron levels, and cholesterol levels. Blood work will also be performed specifically to confirm that the patients are not taking any drugs that will affect the clinical trials by interacting with the pharmacophore of etoposide. Special care will be taken to ensure the blood work and physical examination is precisely and accurately done to ensure the right dosage is being given to each patient. This is to ensure no child receives a dosage that may be lethal to his/her body.
During the duration of the clinical trial, which will extend from approximately 5 to 10 years, regular blood work as well as renal and hepatic, liver, function will be performed every 2 weeks for the duration of the trial. These tests will be performed to closely monitor white blood cells as well as red blood cells to assess the presence of haematological toxicity. Haematological toxicity tests check for neutropenia, anemia, and thrombocytopenia. Neutropenia is an abnormally low count of white blood cells, anemia is an inadequate amount of red blood cells to carry oxygen throughout the body, and thrombocytopenia is a low blood platelet count. Platelets stop bleeding through clumping, which block the blood vessels. The blood work will be performed regularly to monitor the levels of etoposide in the body and its relation to haematological toxicity. If levels of etoposide are detected to be too high, precautions will be taken to ensure the levels do not become lethal. Precautions will also be taken with any detection of high levels in regards to haematological toxicity, which may be a sudden fluctuation of white blood cells, of red blood cells, and/or of blood platelet count. If etoposide levels are detected to be lethal and signs of haematological toxicity are present, the patients will be immediately released from the trial. Dilated eye exams and MRIs will also be taken every 6 months for all patients.

The level of toxicity will be evaluated using the Common Toxicity Criteria, version 2.0, in the case of an adverse event. An adverse event describes any symptom or disease that is a momentary result of the use of the drug during a treatment or procedure that may or may not be considered affiliated with the treatment or procedure. Toxicity levels will be checked at two points during the duration of the trial. The Common Toxicity Criteria is used to categorize the effects of various drugs administered
as part of cancer treatments. There are 6 grades: grade 1 represents no adverse event or within normal limits, grade 1 represents a mild adverse event, grade 2 represents a moderate adverse event, grade 3 represents a severe and undesirable adverse event, grade 4 represents a life-threatening or disabling adverse event, and grade 5 represents a death related to adverse event. The first checkpoint will be in 5 years, the halfway point of the trial. For any patients that are presenting anything greater than grade 2 will receive a lower dosage. Measures will be taken to ensure all patients stay within grades 0 to 2, preferably grade 0.

**Expected Results**

Among the 240 patients that are to participate in this clinical trial, it is assumed approximately 90% will be able to complete the full trial. It is possible that 24 patients, will have to drop out of the trial due to different reasons. The reasons may include moderate to severe side effects to etoposide, such as fainting, hair loss, eye pain, difficulty breathing or swallowing, and seizures.\(^{68}\) Patients that present any signs of tumor growth will be removed immediately to ensure the proper treatment and medications are provided. If the patients have tumors within Group D or E, enucleation will be performed to prevent any further spread of the tumor.

Side effects are expected regardless of the dosage given. The following side effects are commonly seen in patients taking etoposide through oral prescriptions: fever, cough, chills and temporary hair loss.\(^{69}\) Hair is expected to grow back once the etoposide treatments are completed. In the experiment performed by B. Kremens, et. al, patients presented side effects such as diarrhea and severe mucositis, which is commonly seen in
cancer patients undergoing treatments. Mucositis occurs when the rapidly dividing epithelial cells that line the gastro-intestinal tract break down and become vulnerable to ulcerations and infection. The patients received analgesics, or painkillers, as well as antibiotics to mediate the effects. Other side effects present were nausea and vomiting. Similar to the experiment, if any side effects are present in the patients, appropriate treatments will be given to alleviate them. In an extreme case, if the patients are experiencing severe side effects that are hindering the effects of etoposide, they will be removed and treated immediately.

Along with the side effects stated above, the etoposide levels will be thoroughly checked in internal organs, such as the liver and kidneys. If etoposide levels are found to be low, the patients will be left to complete the trial. If etoposide levels are found to be moderate to severely high, the patients will be removed immediately due to the possibility of liver or kidney damage. The patients will be given the appropriate treatments to lower etoposide levels and treat any possible signs of liver and kidney damage.

From the remaining 216 patients that will complete the trial, a success rate will be determined at approximately 85% of patients with reduced tumors. For the children who were admitted at the first site of leucocoria, depending on the type of treatment used with etoposide, the intraocular tumor will be significantly reduced at an increased dosage.

Discussion

The results of this clinical trial may vary depending on the type of patients that participate. To find accurate results of the effect of increasing the dosage of etoposide, an
actual clinical trial will have to be performed. Among the 90% who complete the trial, it is hypothesized that most of the patients who received the elevated dosage presented reduced tumors. Etoposide is an FDA(U.S. Food and Drug Administration)-approved drug.\textsuperscript{70}

Etoposide contains properties that target the source of the tumor growth, the cells. By interrupting the supercoiling of DNA and the cell cycle, the retinoblastoma cells will not divide as quickly, which will lead to control of tumor growth. A higher dosage may result in more control, but, without proper guidance and instructions, the higher etoposide dosage may result in high toxicity levels in the body as well as resistance. It is assumed that the etoposide was given properly throughout the entire clinical trial, which includes all proper medications were taken with etoposide, the etoposide was taken at all proper times, and the etoposide was not mixed with anything that may hinder the effects of the drug or immensely enhance them.

Resistance is an important factor during this clinical trial. Some of the patients that do not show reduction in tumor size may be because of resistance to etoposide. One of the characteristics of etoposide includes possibility of resistance. The resistance may appear earlier in the clinical trial and may possibly appear after the trial. If the resistance appears after the trial, it must be taken into account. The follow-ups after the clinical trial are designed to keep track of the reduction of tumor size without the higher dosage of etoposide. After observing the patients after the trial, the effects of the higher dosage may be prevalent because the tumor will respond to the change in medication dosages. The key results that are obtained will be from the follow-ups as well as the end of the clinical trial.
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