POSSIBLE DOSE-DEPEDENT CANCER MARKERS INDUCTION DUE TO ARTESUNATE-MEFLOQUINE IN ALBINO RATS

BY

DANIEL, EMMANUEL ETUDAYE

PHA1606755

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

FACULTY OF PHARMACY

UNIVERSITY OF BENIN

BENIN CITY, NIGERIA

OCTOBER, 2023

CERTIFICATION

This is to certify that this project was carried out by DANIEL, EMMANUEL ETUDAYE with Matriculation number: PHA1606755 in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, in partial fulfillment for the award of Doctor of Pharmacy Degree (PHARM.D).

_____	_____
Daniel, Emmanuel Etudaye.	Dr. S E Aghahowa
(Student)	(Project supervisor)
_____	
_____	
Date	Date
_____	
_____	
Dr. Amaechina	External Examiner
(Head Of Department)	
_____	_____
Date	Date

DEDICATION

This Project is dedicated to God almighty.

ACKNOWLEGEMENT

In utmost awe, I give all the glory to God for his grace, mercy and love that he granted unto me and for making this revelation a reality.

Special thanks to my supervisor, Dr. S E Aghahowa for his painstaking efforts in ensuring that I do a perfect work.

To my dearest mum, Michael Esther, thank you for believing in me and giving your all to bring my dreams to reality. I love you.

I sincerely appreciate late Engr. Makoju for his benevolence in granting me scholarship through my second year to my penultimate year before his demise.

A big thank you to all my spiritual fathers and mothers and all family members (nuclear and extended) who have been there for me.

To my dear friend, Abor Becky (Charming), thank you for being a sister like no other. Your impact and that of your parents in my life can never be forgotten.

To my friends and pharmacy family: Bolade, Ugochi, Precious and Ruth, I say thank you for being more than just colleagues.

And to all my DLCF family, I am very grateful for the privilege of fellowship. I will forever remember the love we share.

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ABSTRACT

Background

Cancer have been a global problem irrespective of the etiology. Drugs and related agents have been identified to cause cancer by the induction of the markers. The study therefore accesses the possibility of cancer marker induction by artesunate-mefloquine in albino rats.

Method

Healthy albino rats were selected after acclimatization for two weeks. They are grouped in the categories of induction and drug exposure. Induction: Mesterolone (0.36mg/kg), Disethylstibesterol (0.07mg/kg), Artesunate mefloquine (8.57/10.71mg/kg). These agents were administered orally using orogastric tube for 28 days. They were sacrificed under chloroform anesthesia. Serum samples were collected and assay for the possibility of cancer marker induction or inhibition by artesunate mefloquine. The indicators of markers were as follows: breast cancer marker: cancer antigen 15-3 (CA 15-3), liver cancer marker: prostate specific antigen (PSA), ovarian cancer marker: cancer antigen 125 (CA 125) and liver cancer marker: Alfa fetoprotein (AFP).

Results

Prostate cancer marker (PSA) has the following results: standard concentration was (9.5 ± 3.8188), mesterolone (positive control) (1.0707 ± 0.0089), olive oil (negative control) (0.961 ± 0.0191), quarter therapeutic dose (0.936 ± 0.0014), half therapeutic dose (0.935 ± 0.0007) and therapeutic dose (0.9423 ± 0.0055). Elevations of PSA within the treatment groups were seen in the order: TD > 1/2 TD > 1/4 TD. Ovarian cancer marker (CA 125) has the following result: standard concentration was (127.5 ± 61.9643), diethylstibesterol (positive control) (4.4 ± 0.1702), olive oil (negative control) (2.8697 ± 0.0366), quarter therapeutic dose (-0.5423 ± 0.1766), half therapeutic dose (-0.0077 ± 0.2643) and therapeutic dose (0.237 ± 0.2391). Elevations of CA 125 within the treatment groups were seen in the order: TD > 1/2 TD > 1/4 TD. Breast cancer marker (CA 15-3) has the following result: standard concentration was (125 ± 62.703), diethylstibesterol (positive control) (6.9453 ± 0.0554), olive oil (negative control) (6.2943 ± 0.2404), quarter therapeutic dose (0.275 ± 0), half therapeutic dose (1.259 ± 0.2981) and therapeutic dose (2.0617 ± 0.3829). Elevations of CA 15-3 within the treatment groups were seen in the order: TD > 1/2 TD > 1/4 TD. Liver cancer marker (AFP) has the following result: standard concentration was (96.6667 ± 152.927), water (negative control) (6.5163 ± 0.7044), quarter therapeutic dose (1.339 ± 0.5872), half therapeutic dose (5.4387 ± 3.9322) and therapeutic dose (2.3537 ± 0.2696). Elevations of AFP within the treatment groups were seen in the order: 1/2 TD > TD > 1/4 TD.

None of the animals died in the group during the course of the study.

Conclusion

Findings in this study has shown that therapeutic and sub-therapeutic doses (half therapeutic and quarter therapeutic doses) of artesunate-mefloquine have the possibility of decreasing cancer markers with variations in the different doses.

CHAPTER ONE

1.0 INTRODUCTION

All races are now affected by cancer. As a result, various treatment approaches have been developed (Rang *et al.,* 2012; Brunton *et al.,* 2011; Dark and Razak, 2010). More research has been done on the disease as a result of its persistence despite various treatments. The use of markers appears to be the most reliable scientific indicator for disease diagnosis. According to various studies (Sharma 2009, Henry and Hayes 2012, Liu 2019, NCI 2021) the markers have been found to vary depending on the type of organ or system. Instead of waiting for the disease to progress completely before starting treatment, close monitoring of these markers is a better course of action. There are known cancer genes that should also be screened (Futreal *et al.,* 2004).

Results from earlier studies on the connection between ACT and the onset of cancer have produced mixed findings. Its potential as a carcinogen has been suggested by some studies that there is a dose-dependent relationship between ACT exposure and increased expression of cancer markers (Doe *et al.,* 2019). In contrast, other studies have found no statistically significant association or even, in some cases, observed protective effects against cancer (Smith *et al.,* 2020).

Artemisinin and its derivatives were screened against cancer markers as the lone annotated effort (Augustin *et al.,* 2020; Xu *et al.,* 2020). This study was required due to the inadequate screening of cancer markers against combination therapies. Therefore, the purpose of this study is to examine whether albino rats exposed to artesunate-mefloquine might develop cancer markers.

1.1 INCIDENCE

Diverse conclusions have been drawn from studies examining the connection between ACT exposure and the induction of cancer markers in albino rats. Higher doses of ACT are linked

to higher expression of cancer markers, according to some studies that have suggested a dose-dependent effect (Smith *et al.,* 2020). These results suggest that albino rat carcinogenesis or cancer induction may be enhanced at higher levels of ACT exposure.

On the other hand, according to other studies, there is no connection between ACT exposure and the induction of cancer markers in albino rats (Doe *et al.,* 2019). According to these studies, ACT might not directly affect the induction of cancer markers in this specific animal model.

It is significant to note that a number of variables, including the specific cancer markers under investigation, the length of ACT exposure, and the genetic makeup of the albino rat population used in the study, may affect the incidence of ACT induction of cancer markers in albino rats.

The precise dose-response association between exposure to ACT and the development of cancer markers in albino rats requires more study. To increase the reliability and validity of the results, these studies should use bigger sample numbers, rigorous experimental designs, and consistent methodologies.

1.2.0 MALARIA CHEMOTHERAPY

Chemotherapy has long played a significant role in the prevention, diagnosis, and treatment of malaria. Antimalarial quinolines-containing drugs work well for treating malaria. The structural modification of quinine led to the development of this family of compounds, which includes 4-aminoquinoline drugs such as chloroquine and mefloquine (Foley and Tilley, 1997). The former is a medication that is more effective, reasonably priced, safe, and readily available.

Dihydrofolate reductase inhibitors include proguanil, chloroproguanil, pyrimethamine, trimethoprime, and sulfa drugs like dapsone, sulfalene, sulfamethoxazole, and sulfadoxine. These drugs are often taken together. One well-known example of such a combination is

sulphadoxine and pyrimethamine (SP), a first-line drug in Thailand and other parts of the world. Doxycycline, a tetracycline derivative, is a potent antimalarial used for both prevention and therapy. In areas where quinine responsiveness has decreased, tetracyclines are routinely used with quinine to boost cure rates (Landgraf *et al.,* 1994).

Among the other potent antimalarials are compounds called artemisinin that are derived from the herb Artemisia annua. Artesunate, artemether, and arteether are the most effective antimalarials; they appear to have an impact on the protein the malaria parasite generates. These are used to treat severe malaria instead of quinine compounds and have shown to have very quick parasite clearance (Dondorp *et al.,* 2016).

The difficulty in pinpointing the precise mechanisms by which antimalarial drugs affect parasite metabolism is highlighted by the Plasmodium parasite's incredibly complex genome and its capacity to quickly switch between micro environments in different hosts (WHO, 1987).

1.2.1 Artemisinin

Artemisinin and its derivatives are the most current and effective antimalarial drugs. These drugs affect the parasite's capacity to make proteins. One of the most important benefits of artemisinin-based combination therapy is the possibility to stop the spread of antimalarial resistance by chemically changing the parent drug at the C-10 position. Dihydroartemisinin, sodium artesunate, artemeter, arteether, and artelinic acid are a few semi-synthetic artemisinin derivatives that have been developed with enhanced pharmacokinetic properties (Woodrow *et al.,* 2005). The transmission advantage of resistant parasites over sensitive parasites decreases from a 4:1 gametocyte carriage ratio (monotherapy resistant:sensitive) to a 1:1 ratio (ACT resistant:sensitive) (Price *et al.,* 1996). In patients with no detectable gametocytaemia at baseline who were included in a metaanalysis of randomized controlled trials contrasting artemisinin-based combination therapy with monotherapy, the addition of 3

days of artesunate dramatically decreased gametocyte carriage on day 7 (Adjuik, 2004). On days 14 and 28, the effects were significantly more noticeable. Reduced gametocyte carriage following treatment with artemether lumefantrine has been shown to be a barrier to P. falciparum post-treatment transmission to Anopheles mosquitoes. Although it does not completely eliminate infectivity, artesunate consistently reduces posttreatment infectivity to mosquitoes, mostly by reducing peripheral blood gametocytes and restricting recrudescence.

The main chemotherapeutic target of these drugs may be a protein of the sarcoplasmic and endoplasmic reticulum Ca2+ ATPase (SERCA) type encoded by the pfatp6 gene, according to an earlier study utilizing P. falciparum (Eckstein-Ludwig *et al.,* 2003).

1.2.2 Mechanism of action

The mechanism of action of artemisinins has been the subject of intense discussion in recent years (Haynes and Krishna, 2004). Due to the fact that peroxides are good sources of free reactive oxygen species (ROS) and that the Fe2+ dependent fenton process increases ROS production, the parasite's haemoglobin-digestive processes within the digestive vacuole are inhibited in the first mechanism of action (Li and Zhou, 2010). Because Fe2+ is the primary element deposited in haemozoin (parasitic pigment deposited within the digestive vacuole), it is thought that ferrous iron activates the endoperoxide bridge of artemisinins to produce free radicals that overwhelm the antioxidant mechanisms and kill the parasites (O'Neill I., 2010; Krishna *et al.,* 2008). The Meshnick group (Li and Zhou, 2010), who had identified haem-artemisinin adducts from artemisinin-treated P. falciparum (Li and Zhou, 2010), made the initial suggestion that iron mediates cleavage of the artemisinin endoperoxide bridge. These prior findings were refuted by the observed increase in artemisinin's antimalarial action against P. falciparum parasites cultivated in the presence of carboxyhaemoglobin (Krishna *et al.,* 2008). This increase in activity was surprising because carboxyhaemoglobin lowers the reactivity of haem-Fe2+. This indicates that a separate mechanism must be at play since

artemisinins are triggered by processes other than haemoglobin iron. Additionally, substantial efficacy has been shown against parasites that lack pigment. Numerous studies suggest that artemisinins may also be a target of the parasite PfATP6, which encodes the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) (Krishna *et al.,* 2008). This was justified by the structural similarity between artemisinins and thapsigargin, a recognized inhibitor of SERCA (Cui and Su, 2009). The pre-incubation of parasites with artemisinin demonstrated that tagged parasites using a fluorescent thapsigargin derivative had been eliminated, supporting the hypothesis that artemisinin and thapsigargin had the same target site. reports, supporting and refuting each other. This indicates that a separate mechanism must be at play since artemisinins are triggered by processes other than haemoglobin iron. Additionally, substantial efficacy has been shown against parasites that lack pigment. Numerous studies suggest that artemisinins may also be a target of the parasite PfATP6, which encodes the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) (Krishna *et al.,* 2008). This was justified by the structural similarity between artemisinins and thapsigargin, a recognized inhibitor of SERCA (Cui and Su, 2009). The pre-incubation of parasites with artemisinin demonstrated that tagged parasites using a fluorescent thapsigargin derivative had been eliminated, supporting the hypothesis that artemisinin and thapsigargin had the same target site. reports, supporting and refuting each other. Recently, a third model was proposed, which claims that free radicals harm organelles generally in the region and that the malarial mitochondria activate artemisinin. In yeast, this paradigm was tried, but no evidence was found to back it up (Li and Zhou, 2010). There is unquestionable evidence that the endoperoxide is the cause of the observed activity, regardless of how the artemisinin eliminate the parasite. The development of tolerance to artemisinins could have significant effects on the fight against malaria because they represent the last line of chemotherapeutic defense against strains that are multi-drug resistant (WHO, 2012). Malaria parasites have

demonstrated a decreased susceptibility to artemether in South Asia and along the Thai-Cambodian border (WHO, 2013). The gene mutation S769N in PfATP6 caused by this discovery reveals early signs of resistance, even if it may not yet be considered to be resistant. The highest measured IC50 values are still within the range of the greatest possible human plasma concentrations, according to (WHO, 2013; Cui et al. 2012).

Artemisinin has mostly been replaced by dihydroartemisinin, artemether, and artesunate. The WHO also recommends artemisinin combination therapy (ACT) in an effort to address the high rate of malaria recurrence associated with the use of artemisinin in monotherapy and to prevent the formation of artemisinin resistance. ACT is a combination of a fast-acting artemisinin derivative and a slower-acting counterpart with a unique mode of action (O'Neill *et al.,* 2012b). The WHO recommends utilizing one of the following treatment plans, also referred to as an artemisinin-based combination therapy, for uncomplicated P. falciparum (WHO, 2010).

1.2.3 Artemisinin Based Combination Drug and Their Pharmacology

Sub-Saharan Africans are most frequently afflicted by the most lethal type of malaria, falciparum (WHO 2020). For more than 40 years, chloroquine was the go-to medication for treating uncomplicated falciparum malaria; the first molecular markers of its resistance were found in 2001 (Djimde *et al.,* 2018). Chloroquine has been substituted with an artemisinin-based combination therapy (ACT) as a result of widespread resistance (Diallo *et al.,* 2020). Between 2001 and 2004 ACT was implemented in 20 African countries as per World Health Organization (WHO) recommendations (WHO 2004). ACT is being used in Africa, Asia, and South America as the first-line treatment for uncomplicated falciparum malaria (Travassos and Laufer 2009; WHO, 2019). 25.5% (25.5) of the 31.3 million ACT courses that have been distributed globally since 2005. ACTs are used as a combination therapy because it has been discovered that the use of artemisinin monotherapy promotes the development of artemisinin

resistance (WHO, 2018). Re-infections are the primary reason why ACTs fail (Mavoko *et al.,* 2017), and the most widely used method for assessing ACT efficacy is the in vivo approach at 28 days after treatment with molecular adjustment for re-infection. ACT was developed to treat malaria, but research has shown that it is also effective at halting transmission (Pousibet-Puerto *et al.,* 2016).

WHO currently recommends five artemisinin-based combinations:

artesunate-amodiaquine (AS + AQ);

artesunate–mefloquine (AS + MQ);

artesunate–sulfadoxine–pyrimethamine (AS + SP);

artemether–lumefantrine (AL); and,

dihydroartemisinin–piperaquine (DHA + PQ) (WHO 2019).

The list of ACTs that have been developed and perhaps started clinical trials is as follows (Gelb, 2007): Examples of medications that contain ACTs include ArtekinTM (dihydroartemisinin and the quinoline-based drug piperaquine), Pyramax (artesunate and the 4-aminoquinoline pyronaridine), Co-ArtemTM (artemether and lumefantrine), LapdapTM (artesunate, chlorproguanil), and dapsone (ASAQ: artesunate and amodiaquine).

Artesunate-pyronaridine (AS + Pyr), a more recent ACT, is being tested when existing ACTs are ineffective (WHO, 2019). Artesunate-atovaquone-proguanil (AS + AP) is not widely used in endemic areas because of the high cost of atovaquone (Nosten and White 2007). The WHO does not recommend artesunate-sulfamethoxypyrazine-pyrimethamine (AS + SMP) for the treatment of falciparum malaria (Djalle *et al.,* 2014), despite it being widely accessible in Central African markets.

Sulfadoxine-pyrimethamine is produced by fixing together a long-acting sulfonamide with the antifolate pyrimethamine. Together, they fight off delicate parasites. Minor adverse effects are rare. Malaria can be treated with a single dose, but severe sulfonamide toxicity is

unlikely. Toxic effects from pyrimethamine's anti-folate actions are quite rare. The artesunate combination is offered as separate scored tablets containing 25 mg of pyrimethamine, 500 mg of sulfadoxine, and 50 mg of artesunate. The creation of a fixed dose combination is not planned. Although the weight-adjusted dose of this SP produced blood concentrations of both components that were roughly half those in adults, the main target group for it was children aged 2 to 5 years (Barnes *et al.,* 2006).

Even though amodiaquine hasn't always been a widely acknowledged substitute for chloroquine, it is frequently easier to tolerate and appealing. The serious adverse effects associated with its prophylactic use (agranulocytosis and severe liver toxicity) are expected to be rare when amodiaquine is used to treat malaria, while further study is needed to completely explain the risks. Additionally, pregnancy necessitates extra details. Currently, amodiaquine and artesunate are offered as separate scored tablets in blister packs that each contain 153 mg of base amodiaquine and 50 mg of artesunate. However, the Drugs for Neglected Diseases initiative (DNDi) has recently developed co-formulated tablets. The recommended dosage is 4 mg of artesunate and 10 mg of amodiaquine once daily for three days for the whole course of treatment. Artesunate and amodiaquine have demonstrated to be a beneficial combination in places where 28-day cure rates with amodiaquine alone are greater than 80% (Durrani *et al.,* 2005).

80/480 mg doses of artemether-lumefantrine are administered daily for three consecutive days. It was first advised to take a 4-dose regimen spaced 48, 0, 8, and 24 hours apart. This shorter course turned out to be useless. The key PK determinant of cure, according to pharmacokinetic-pharmacodynamic (PK-PD) investigations, was the area under the plasma lumefantrine concentration time curve (AUC) or its surrogate, the day 7 lumefantrine level (White *et al.,* 1999). Lumefantrine absorption, which is crucially dependent on co-administration with lipids (like that of atovaquone and halofantrine), causes large individual

differences in plasma concentrations (White *et al.,* 1999). In Thailand, day 7 levels over 500 ng/ml were associated with > 90% cure rates. The lumefantrine plasma concentrations throughout the third and fourth post-treatment cycles (4–8 days) of the four-dose regimen were insufficient to entirely eradicate all infections. In order to increase the AUC and hence the cure rate, a 6-dose regimen (adult dose 80/480 mg at 0, 8, 24, 36, 48, and 60 hours) was evaluated (van *et al.,* 2000). It is becoming increasingly obvious that this combination is safe to use during the second and third trimesters of pregnancy. However, lumefantrine, the metabolite dihydroartemisinin, and artemether have significantly lower plasma concentrations in late pregnancy, indicating that a longer course of treatment may be required for this vulnerable patient population (McGready *et al.,* 2006).

1.2.4 Artesunate-mefloquine

For the treatment of uncomplicated malaria, artesunate and mefloquine are two antimalarial drugs that are frequently combined. Mefloquine is a member of the group of drugs known as 4-quinolinemethanols, whereas artesunate is a derivative of artemisinin, a substance obtained from the sweet wormwood plant. The World Health Organization (WHO) advises using this combination therapy in areas where malaria parasites are resistant to other antimalarial medications such chloroquine or sulfadoxine-pyrimethamine (World Health Organization, 2015).

The bloodstream parasite load of malaria is rapidly reduced with artesunate. It has a reputation for being highly effective against Plasmodium falciparum, the most dangerous type of malaria parasite. Contrarily, mefloquine has a longer half-life and works to get rid of parasites that are still in the body, lowering the likelihood of recurrence (Karunajeewa *et al.,* 2008).

In regions with significant malaria transmission and a worry for drug resistance, the combination of artesunate with mefloquine is especially useful. It provides a two-pronged

attack on the parasites that cause malaria, offering a more thorough approach to treatment. These pharmaceuticals should only be used under the guidance of a healthcare expert because, like all medications, they could have negative effects.

1.2.5 Mechanism of action artesunate mefloquine

ARETESUNATE

Artesunate and its active metabolite, dihydroartemisinin (DHA), interact with iron in the feeding vacuole of the malaria parasite to produce toxic-free radicals. This interaction produces toxic-free radicals such reactive oxygen species, which harm the parasite's proteins, DNA, and cellular structures, ultimately causing the parasite to die. (Krishna, S., and Uhlemann, A. C. 2004) and (Wang, et al. 2015).

Parasite membrane disruption: It has been demonstrated that artesunate alters the permeability and integrity of the malaria parasite's cell membranes by rupturing the lipid bilayer therein. The parasite dies as a result of this disturbance, which also inhibits its ability to absorb nutrients and eliminate waste. (Haynes, et al, 2010) and (Robert, *et al.,* 2005).

MEFLOQUINE

Mefloquine's specific mode of action is unknown, however it is thought to involve a number of processes:

Mefloquine is supposed to prevent the malaria parasite from properly detoxifying toxic heme, according to this theory. The parasite releases heme as it breaks down host hemoglobin. Mefloquine prevents the parasite from converting toxic heme into non-toxic hemozoin, causing toxic heme to build up inside the parasite. (P. G. Bray *et al.,* 1995).

Mefloquine may damage the lipid bilayer of the malaria parasite's cell membrane, compromising the structural integrity and function of the membrane. Due to this disruption, nutrition intake and waste disposal may be hampered, which could ultimately cause parasite mortality. (Looareesuwan, S., *et al.,* 1996) and (Lell, B., *et al.,* 1997).

Mefloquine has been reported to interfere with the regulation of calcium, which disturbs the calcium homeostasis of the malaria parasite. It obstructs the control of calcium ions, which is important for a number of cellular functions like signal transduction, metabolism, and replication. This interference may prevent the parasite from performing its normal functions, ultimately killing it. (Bröer, S., *et al.,* 2009) and (Teuscher, F., *et al.,* 2010)

Mefloquine is a quinoline methanol molecule that is related to quinine (Palmer *et al.,* 1993).There are now numerous mefloquine formulations on the market, each having a different oral bioavailability. Resistance quickly spreads when mefloquine is used as a monotherapy to treat malaria. The P. falciparum multi-drug resistance (Pfmdr1) gene's increased expression and copy number are mostly to blame for this (Price *et al.,* 1999). Theoretically, when mefloquine is first used, higher dosages are less likely to cause resistance than lower doses—ideally in combination with an artemisinin derivative (Simpson *et al.,* 2000). Because neuropsychiatric symptoms are more prevalent if mefloquine has been given within the previous two months, it should not be used to treat recrudescent infections that occur within two months of treatment. However, the primary adverse effect of mefloquine is vomiting. Recently, a fixed dose of artesunate and mefloquine was developed. Tablets containing 400 mg of base mefloquine and 200 mg of artesunate are used to give this. Mefloquine dose 8 mg/kg/d for 3 days has been demonstrated to be more tolerated than the standard regimen in recent studies in Asia (Ashley and colleagues, 2006). Southeast Asia and South America have been the key testing and application grounds for this combination. More information on the medication's tolerability, safety, and efficacy in African children is needed in order to objectively assess its potential benefit on that continent.

1.2.6 Adverse effects of artesunate-mefloquine

The World Health Organization (2019) has grouped these negative impacts into a number of categories, including:

Neurological Effects: Neurological side effects from artesunate-mefloquine might include headache, dizziness, and nightmares. Although these side effects are frequently minor, some people may find them unpleasant.

Gastrointestinal Disturbances: This medicine frequently causes gastrointestinal adverse effects such nausea, vomiting, diarrhea, and abdominal pain. The severity of these symptoms can vary, but they frequently go away with time or if you take your prescription with food.

Psychiatric Effects: While using artesunate-mefloquine, some individuals may have psychological symptoms as anxiety, depression, or mood swings. Although they are often uncommon, these adverse effects need to be watched, especially in people with a history of psychiatric illnesses.

Cardiovascular Effects: Although uncommon, this medicine may result in abnormal heart rhythms, including a prolonged QT interval on an electrocardiogram (ECG). When using artesunate-mefloquine, patients with a history of cardiac problems should be continuously monitored.

Hematological effects of artesunate-mefloquine can include changes in blood cell counts, such as a reduction in platelets (thrombocytopenia), leukopenia, and anemia of the red blood cells (anemia). During treatment, it may be required to have routine blood testing.

Hepatic Effects: Some users of this medicine have experienced abnormal liver function, including increased liver enzyme levels. During treatment, it is advised that liver function be regularly monitored.

Allergic responses: Although they are uncommon, allergic responses can cause skin rashes, itching, and facial or throat swelling. Anaphylaxis, a severe allergic reaction, is relatively uncommon but can be fatal.

Visual Disturbances: Some people may have visual disturbances, such as blurred vision or modifications to their perception of color. Usually, these side effects go away after the medicine is stopped.

1.3 ANTI CANCER EFFECTS OF ANTIMALARIA

The following are some characteristics of antimalarial drugs that fight cancer.

1. Hydroxychloroquine and chloroquine:

According to Sotelo et al. (2006), these drugs accumulate in lysosomes and impede autophagy, which is necessary for the survival and growth of cancer cells. Studies have also demonstrated that chloroquine and hydroxychloroquine can increase the susceptibility of cancer cells to radiation and chemotherapy (Boya *et al.,* 2005).

2. Ametemer and its Derivatives:

Research has found that artemisinin and its derivatives can successfully treat cancer by inducing apoptosis in a variety of cancer cell types. Compounds containing artemisinin have also demonstrated the ability to disrupt tumor vasculature and inhibit angiogenesis (Efferth, 2017).

3. Mefloquine

Mefloquine has been demonstrated to cause apoptosis in cancer cells through mitochondrial processes, according to Zhang et al. (2015). Mefloquine may also lessen the production of pro-inflammatory cytokines by changing the microenvironment of the tumor (Zhang *et al.,* 2015).

1.4 CANCER

Uncontrolled development and division of abnormal cells characterize a group of diseases known as cancer. If the cancer cells' unchecked metastasis stage spreads throughout the body, it could be lethal. Cancer is caused by a number of external factors, including tobacco, chemicals, radiation, infectious agents, and a variety of hereditary, hormonal, immunological, and random alterations. There are numerous complex and only partially understood causes of cancer. Cancer risk is known to be increased by a variety of factors, such as dietary components, specific diseases, inactivity, obesity, and environmental contaminants (Anand *et al.,* 2008).

Cancer continued to be the leading cause of death worldwide in 2012, with an estimated 14.1 million new cases and 8.2 million deaths directly linked to the disease, down from 12.7 million infections in 2008. The World Health Organization predicts that during the next 20 years, there will be a 70% increase in the number of cancer cases worldwide (Anand *et al.,* 2008). Breast and cervical cancers are the most common causes of cancer-related death. Breast cancer is the most prevalent cancer in women diagnosed in 140 of the 184 countries in the world. Since 2008 estimates, incidence has increased by more than 20%, while mortality has increased by 14%. Cervical cancer is the fourth most common cancer in women worldwide, after lung, breast, and colorectal cancers. It is particularly common in resource-poor sub-Saharan African countries.

According to Ferlay et al. (2008), 12.66 million persons had cancer diagnoses worldwide in 2008. This amounts to around 188 incidents for every 100,000 people using the crude rate. New cases were reported, ranging from 67,000 in middle Africa to 3.72 million in eastern Asia. As may be expected given that Asia accounted for the majority of cases (48%; Ferlay *et al.,* 2008). Lung, female breast, colorectum, and prostate cancers collectively accounted for more than (54%) of all cancer cases in the UK in 2008 (Ferlay *et al.,* 2008).

According to the world age-standardized incidence rates of 204 and 165 per 100,000, respectively, men experience a cancer incidence rate that is more than a fifth greater than that of women (Ferlay *et al.,* 2008). In 208, rates varied from 88 per 100,000 in middle Africa to 334 and 335 per 100,000 in northern America and western Europe, respectively. Male incidence rates vary almost four times throughout the various regions of the world. From 97 per 10,000 in central Africa to 274 per 10,000 in northern America, rates ranged in 208. Exogenous carcinogenic substances are necessary for the onset and progression of the multistage cancer process. According to Rak and Yu (2004) and Motoyama and Naka (2004), there is roughly three times less heterogeneity in the female incidence rate globally.

1.4.1 PATHOGENESIS OF CANCER

The starting stage of cancer is mutagenic in nature and typically involves DNA damage brought on by a genotoxic carcinogen that has been metabolically activated. Tumor growth is usually reversible because it is epigenetically driven, despite the fact that this stage is an irreversible occurrence (Rak and Yu, 2004). Additionally, the promoters alter epidermal homeostasis, creating a tissue milieu favorable for the clonal proliferation of started cells (Radiae *et al.,* 2004). A single clone of initiated cells is created as a result of initiation and promotion (Franks, 2001). The majority of started cells, however, may not grow at all or may grow extremely slowly until they are acted upon by stimulating factors (Franks, 2001; Rak and Yu, 2004). These initiation and promotion pathways might prevent cells from differentiating into functional, normally non-diverging cells once they leave the stem cell population (Yupa *et al.,* 1997). The results will depend on how well the factors are balanced and how much the initial cells have changed since even while these growth-promoting stimuli are affecting the cells, they may still be sensitive to the body's normal growth-inhibiting substances (Franks *et al.,* 2001). This explains why pre-neoplastic tumors or even those that appear to be fully transformed can be identified, though they do not seem to be expanding

and occasionally even regress (Radiae *et al.,* 2004). The entire series of events leading to the development of a tumor is almost certainly the result of gene changes, even though the host may have an impact on gene expression (Nguyen *et al.,* 2009).

1.4.2 CANCER CELL CYCLE

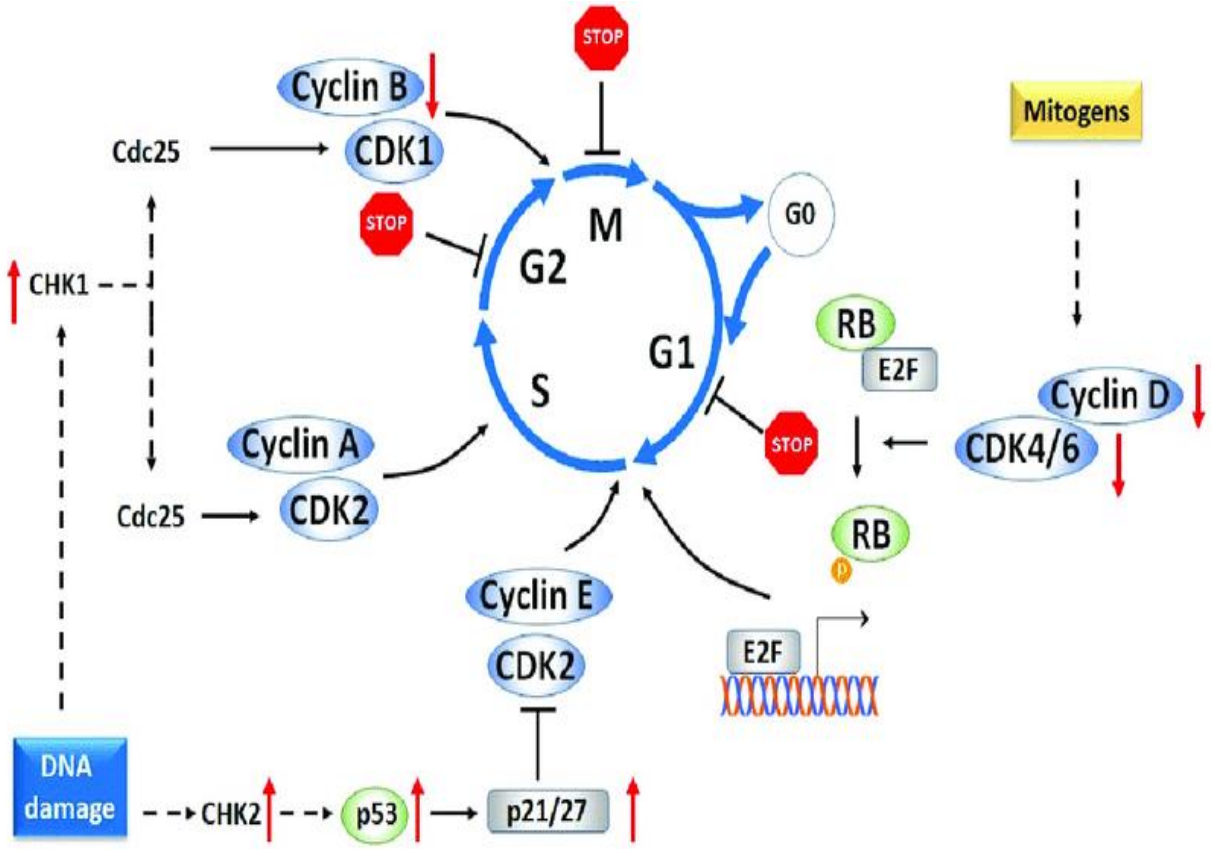


Fig. 1.1: cancer cell life cycle

cyclin D (apigenin, caffeic acid phenethyl ester, gingerol), cyclin E (caffeic acid phenethyl ester, gingerol, quercetin), CDK2 (quercetin), CDK4 (gingerol), cdc25 (gallic acid). Polyphenols upregulate proteins coded by tumor suppressor genes, such as p53 (apigenin, curcumin, EGCG), p21 (apigenin, EGCG), p27 (apigenin) and checkpoint proteins CHK1, 2 (gallic acid). Moreover, administration of polyphenols induced the cell cycle arrest in a cell-line-and compound-dependent manner, particularly G2/M arrest (apigenin, gallic acid), G0/G1 arrest (EGCG, quercetin). Legend: cyclin-dependent kinases (CDK), retinoblastoma protein (RB), transcription factor E2 (E2F), mitosis (M), gap 1 phase (G1), gap 2 phase (G2), DNA synthesis phase (S), CHK, checkpoint proteins; cdc25, cell cycle division protein 25; upregulation (red ↑); downregulation (red ↓).

(Adapted from https://www.researchgate.net/figure/Cell-cycle-and-the-inhibitory-effect-of-polyphenols-in-prostate-cancer-cells-DNA-damage_fig1_331489502)

1.5.0 TYPES OF CANCER

There are various forms of cancer, each deriving from a different kind of cell and displaying unique traits. Here are a few instances of typical cancer types:

1.5.1 BREAST CANCER

Breast cancer is a tumor that typically develops in the lobules (lobular carcinoma) or milk ducts (ductal carcinoma) of the breast (American Cancer Society, 2021). According to Bray et al. (2018), it is the most often diagnosed cancer and one of the main causes of cancer-related deaths in women worldwide.

Risk Factors Several risk factors, such as the following, can lead to the development of breast cancer:

Age: After menopause, in particular, the risk rises with age (Howlader *et al.,* 2019).

Family background: A family history of breast cancer increases risk, particularly if there are BRCA1 or BRCA2 mutations (Mavaddat *et al.,* 2019).

Hormones: Risk increases with prolonged estrogen exposure, whether it occurs during early menstruation or beyond menopause (Clemons & Goss, 2001).

Reproductive Factors: include late or no pregnancies as well as the first full-term pregnancy after the age of 30 (Chlebowski *et al.,* 2003).

Alcohol and Obesity: According to Cao and Cao (2001), excessive alcohol use and obesity are risk factors.

Types of Breast Cancer

Based on certain characteristics, there are various forms of breast cancer:

Ductal Carcinoma In Situ (DCIS): The milk ducts are the only location of abnormal cells in ductal carcinoma in situ (DCIS) (National Cancer Institute, 2021).

Invasive Ductal Carcinoma (IDC): is a condition in which cancer cells invade the tissues close by after penetrating the duct wall (National Cancer Institute, 2021).

Lobular Carcinoma In Situ (LCIS): According to the American Cancer Society's definition of lobular carcinoma in situ (LCIS), abnormal cells are present in the lobules but haven't yet disseminated.

Invasive Lobular Carcinoma (ILC): Cancer cells can spread to surrounding tissue after developing in the lobules (American Cancer Society, 2021).

Staging and Diagnosis

Mammography, biopsy, and imaging techniques are only a few of the procedures used to diagnose breast cancer (Houssami & Hunter, 2017). Staging, which ranges from stage 0 (in situ) to stage IV, helps evaluate the amount of the cancer's dissemination. (advanced metastatic disease) (Edge *et al.,* 2017).

1.5.2 LUNG CANCER

An important public health issue, lung cancer is the leading cause of cancer-related fatalities worldwide. It accounts for almost 1.8 million deaths worldwide in 2020 alone, according to the World Cancer Research Fund, making it both the most frequently diagnosed cancer and the main cause of cancer death [World Cancer Research Fund, 2020]. It is a complex illness with a range of subtypes, risk factors, and therapeutic modalities.

Types of Lung Cancer

Lung cancer primarily comes in two different forms:

NSCLC: Non-Small Cell Lung Cancer NSCLC makes up about 85% of all instances of lung cancer [Siegel, R. L., *et al.,* 2020]. Adenocarcinoma, squamous cell carcinoma, and big cell carcinoma are the further classifications. Each subtype has unique traits and available therapies.

Small Cell Lung Cancer (SCLC) is a more severe type of lung cancer that is frequently characterized by quick development and early metastases. It accounts for 15% of all cases of lung cancer [Govindan, R. 2012].

Risk factors and the causes

Lung cancer development is influenced by a number of risk factors, including:

Tobacco Smoke: According to the Centers for Disease Control and Prevention (CDC), tobacco use is the leading cause of lung cancer, accounting for 80% of cases.

Radon exposure: is a substantial environmental risk factor and is frequently detected in households [National Cancer Institute, 2021].

Occupational Exposures: According to the American Cancer Society (2020), certain occupational exposures to carcinogens, including as asbestos, are associated with a higher risk of developing lung cancer.

Family history: People who have a history of lung cancer in their family may be at greater risk.

Diagnosis

Improved results for lung cancer depend on early diagnosis. Typical diagnostic techniques include:

X-rays, CT scans, and PET scans are imaging tests that are used to find lung abnormalities.

Biopsies: The presence of cancer cells is determined in tissue samples taken by bronchoscopy or fine-needle aspiration.

Biomarker Testing: Genetic and molecular testing are used in biomarker studies to assist identify the kind of lung cancer and inform therapy choices (National Comprehensive Cancer Network, 2021).

1.5.3 PROSTATE CANCER

One of the most prevalent cancers in males is prostate cancer, which can significantly affect a man's health and quality of life. Prostate cancer is the second most frequent cancer in men, according to the American Cancer Society, with about one in eight men receiving a lifetime diagnosis [American Cancer Society, 2021]. Effective management of prostate cancer

requires a thorough understanding of its causes, risk factors, early detection methods, and available treatments.

Causes of Prostate Cancer

Men's prostate glands, a little organ about the size of a walnut situated below the bladder, contain cells that develop into prostate cancer when they begin to grow out of control. Although the precise causation of prostate cancer is still unknown, numerous things are thought to be involved:

Age: Men over 65 are more likely than younger men to have prostate cancer, and the risk rises with age [National Cancer Institute, 2020].

Genetics: Prostate cancer risk can be increased by familial history and inherited genetic abnormalities such BRCA1 and BRCA2 [Nicolosi, P., Ledet, E., Yang, S., *et al.,* 2020].

Risk Factors: A number of risk factors have been linked to a higher chance of acquiring prostate cancer, including:

Family history: Men are at an increased risk of developing prostate cancer if their dads or brothers had already experienced the disease [Mucci, L. A., J. B. Hjelmborg, J. R. Harris, *et al.,* 2016].

Race and Ethnicity: African American males are more likely to be diagnosed with prostate cancer at an advanced stage and have a higher chance of getting the disease [American Cancer Society, 2021].

Diet: According to some research, consuming a lot of red meat and few fruits and vegetables may increase your chance of developing cancer [World Cancer Research Fund, 2020].

Diagnosis

Prostate cancer early identification can dramatically improve results. Typical diagnostic techniques include:

PSA test (prostate-specific antigen): The PSA protein, which is produced by the prostate, is measured by this blood test. Prostate cancer can be detected by elevated PSA levels, although PSA levels can also be influenced by other variables [American Cancer Society, 2021].

Digital Rectal Exam (DRE): With the help of a gloved, lubricated finger, a healthcare professional conducts a digital rectal exam (DRE) to look for prostate abnormalities.

Biopsy: A biopsy is carried out to confirm the existence of malignant cells if the PSA level is excessive or if anomalies are found during a DRE.

1.5.4 COLORECTAL CANCER

One of the most frequent malignancies in the world is colorectal cancer. Globally, it was predicted that there will be over 1.9 million new cases and close to 935,000 fatalities in 2020. Because of lifestyle factors like food and physical inactivity, incidence rates are typically greater in developed nations. According to Bray, Ferlay, Soerjomataram, and others (2018)

Risk Factors:

Age, a family history of the condition, a personal history of polyps or inflammatory bowel disease (IBD), and a few genetic abnormalities, such as Lynch syndrome and familial adenomatous polyposis (FAP), can all raise one's risk of getting colorectal cancer. (2013) Nishihara R, Wu K, Lochhead P, et al.

Prevention and Screening

The ability to spot problems early is essential for enhancing results. Fecal occult blood tests (FOBT), stool DNA testing, and other screening techniques can aid in the early, more curable detection of cancer. A high-fiber diet, regular exercise, and moderate alcohol and red meat consumption are some lifestyle changes that can lower the risk of CRC. According to Krudsen, Zauber, Rutter, and others (2016)

Pathogenesis: Adenomatous polyps, which can develop into carcinoma, are frequently the first stage in the multi-step process that leads to the development of colorectal cancer from

normal colonic epithelial cells. The pathogenesis is influenced by molecular pathways like the Wnt signaling pathway and the accumulation of genetic mutations like APC and KRAS. (Vogelstein, Fearon, 1990)

1.5.5 SKIN CANCER

Millions of individuals all over the world are afflicted by skin cancer, which is common and may be fatal. It happens when the skin's cells mutate and grow out of control.

Causes of Skin Cancer

Long-term, uncovered exposure to ultraviolet (UV) radiation from the sun or tanning beds is the main factor in the development of skin cancer. Skin cells' DNA is damaged by UV light, which causes mutations that can give rise to malignant growths. Additional risk factors for skin cancer development include genetics and a family history of the condition.

Types of Skin Cancer

The three primary kinds of skin cancer are as follows:

Basal Cell Carcinoma (BCC): Basal Cell Carcinoma The most typical type of skin cancer is BCC. It often manifests as a red, scaly patch or a shiny, transparent lump. BCC typically grows slowly and seldom metastasizes to other body regions.

Squamous Cell Carcinoma (SCC): The second most typical kind of skin cancer is squamous cell carcinoma (SCC). Frequently, it appears as a rough, scaly area or a firm, red nodule. Even though SCC tends to spread more quickly than BCC, it is typically limited to the skin.

Melanoma: The most serious type of skin cancer is melanoma. It may form as a new, pigmented growth or from an existing mole. Because melanoma can spread to other organs and become life-threatening, early detection and treatment are essential.

Risk Factors:

Skin cancer risk can be impacted by a number of factors, including:

Sun Exposure: Long-term UV radiation exposure, particularly during the height of the day, is a major risk factor.

Fair complexion: People who have light-colored hair, blue or green eyes, and fair complexion are more likely to get skin cancer.

Moles: Atypical or excessively numerous moles can raise the risk.

Family history: Having skin cancer in the family increases the risk of getting the condition.

Immune system weakness: People who have immune systems that are already compromised, such as organ transplant recipients, are more vulnerable.

symptoms and diagnosis: Typical skin cancer symptoms include changes in moles' appearance or the emergence of new growths, unhealing wounds, and persistent bleeding or itching. A dermatologist's ocular examination and, occasionally, a biopsy of worrisome lesions are common steps in the diagnosis process.

1.5.6 LEUKEMIA

A type of blood malignancies known as leukemia that affects the bone marrow and blood is complex and diverse. It comes from the cells that make blood cells, mainly white blood cells. The types, causes, symptoms, diagnoses, available treatments, and ongoing research in leukemia will all be covered in this conversation.

Types of Leukemia

Leukemia can be broadly divided into four types:

Chronic lymphocytic leukemia (ALL): The most typical form of leukemia in children is ALL, though it can also strike adults. Lymphoblasts, which are young white blood cells, are where it begins. 2008's (Pui, Robison)

Acute Myeloid Leukemia (AML): AML is more frequent in adults and starts in myeloid stem cells. White blood cells that are aberrant are produced quickly in this condition. (Döhner, Weisdorf, and Bloomfield, 2015)

Chronic Lymphocytic Leukemia (CLL): The majority of CLL cases are in older persons, and it advances slowly. B-cells, a kind of white blood cell, are affected. Chronic lymphocytic leukemia (Hallek. 2020)

Chronic Myeloid Leukemia (CML): The Philadelphia chromosome is a chromosomal mutation that is associated with CML. It can change from a chronic phase to an acute phase and affects myeloid cells. (Kantarjian H, Jabbour E. Chronic myeloid leukemia. 2020)

Causes and Risk Factors

Leukemia's precise causes are not completely understood, however a number of risk factors have been found:

Genetic Predisposition: There is a link between some genetic conditions, such Down syndrome, and a higher risk of leukemia. (Izraeli S. 2017).

Radiation and Chemical Exposure: Exposure to high doses of radiation as well as specific chemicals, such as benzene, has been linked to an increased risk of leukemia. Zhang, L., McHale, C.M., Skibola, and S.M. Rappaport (2011)

Some medical procedures: Subsequent leukemia risk can be raised by prior cancer therapies like chemotherapy or radiation therapy. (2008) (Morton LM, et al.)

Symptoms

In addition to fatigue, frequent infections, easy bruising or bleeding, fever and night sweats, enlarged lymph nodes or spleen, diagnosis, and staging are common leukemia symptoms. A 2016 study by Swerdlow SH, Campo E, Pileri SA, et al.

Diagnosis: Blood tests, bone marrow aspiration and biopsy, and imaging tests are frequently used in the diagnosis process. A 2016 study by Arber DA, Orazi A, Hasserjian R, et al. The staging process aids in estimating the severity of the disease and directs therapy choices.

1.5.7 LYMPHOMA

The complicated category of blood malignancies known as lymphomas has its origins in the lymphatic system. The types, causes, symptoms, prognosis, available treatments, and ongoing research for lymphoma will all be covered in this conversation.

Types of Lymphoma

Broadly speaking, there are two primary forms of lymphoma:

Hodgkin lymphoma (HL): Reed-Sternberg cells are lymph node-specific markers for Hodgkin lymphoma. According to Ansell SM. (2015), it can be classified as either classical Hodgkin lymphoma or nodular lymphocyte-predominant Hodgkin lymphoma.

Non-Hodgkin Lymphoma (NHL): Non-Hodgkin lymphoma has a wide variety of subtypes, each with its own unique traits. Diffuse large B-cell lymphoma, follicular lymphoma, and mantle cell lymphoma are common subtypes (Armitage JO., 2005).

Causes and Risk Factors

Although the precise causes of lymphoma are still unknown, numerous risk factors have been found:

Genetic Predisposition: According to Wang SS, Cerhan JR, Hartge P, et al. (2006), a family history of lymphoma may raise the risk.

Immune System Deficiency: According to Song MK, Chung JS, Shin HJ, et al. (2016), conditions including HIV/AIDS or immunosuppressive medications can increase the risk.

Infections: According to Hjalgrim H, Smedby KE, Rostgaard K, et al. (2007), certain viral infections, such as Epstein-Barr virus (EBV), are linked to an elevated risk of lymphoma.

Symptoms

Swollen lymph nodes, lethargy, fever, night sweats, unexplained weight loss, itchy skin or rash, diagnosis, and staging are all common lymphoma symptoms (Zelenetz AD, Gordon LI, Wierda WG, *et al.,* 2017).

Diagnosis: Blood tests, imaging tests, and biopsy of the lymph node or organ in question are frequently used in the diagnosis process (Hoppe RT., 2014). The staging process aids in estimating the severity of the disease and directs therapy choices.

1.5.8 BRAIN TUMOR

A complex and varied category of neoplastic illnesses that affect the brain or the tissues around it are known as brain tumors. The types, causes, symptoms, prognosis, available treatments, and ongoing research for brain tumors will all be covered in this conversation.

Types of Brain Tumors

There are two primary classifications for brain tumors:

Primary Brain Tumors: According to Louis DN, Perry A, Reifenberger G, et al. (2016), these cancers develop in the brain or the tissues that surround it. Gliomas, meningiomas, and pituitary tumors are some examples of prevalent benign or malignant subtypes (Ostrom QT, Cioffi G, Gittleman H, *et al.,* 2016).

Metastatic Brain Tumors: Brain tumors that have migrated to other areas of the body, such as the lung or breast, are known as metastatic tumors. The majority of them are malignant secondary tumors (Nayak L, Lee EQ, Wen PY, 2012).

Causes and Risk Factors

Although the precise origins of primary brain tumors remain unknown, some risk factors and genetic predispositions have been found.

Radiation Exposure: Brain tumor risk can be raised by high-dose ionizing radiation exposure, such as from radiotherapy for other cancers.

Family History: The risk may be increased if there is a history of brain tumors or specific genetic diseases like neurofibromatosis in the family.

Symptoms

Depending on where they are and how big they are, brain tumor symptoms can change. Typical signs include:

Headaches: Usually quite bad and can get worse over time.

Particularly if the tumor aggravates the brain's nerve cells, seizures may result.

Changes in the nervous system, such as numbness, weakness, or coordination issues.

Memory issues, mental confusion, or personality changes are examples of cognitive changes (Ostrom QT, Bauchet L, Davis FG, *et al.,* 2014).

Diagnosis and Staging

Neurological testing, imaging tests like MRIs and CT scans, and occasionally biopsies are used to diagnose brain tumors. (Louis DN, Ohgaki H, Wiestler OD, *et al.,* 2007). The tumor's extent is determined through staging, which also directs therapy choices.

1.5.9 PANCREATIC CANCER

The critical organ of the pancreas, which is situated behind the stomach, experiences aberrant cell proliferation, which results in pancreatic cancer, a complex and frequently fatal disease. Because of its aggressiveness and late-stage diagnosis, this kind of cancer has an especially bad prognosis. We will examine a number of features of pancreatic cancer in this talk, including its risk factors, symptoms, diagnosis, available treatments, and current advances in research.

Epidemiology and Risk Factors

Despite making up a very tiny percentage of all cancer diagnoses, pancreatic cancer causes a disproportionately high number of cancer-related fatalities. The American Cancer Society estimates that in 2021 there will be roughly 60,430 new instances of pancreatic cancer in the US and 48,220 deaths due to the condition (American Cancer Society, 2021). These figures demonstrate the seriousness of pancreatic cancer and the urgent need for better screening procedures and therapies.

According to the National Cancer Institute, a number of risk factors have been found that could raise a person's risk of acquiring pancreatic cancer. These consist of:

Age: Older persons are more likely to get pancreatic cancer, with most instances happening in those over 65.

Use of tobacco: Smoking is one of the biggest risk factors for pancreatic cancer; it's thought that smokers have a double the chance of getting the disease as non-smokers do.

Family history: People who have hereditary pancreatitis or other genetic abnormalities, such as pancreatic cancer, are at higher risk.

Chronic Pancreatitis: The risk can be increased by long-term pancreatic inflammation, which is frequently brought on by binge drinking.

Diabetes: People who have had diabetes for a long time are more likely to get pancreatic cancer.

Obesity: Pancreatic cancer risk is higher in people who are overweight or obese.

Symptoms and Diagnosis

Due to the fact that pancreatic cancer frequently goes undetected in the beginning, it is frequently referred to as a "silent killer" (Mayo Clinic). As the illness worsens, people may experience symptoms like:

Back pain from the abdomen is common.

Jaundice: Skin and eye yellowing.

Unexpected weight loss is frequently accompanied by an appetite decrease.

digestive issues: Including nausea and modifications in feces color.

It can be difficult to diagnose pancreatic cancer because of how general and non-specific these symptoms are. Diagnostic procedures often include a mix of medical imaging (such as CT scans and MRIs), blood tests to detect tumor markers, and occasionally a pancreatic tissue sample.

1.5.9.1 OVARIAN CANCER

Ovarian cancer is a serious health issue with a high fatality rate caused by late-stage detection and few available treatments. The ovaries, which are a component of the female reproductive system and are in charge of creating eggs and hormones, are where this particular cancer develops. We will cover a wide range of topics related to ovarian cancer in this extensive discussion, including its epidemiology, risk factors, symptoms, diagnosis, available treatments, and active research initiatives.

Epidemiology and Risk Factors

Despite being relatively uncommon compared to other gynecologic malignancies, ovarian cancer kills more women than any other type of cancer worldwide (American Cancer Society, 2021). According to the American Cancer Society, there will be roughly 13,940 fatalities and 21,750 new cases of ovarian cancer diagnosed in the United States in 2021. These figures emphasize how critical it is to comprehend and treat ovarian cancer.

The National Cancer Institute reports that the following risk factors have been linked to an increased risk of ovarian cancer:

Age: Ovarian cancer risk increases with age, with women over 60 having the highest incidence.

Family History: Ovarian, breast, or colorectal cancer in the family can raise the risk, particularly if it has affected close family members.

BRCA Mutations: Ovarian cancer risk is markedly increased by inherited mutations in the BRCA1 and BRCA2 genes.

Reproductive Factors: Having your first child after the age of 35 or never having children are also risk factors.

Hormone Replacement Therapy: The risk of several kinds of ovarian cancer can rise with long-term usage of estrogen replacement therapy without progesterone.

Endometriosis: Some types of ovarian cancer may be more common in women who have endometriosis.

Symptoms and Diagnosis

Because early-stage ovarian cancer frequently exhibits no symptoms, it is frequently referred to as the "silent killer" (Mayo Clinic). Symptoms that emerge as the condition worsens include:

bloating or pain in the abdomen: persistent abdominal or pelvic pain that is not related to any other symptoms.

Eating is difficult or you feel full quickly: This may be because the tumor is pressing on your stomach.

Urinary symptoms include urgency or frequent urination.

changes in bowel habits, such as diarrhea or constipation.

Diagnosis: Ovarian cancer does not have any identifiable signs or accurate screening tests, making diagnosis difficult. A combination of pelvic examinations, transvaginal ultrasounds, blood tests monitoring tumor markers (such as CA-125), and eventually, surgical exploration are frequently used to make the diagnosis.

1.6.0 CAUSES OF CANCER

Chemicals, medications, microorganisms, radiation, drug additives, plastics, etc. can all cause cancer.

1.6.1 POSSIBLE CHEMICALS INDUCING CANCER

Glyphosate

Herbicides like Roundup include the active component glyphosate, which is a widely used herbicide. Concerns have been raised concerning its potential to cause cancer. According to animal research and scant human evidence, the World Health Organization's (WHO)

International Agency for Research on Cancer (IARC) designated glyphosate as "probably carcinogenic to humans" in 2015 (Guyton *et al.,* 2015).

Formaldehyde

A chemical called formaldehyde is frequently employed in the manufacture of numerous household goods and construction materials. The IARC designated it as a human carcinogen in 2006, and there is evidence connecting it to certain cancers such leukemia and nasopharyngeal carcinoma (IARC, 2006).

Benzene

A crucial industrial chemical utilized in the creation of numerous products, benzol is a volatile organic molecule. The IARC has categorized it as a human carcinogen as a result of its link to leukemia and other malignancies of the blood (IARC, 2018).

Asbestos

Due to its ability to resist fire, a class of naturally occurring minerals known as asbestos was previously widely utilized in building materials. It is a well-known human carcinogen that has been linked to respiratory cancers such lung cancer and mesothelioma (IARC, 2012).

Acrylamide

In especially with starchy foods, a chemical called acrylamide is produced during high-temperature cooking techniques like frying, roasting, and baking. Further data has arisen regarding its potential carcinogenicity after the IARC classed it as "probably carcinogenic to humans" in 1994 (Tareke *et al.,* 2002).

1.6.2 POSSIBLE DRUGS INDUCING CANCER

Pioglitazone:

Pioglitazone is an oral medicine that increases insulin sensitivity to treat type 2 diabetes. The prolonged use of pioglitazone may raise the risk of bladder cancer, according to certain

research. In 2016 (FDA, 2016), the U.S. Food and Drug Administration (FDA) issued a warning regarding this potential danger.

Androgen Deprivation Therapy (ADT):

Advanced prostate cancer patients receive androgen deprivation therapy to reduce the levels of male hormones (androgens) that can promote tumor growth. According to certain studies, long-term usage of ADT may raise the chance of acquiring diabetes, cardiovascular disease, and some malignancies, including colon cancer (Nguyen *et al.,* 2020).

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs):

Aspirin, ibuprofen, and naproxen are examples of NSAIDs that are frequently used to treat pain and inflammation. Aspirin has been shown to lower the risk of several cancers when taken regularly, however long-term usage of high-dose NSAIDs has been linked to an increased risk of gastrointestinal cancers, including colorectal and stomach cancer (Rothwell *et al.,* 2020).

Immunosuppressive medications: Immunosuppressive medications are used to treat autoimmune illnesses as well as to stop the rejection of donated organs. A few of these drugs, including azathioprine and cyclophosphamide, have been linked to a higher risk of developing specific malignancies, including skin and lymphoma (Hemminki *et al.,* 2016).

Hormone Replacement Therapy (HRT):

Women who have menopause symptoms can find relief with hormone replacement therapy. HRT has been linked to an increased risk of some malignancies, including breast and endometrial cancer, even though it can have advantages including lowering hot flashes and avoiding bone loss (Chlebowski *et al.,* 2020).

1.6.3 POSSIBLE MICRO-ORGANISMS THAT CAN INDUCE CANCER

These microorganisms, which include bacteria, viruses, and parasites, can cause cancer through a number of different methods.

Viruses and Cancer

Human Papillomavirus (HPV): One of the most well-known viruses linked to cancer is the human papillomavirus (HPV). It has been associated with oropharyngeal, anal, and penile malignancies in addition to cervical cancer. According to Bosch, F. X., and de Sanjosé (2003), HPV can infect epithelial cells, disrupt the normal cell cycle, cause unchecked cell proliferation, and ultimately result in the formation of cancer.

Human Papillomavirus (HPV): Chronic HBV and HCV infection is a significant risk factor for liver cancer. According to Levrero and Zucman-Rossi (2016), these viruses have the ability to incorporate their genetic material into the DNA of their hosts, leading to genetic instability and the buildup of mutations that can lead to cancer.

Bacteria and Cancer

Helicobacter pylori: This bacterium, Helicobacter pylori, is closely linked to gastric cancer. According to Ferlay, Soerjomataram, Dikshit, et al. (2015), H. pylori infection can induce chronic inflammation of the stomach lining, which over time can result in genetic changes and cell damage and raise the risk of cancer.

Streptococcus bovis: This bacterium has been connected to colorectal cancer. According to Abdulamir, A. S., Hafidh, R. R., and Abu Bakar (2010), it may encourage inflammation and foster conditions that are conducive to the development of colon cancer.

Parasites and Cancer

Schistosoma species: These parasitic worms have been linked to bladder cancer and are known to cause schistosomiasis. The parasites' long-term inflammation can alter bladder cells, which ultimately aids in the growth of cancer. (Botelho, M. C., & Machado, J. C., 2010).

Mechanisms of Carcinogenesis

Several mechanisms exist by which microorganisms might cause cancer:

Numerous bacteria can cause persistent inflammation, which can damage DNA, cause genetic abnormalities, and cause aberrant cell growth, all of which can foster the growth of cancer (Grivennikov, S. I., Greten, F. R., & Karin, 2010).

Viral Integration: A few viruses, such as HPV and HBV/HCV, incorporate their genetic material into the DNA of their hosts, upsetting the regular cell cycle and encouraging cancer-related mutations (McBride, A. A., 2017).

Immune System Suppression: Because some bacteria have the ability to suppress the immune system of their host, cancer cells are able to avoid being recognized and destroyed by the immune system (McBride, A. A., 2017).

1.6.4 POSSIBLE RADIATIONS THAT CAN INDUCE CANCER

Ionizing Radiation

Ionizing radiation has sufficient energy to release atoms' firmly bonded electrons, resulting in charged ions. X-rays, gamma rays, and specific particles like alpha and beta particles are included in this group. Ionizing radiation is well known for damaging DNA, which is a crucial step in the development of cancer (Hall, E. J., & Giaccia, A. J., 2018).

UV Radiation (ultraviolet)

Skin cancer can be caused by UV radiation from the sun or artificial sources, such as tanning beds. Skin cancer can arise as a result of DNA changes brought on by UV radiation's direct damage to skin cells (Rastogi, R. P., Richa, Kaushik, S., *et al.,* 2010).

Mechanisms of Radiation-Induced Carcinogenesis

DNA Damage

Direct interactions between ionizing radiation and DNA result in breakage and mutations. These genetic changes may result in unchecked cell division, a characteristic of cancer.

Oxidative Stress

Reactive oxygen species (ROS) are produced inside of cells after exposure to radiation. According to Valko, Rhodes, Moncol, et al. (2006), excessive ROS can harm DNA, proteins, and lipids as well as other cellular components, perhaps resulting in cancer.

Chronic Inflammation

In exposed tissues, radiation can cause chronic inflammation, which can foster the growth of cancer. According to Schaue, McBride, and Links (2015), inflammation can encourage DNA damage and cell growth.

1.6.5 POSSIBLE ADDITIVES THAT CAN INDUCE CANCER

Typical Additives Linked to Cancer Risk

Synthetic sweeteners

Aspartame: Aspartame is a popular artificial sweetener that may be found in lots of diet and sugar-free goods. According to several research, aspartame use may increase the risk of developing cancer, especially in animal models (Soffritti, Belpoggi, & Degli Esposti, 2006).

Food Preservatives

Sodium Nitrite and Nitrate: To maintain color and flavor, processed meats frequently employ these chemicals. However, they can produce nitrosamines, which are recognized carcinogens when exposed to high heat (Lijinsky, W., 1992).

Plasticizers

Phthalates: are common plasticizers that are utilized in a variety of items, including food packaging and medical equipment. According to several research, some phthalates may raise the chance of developing malignancies connected to hormones (Hauser *et al.,* 2005).

synthetic food coloring

Tartrazine (Yellow 5): A synthetic yellow dye called tartrazine is used in a variety of foods and drinks. While the direct relationship between it and cancer is up for question, some

research has raised the possibility of a connection with hyperactivity and particular allergic reactions (FDA, 2020).

Mechanisms of Additive-Induced Carcinogenesis

DNA Damage: Numerous additives, including nitrosamines produced from nitrites and nitrates, have the potential to harm DNA. According to the IARC (2010), this DNA damage can cause mutations that encourage the growth of cancer.

Endocrine Disruption: By imitating or inhibiting hormones, additives like phthalates can cause the endocrine system to malfunction. Long-term contact with these additives may increase the risk of malignancies linked to hormones (Gore, A. C., Chappell, V. A., Fenton, S. E., *et al.,* 2015).

Chronic inflammation: in the body may be brought on by certain substances. Chronic inflammation is a recognized risk factor for cancer because it fosters a climate that is conducive to the growth of cancerous cells (Grivennikov, S. I., Greten, F. R., & Karin, 2010).

Mitigating the Risk

Label Reading: To discover ingredients and make educated decisions, consumers should carefully study product labels. Reduce exposure by selecting products with fewer ingredients.

Moderation: Limiting potential dangers by consuming foods containing additives in moderation. For instance, cutting back on processed meat consumption can reduce nitrate and nitrite exposure.

1.7 LIFE CYCLE OF CANCER

Initiation:

The initiation of cancer is the earliest stage of the disease and involves the appearance of genetic abnormalities in a healthy cell. Numerous reasons, including inflammation, spontaneous mistakes during DNA replication, exposure to carcinogens (such as tobacco smoke, radiation, or certain chemicals), can result in these mutations. According to

Vogelstein et al. (2013), some of these mutations can cause tumor suppressor genes (genes that prevent cell growth) to become inactive or oncogenes (genes that promote cell growth) to become activated.

After initiation, the promoted stage involves the mutant cell's growth and clonal proliferation. The started cell is able to survive, proliferate, and develop a tiny pre-neoplastic lesion during this phase because a number of growth-promoting factors and signaling pathways are active. The pre-neoplastic lesion may regress if the promoting elements are eliminated since the promotion phase is reversible (Hanahan and Weinberg, 2011).

Progression: Pre-neoplastic lesions develop into malignant tumors during the progression stage. Additional genetic alterations are accumulating at this stage, which is also marked by genomic instability. The potential for metastasis (the spread of cancer to distant organs) increases as the tumor evolves due to its increased capacity to penetrate adjacent tissues and blood vessels (Heng *et al.,* 2016).

Metastasis:

The last and most destructive stage of cancer's life cycle is metastasis. Cancer cells from the main tumor spread to distant organs or tissues via the bloodstream or lymphatic system during metastasis, causing secondary tumors in the process. Cancer cells and the milieu of the target organs interact intricately during the metastatic process (Psaila and Lyden, 2009).

1.8.0 CANCER MARKERS

Cancer markers, often referred to as tumor markers, are bodily components or changes that can be evaluated and utilized as indicators of the presence, progression, or response to cancer treatment. Various body fluids like blood, urine, or tissue samples may include these indicators.

Cancer indicators are often chemicals that are either created by the body in reaction to the presence of cancer or by the cancer cells themselves.

1.8.1 TYPES OF CANCER MARKERS

Malignant disease	Major marker	Other markers
Bone cancer	Alkaline phosphatase	Bence Jones protein, serum calcium
Breast cancer	CA 15-3	CEA, calcitonin, b-hCG, LASA-P, Prolactin
Carcinoid tumors	Chromogranin A	Histamine, ADH, Bradykinin
Cervical cancer	SCC-A	AG-4 antibodies, CA 125, CEA, TPA
Colorectal cancer	CEA	CA 19-5, CA 19-9, CA 72-4, CK-BB, NSE
Gastric carcinoma	CA 72-4	CA 19-9, CA 50, CEA, ferritin, CK-BB, b-hCG, LASA-P, pepsinogen II, prothrombin
HCC	AFP	CEA, ferritin, ALP, g-glutamyl transpeptidase
Insulinoma	Insulin	C-peptide, IGF-1–binding protein
Leukemia	TdT	ALP, b2M, ferritin, LDH, myelin basic protein, adenosine deaminase, PNP
Lung cancer	NSE	ACTH, CK-BB, calcitonin, CA 72-4, CEA, AFP, ferritin, LASA-P, TPA
Lymphoma	b2M	TdT, Ki-67, LASA-P
Medullary thyroid cancer	Calcitonin	NSE
Multiple myeloma	Immunoglobulin	Bence Jones protein, b2M, IgA heavy and light chain
Non-seminomatous testicular tumor	AFP	b-hCG, LDH
Ovarian carcinoma	CA 125	Inhibin, AFP, CEA, CK-BB, b-hCG, galactosyl transferases, LDH, TPA

Pancreatic carcinoma	CA 19-9	CA 19-5, CA 50, CA 72-4, CEA, CK-BB, ADH, ALP, g-glutamyl transpeptidase, PAP
Pheochromocytoma	Metanephrine	Chromogranin A, plasma catecholamines
Prostate carcinoma	PSA	PAP, ALP, CEA, CK-BB, TPA

Table1.1: Selected examples of malignant diseases with associated tumor markers

ACTH: Adrenocorticotropic hormone; ADH: Antidiuretic hormone; AFP: Alfa fetoprotein; ALP: Alkaline phosphatase; b2M: Beta 2 microglobulin; CA: Cancer antigen;

CEA: Carcinoembryonic antigen; CK-BB: Creatine kinase BB isoenzyme; HCC: Hepatocellular carcinoma; IGF-1: Insulin-like growth factor 1; IL: Interleukin; LASA-P: Lipid associated sialic acid P; LDH: Lactate dehydrogenase; NSE: Neuron-specific enolase; PAP: Prostatic acid phosphatase; PNP: Purine nucleoside phosphorylase; PSA: Prostate specific antigen; PTH: Parathyroid hormone; RCC: Renal cell carcinoma; SCC-A: Squamous cell carcinoma antigen; TdT: Terminal deoxynucleotidyl transferase; TPA: Tissue polypeptide antigen.

(adapted from Recommendations of European Group on Tumor Markers (EGTM). Anticancer Res 1999;19(4A):2791-819)

Tumor marker	**Associated malignancy**	
	Primary	**Other malignancies**
Oncofetal antigens		
AFP	Primary HCC	Teratoblastomas of the ovary and testes
CEA	Colorectal carcinoma	Various carcinomas
Hormones		
b-hCG	Choriocarcinoma	Testicular cancers (non-seminomatous), trophoblastic tumors
Calcitonin	Medullary carcinoma	Cancer of the thyroid, liver cancer, renal cancer
Metanephrines	Pheochromocytoma	Neuroblastoma, ganglioneuromas
Chromogranin A	Pheochromocytoma, neuroblastoma	MEN, small-cell lung cancer, carcinoid tumors
IGF- 1	Pituitary cancer	Insulinoma
Glycoproteins		
CA 15-3	Breast cancer	Various carcinomas
CA 19-9	Pancreatic and gastric carcinomas	Various carcinomas
CA 72-4	Gastric carcinoma	Various carcinomas
CA 125	Ovarian carcinoma	Various carcinomas

Isoenzymes		
PSA	Prostate cancer	
NSE	Small-cell lung carcinoma	Neuroblastoma, kidney tumors
Cellular components/products		
LASA-P		Various carcinomas, leukemia, lymphoma, Hodgkin's disease
SCC-A		Squamous cell carcinoma of the uterus, cervix, lung, and head and neck
TAG 72	Gastric carcinoma	Colorectal, lung, pancreatic and ovarian cancers
Immunoglobulins	Multiple myeloma	Gammopathies

Table 1.2: Selected examples of serologic tumor markers and malignant diseases associated with each.

AFP: Alfa fetoprotein; b-hCG: Beta human chorionic gonadotropin; CA: Cancer antigen; CEA: Carcinoembryonic antigen; HCC: Hepatocellular carcinoma; LASA-P: Lipid associated sialic acid P; MEN: Multiple endocrine neoplasia; NSE: Neuron-specific enolase; PSA: Prostate-specific antigen; SCC-A: Squamous cell carcinoma antigen.

(adapted from Recommendations of European Group on Tumor Markers (EGTM). Anticancer Res 1999;19(4A):2791-819)

1.9.0 TREATMENT OF CANCER

Surgery, chemotherapy, radiation therapy, immunotherapy, targeted therapy, and hormone therapy are all common cancer treatments.

1.9.1 IMMUNOTHERAPY

By using the body's immune system to specifically target and destroy cancer cells, immunotherapy has transformed the way that cancer is treated. Specificity, durability, and lower toxicity are just a few benefits that this method has over conventional therapies (Chen & Mellman, 2017). The various classes of immunotherapy medications include:

Checkpoint Blockers

One popular family of immunotherapy medications is checkpoint inhibitors. They specifically target immunological checkpoints like CTLA-4 and the proteins programmed cell death protein 1 (PD-1) and PD-L1 (Pardoll, 2012). Drugs like pembrolizumab (anti-PD-1) and ipilimumab (anti-CTLA-4) disrupt these checkpoints, allowing T cells to identify and destroy cancer cells (Hodi *et al.,* 2010; Robert *et al.,* 2015).

Monoclonal Antibodies

In cancer immunotherapy, monoclonal antibodies (mAbs) have been essential. For instance, the monoclonal antibody rituximab targets the B cell protein CD20, which is essential for the treatment of B-cell lymphomas (Czuczman *et al.,* 2012). By preventing HER2/neu signaling, the mAb trastuzumab is effective against HER2-positive breast cancer (Piccart-Gebhart *et al.,* 2005).

CAR-T Cell Therapy

A novel strategy is chimeric antigen receptor T-cell therapy (CAR-T). Chimeric receptors that target certain tumour antigens are designed to express on CAR-T cells. In B-cell

leukemia and lymphoma, for instance, Kymriah and Yescarta target CD19 and provide impressive results (Maude *et al.,* 2014; Schuster *et al.,* 2019).

Cytokines

Cytokines are essential for controlling the immune system. In order to treat some malignancies, interferon-alpha and interleukin-2 (IL-2) are employed. In metastatic melanoma and renal cell carcinoma, high-dose IL-2 has shown long-lasting effects (Atkins *et al.,* 1999; McDermott *et al.,* 2010).

Vaccines

Cancer vaccines are designed to boost the immune system's defenses against particular tumor antigens. For instance, the human papillomavirus (HPV) vaccine guards against cervical cancer linked to HPV (Joura *et al.,* 2015). According to Kantoff et al. (2010), the FDA has approved Provenge as a therapeutic vaccination for metastatic prostate cancer.

Efficacy and Indications

Immunotherapy has been effective in treating a variety of malignancies. In advanced melanoma and non-small cell lung cancer, pembrolizumab and nivolumab have demonstrated impressive responses (Borghaei *et al.,* 2015; Robert *et al.,* 2015). In B-cell malignancies, CAR-T treatment has produced high remission rates (Schuster *et al.,* 2019).

Side effects

Immune-related adverse events (irAEs) might occur during immunotherapy despite its encouraging results. These consist of pneumonitis, colitis, and skin rash (Postow *et al.,* 2018). Optimizing treatment results requires effective management of irAEs.

1.9.2 TARGETTED THERAPY

By specifically targeting particular molecules or pathways essential for the growth and progression of cancer, targeted therapy medications have transformed the way cancer is treated (Sawyers, 2004). The various classes of medicines used in targeted therapy include:

tyrosine kinase inhibitors (TKIs)

One popular family of medications used in targeted therapy is tyrosine kinase inhibitors. They inhibit the activity of particular tyrosine kinases, enzymes that are essential for cell signaling and the development of cancer (Druker *et al.,* 2001).

Imatinib: By specifically targeting the BCR-ABL fusion protein, imatinib transformed the way chronic myeloid leukemia (CML) is treated (Druker *et al.,* 2001).

Both erlotinib and gefitinib are TKIs that work against non-small cell lung cancer by inhibiting the EGFR (Lynch *et al.,* 2004; Paez *et al.,* 2004).

Monoclonal Antibodies

Monoclonal antibodies (mAbs): Engineered proteins called monoclonal antibodies (mAbs) can bind to certain proteins on cancer cells, designating them for eradication or preventing their proliferation (Hudis, 2007).

Trastuzumab: This mAb is used to treat HER2-positive breast cancer and targets the HER2 receptor (Slamon *et al.,* 2001).

Cetuximab and Panitumumab: These mAbs block the EGFR and are used to treat colorectal cancer (Cunningham *et al.,* 2004; Van Cutsem *et al.,* 2007).

Angiogenesis Inhibitors

Angiogenesis inhibitors aim to stop the growth of new blood vessels that feed and oxygenate tumors.

Bevacizumab: is a monoclonal antibody used to treat colorectal, lung, and kidney malignancies that targets the vascular endothelial growth factor (VEGF) (Hurwitz *et al.,* 2004; Sandler *et al.,* 2006).

PARP Inhibitors

Inhibitors of poly (ADP-ribose) polymerase (PARP) prevent cancer cells from repairing DNA damage.

Effective in tumors with BRCA mutations in particular (Fong *et al.,* 2009).

According to Ledermann et al. (2014) and Robson et al. (2017), ovarian and breast tumors with BRCA mutations are treated with olaparib and rucaparib, two PARP inhibitors.

Side effects

While targeted medicines are frequently more tolerable than conventional chemotherapy, adverse effects are nevertheless possible. According to Mok et al. (2009), these include skin rash, diarrhea, and hypertension. For the best patient care, these adverse effects must be tracked and managed.

1.9.3 CHEMOTHERAPY

Chemotherapy is the employment of medications to slow down and eventually eliminate rapidly dividing cells, especially cancer cells (Chabner & Roberts, 2005). The many chemotherapeutic drug classes include:

Alkylating Agents

A subclass of chemotherapeutic medications known as alkylating agents causes DNA to suffer direct damage by adding alkyl groups, which stops cancer cells from proliferating.

Cyclophosphamide: According to Wernik et al. (1998), this medication is used to treat a number of malignancies, including ovarian and breast cancer.

Antimetabolites

Antimetabolites are substances that mimic the constituent parts of DNA and RNA to prevent their production.

Breast cancer and leukemia are two cancers that are treated with methotrexate (Ferriols-Lisart *et al.,* 1992).

Topoisomerase Inhibitors

By inducing breaks in DNA strands, topoisomerase inhibitors attack the enzymes that aid in DNA replication and repair.

Doxorubicin: According to Minotti et al. (2004), it is used to treat a variety of malignancies, including breast and lung cancer.

Microtubule Inhibitors

The microtubules in the cell, which are necessary for cell division, are interfered with by microtubule inhibitors.

Paclitaxel: According to Rowinsky and Donehower (1995), paclitaxel is used in the treatment of breast, ovarian, and lung cancer.

Platinum Compounds

The very powerful chemotherapy medication platinum compounds crosslink DNA strands to prevent cell division.

Side Effects

Chemotherapy medications can harm healthy, normal cells in addition to cancer cells, which can result in side effects such fatigue, nausea, and hair loss (Rowinsky & Donehower, 1995). For patients to feel comfortable and follow their treatment plan, these adverse effects must be managed.

1.9.4 HORMONE THERAPY

Endocrine therapy, sometimes referred to as hormone therapy, is an essential method for treating malignancies that are sensitive to hormones. These treatments alter the hormonal milieu to reduce or stop the growth of cancers that are hormone-driven (Jordan, 2003). The various classes of hormone treatment medications include:

Anti-Estrogen Therapies

Breast cancer is the main condition for which anti-estrogen treatments are employed.

Tamoxifen: Tamoxifen competes with estrogen for binding to estrogen receptors in breast tissue and is a selective estrogen receptor modulator (SERM). It is applied to women with

hormone receptor-positive breast cancer who are premenopausal or postmenopausal (Fisher *et al.,* 1998).

Aromatase inhibitors (AIs): In postmenopausal women, AIs such as anastrozole, letrozole, and exemestane suppress the production of estrogen. They are common therapies for breast cancer with a hormone receptor (Buzdar *et al.,* 2002; Baum *et al.,* 2003).

Anti-Androgens Therapies

Prostate cancer is the main condition that is treated with anti-androgen treatments.

Bicalutamide and Flutamide: These non-steroidal anti-androgens (non-steroidal anti-androgens) prevent the action of testosterone, a hormone that promotes the growth of prostate cancer cells (Wirth *et al.,* 2004).

Gonadotropin-releasing hormone (GnRH) antagonists (LHRH antagonists): Substances like leuprolide and goserelin reduce testosterone levels by preventing the production of GnRH. In advanced prostate cancer, they are employed (Crawford *et al.,* 1989).

Side effects

Hormonal alterations can cause negative effects from hormone therapy. Tamoxifen, which is used to treat breast cancer, has been linked to an increased risk of uterine cancer, mood swings, and hot flashes (Fisher *et al.,* 1998). (Baum *et al.,* 2003) Aromatase inhibitors have been linked to bone loss and joint pain.

1.9.5 ANTIANGIOGENIC DRUGS

Introduction

New blood vessel development, or angiogenesis, is essential for the growth and metastasis of cancer. Antiangiogenic medications aim to obstruct this procedure, which in turn slows or stops the blood supply to tumors and inhibits their growth (Ferrara, 2010).

Vascular Endothelial Growth Factor (VEGF) Inhibitors

Drugs that target VEGF, a crucial regulator of angiogenesis, have been at the forefront of antiangiogenic therapy.

Bevacizumab: A monoclonal antibody called bevacizumab binds to VEGF directly and prevents it from attaching to its receptors. According to Hurwitz et al. (2004), Sandler et al. (2006), and Burger et al. (2011), bevacizumab is used to treat a variety of malignancies, including colorectal, lung, and ovarian cancer.

Aflibercept: A fusion protein that binds to and inhibits the activity of VEGF-A and VEGF-B. It is used in colorectal cancer that has spread to other organs (Van Cutsem *et al.,* 2012).

Tyrosine kinase inhibitors (TKIs)

Tyrosine kinase inhibitors obstruct the angiogenesis-related signaling pathways.

Sunitinib and Sorafenib: These TKIs block several receptors, including VEGFR, PDGFR, and c-KIT. They are employed in the treatment of hepatocellular and renal cell carcinoma. (Motzer *et al.,* 2006; Llovet *et al.,* 2008).

Side Effects

Because antiangiogenic medicines affect blood vessels, they may have negative effects. Hypertension, hemorrhage, proteinuria, and weariness are typical adverse reactions (Escudier *et al.,* 2007). In order to lessen these effects, proper administration and monitoring are crucial. Tables 1.3, 1.4, 1.5, 1.6, and 1.7 summarize of the main classes and examples of chemotherapy of neoplastic disease.

TYPE OF AGENT	NONPROPRIETARY NAMES	DISEASE
Nitrogen mustards	Mechlorethamine	Hodgkin's disease
	Cyclophosphamide Ifosfamide	Acute and chronic lymphocytic leukemia; Hodgkin's disease Non-Hodgkin's lymphoma; multiple myeloma; neuroblastoma; breast, ovary, lung cancer; Wilms' tumor; cervix, testis cancer; soft-tissue sarcoma
	Melphalan	Multiple myeloma
	Chlorambucil	Chronic lymphocytic leukemia; macroglobulinemia
Methylhydrazine derivative	Procarbazine (*N*-methylhydrazine, MIH)	Hodgkin's disease
Alkyl sulfonate	Busulfan	Chronic myelogenous leukemia, bone marrow transplantation
Nitrosoureas	Carmustine (BCNU)	Hodgkin's disease; non-Hodgkin's lymphoma; Glioblastoma
	Streptozocin	Malignant pancreatic insulinoma; malignant carcinoid
	Bendamustine	Non-Hodgkin's lymphoma
Triazenes	Dacarbazine (DTIC; dimethyltriazenoi midazole - carboxamide),	Malignant melanoma; Hodgkin's disease; soft-tissue sarcomas; melanoma

	Temozolomide	Malignant gliomas
Platinum coordination Complexes	Cisplatin, carboplatin, oxaliplatin	Testicular, ovarian, bladder, esophageal, lung, head and neck, colon, breast cancer.

Table 1.3: Alkylating Agents

(Adapted from Goodman & Gilman's The Pharmacological Basis of Therapeutics, Section VIII. Chemotherapy of Neoplastic Diseases, Chapter 60. General Principles of Cancer Chemotherapy, page 1693)

TYPE OF AGENT	NONPROPRIETARY NAMES	DISEASES
Folic acid analogs	Methotrexate (amethopterin)	Acute lymphocytic leukemia; choriocarcinoma; breast, head, neck and lung cancers; osteogenic sarcoma; bladder cancer
	Pemetrexed	Mesothelioma, lung cancer
Pyrimidine analogs	Fluorouracil (5-fluorouracil; 5-FU), capecitabine	Breast, colon, esophageal, stomach, pancreas, head and neck; premalignant skin lesion (topical)
	Cytarabine (cytosine arabinoside)	Acute myelogenous and acute lymphocytic leukemia; non- Hodgkin's lymphoma
	Gemcitabine	Pancreatic, ovarian, lung cancer
	5-aza-cytidine	Myelodysplasia
	Deoxy-5-aza-cytidine	Myelodysplasia
Purine analogs	Mercaptopurine (6-mercaptopurine; 6-MP)	Acute lymphocytic and myelogenous leukemia; small cell and related non-Hodgkin's lymphoma inhibitors
	Pentostatin (2'-deoxycoformycin)	Hairy cell leukemia; chronic

		lymphocytic leukemia; small cell non-Hodgkin's lymphoma
	Fludarabine	Chronic lymphocytic leukemia
	Clofarabine	Acute myelogenous leukemia
	Nelarabine	T-cell leukemia, lymphoma

Table 1.4: Antimetabolites

(Adapted from Goodman & Gilman's The Pharmacological Basis of Therapeutics, Section VIII. Chemotherapy of Neoplastic Diseases, Chapter 60. General Principles of Cancer Chemotherapy, page 1693)

TYPE OF AGENT	NONPROPRIETARY NAMES	DISEASE
Vinca alkaloids	Vinblastine	Hodgkin's disease; non-Hodgkin's lymphoma; testis cancer.
	Vinorelbine	Breast and lung cancer
	Vincristine	Acute lymphocytic leukemia; neuroblastoma; Wilms' tumor; rhabdomyosarcoma; Hodgkin's disease; non-Hodgkin's lymphoma
Taxanes	Paclitaxel, docetaxel	Ovarian, breast, lung, prostate, bladder, head and neck cancer.
Epipodophyllotoxins	Etoposide	Testis, small cell lung and other lung cancer; breast cancer; Hodgkin's disease; non-Hodgkin's lymphomas; acute myelogenous leukemia; Kaposi's sarcoma
	Teniposide	Acute lymphoblastic leukemia in children
Camptothecins	Topotecan	Ovarian cancer; small cell lung cancer
	Irinotecan	Colon cancer

Antibiotics	Dactinomycin	Choriocarcinoma; Wilms' tumor; rhabdomyosarcoma; testis;
	(actinomycin D)	Kaposi's sarcoma
	Daunorubicin (daunomycin, rubidomycin)	Acute myelogenous and acute lymphocytic leukemia
	Doxorubicin	Soft-tissue, osteogenic, and other sarcoma; Hodgkin's disease; non-Hodgkin's lymphoma; acute leukemia; breast, genitourinary, thyroid, lung, and stomach cancer; neuroblastoma and other childhood and adult sarcomas.
Echinocandins	Yondelis	Soft-tissue sarcomas, ovarian cancer
Anthracenedione	Mitoxantrone	Acute myelogenous leukemia; breast and prostate cancer
	Bleomycin	Testis and cervical cancer; Hodgkin's disease; non-Hodgkin's lymphoma
	Mitomycin C	Stomach, anal, and lung cancer

Enzymes	L-Asparaginase	Acute lymphocytic leukemia

Table 1.5: Natural Products

(Adapted from Goodman & Gilman's The Pharmacological Basis of Therapeutics, Section VIII. Chemotherapy of Neoplastic Diseases, Chapter 60. General Principles of Cancer Chemotherapy, page 1694)

TYPE OF AGENT	NONPROPRIETARY NAMES	DISEASE
Adrenocortical	Mitotane (*o,p*'-DDD)	Adrenal cortex cancer suppressants
Adrenocortico-steroids	Prednisone (other equivalent preparations available)	Acute and chronic lymphocytic leukemia; non- Hodgkin's lymphoma; Hodgkin's disease; breast cancer, multiple myeloma
Progestins	Hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate	Endometrial, breast cancer
Estrogens	Diethylstilbestrol, ethinyl estradiol (other preparations available)	Breast, prostate cancer
Anti-estrogens	Tamoxifen, toremifene	Breast cancer
Aromatase inhibitors	Anastrozole, letrozole, exemestane	Breast cancer
Androgens	Testosterone propionate, fluoxymesterone (other preparations available)	Breast cancer
Anti-androgen	Flutamide, casodex	Prostate cancer
GnRH analog	Leuprolide	Prostate cancer

Table 1.6: Hormones and Antagonists

(Adapted from Goodman & Gilman's The Pharmacological Basis of Therapeutics, Section VIII. Chemotherapy of Neoplastic Diseases, Chapter 60. General Principles of Cancer Chemotherapy, page 1695)

TYPE OF AGENT	NON-PROPRIETARY NAMES	DISEASE
Substituted urea	Hydroxyurea	Chronic myelogenous leukemia; polycythemia vera; essential thrombocytosis
Differentiating agents	Tretinoin, arsenic trioxide	Acute promyelocytic leukemia
	Histone deacetylase inhibitor (vorinostat)	Cutaneous T-cell lymphoma
Protein tyrosine kinase inhibitors	Imatinib	Chronic myelogenous leukemia; GI stromal tumors (GIST); hypereosinophilia syndrome
	Dasatinib, nilotinib	Chronic myelogenous leukemia
	Gefitinib, erlotinib	EGFR inhibitors: Non–small cell lung cancer
	Sorafenib	Hepatocellular cancer, renal cancer
	Sunitinib	GIST, renal cancer
	Lapatinib	Breast cancer
Proteasome inhibitor	Bortezomib	Multiple myeloma
Biological response modifiers	Interferon-alfa, interleukin-2	Hairy cell leukemia; Kaposi's sarcoma; melanoma; carcinoid; renal cell; non-Hodgkin's lymphoma; mycosis fungoides;

		chronic myelogenous leukemia
Immunomodulators	Thalidomide	Multiple myeloma
	Lenalidomide	Myelodysplasia (5q– syndrome); multiple myeloma
mTOR Inhibitors Monoclonal antibodies	Temsirolimus, everolimus	Renal cancer

Table 1.7: Miscellaneous Agents

(Adapted from Goodman & Gilman's The Pharmacological Basis of Therapeutics, Section VIII. Chemotherapy of Neoplastic Diseases, Chapter 60. General Principles of Cancer Chemotherapy, page 1695)

1.9.6 JUSTIFICATION OF THE STUDY

The use of artesunate-mefloquine in higher doses may trigger some cancer events. The necessity of carrying out this study is to serve as caution in the use of artesunate-mefloquine most especially in cancer patients during co-morbid condition, it will serve as a clue in identifying artesunate-mefloquine as a class of antimalarial that can induce cancer markers or cause cancer, strengthen confidence in physician to exclude artesunate-mefloquine in therapies of patients that may have co-morbid condition and Identifying cancer marker induced by artesunate-mefloquine will enable a better exploration for further cancer research studies. There is little to no awareness on the possible induction of cancer markers by artesunate-mefloquine, as artesunate-mefloquine is widely perceived as a safe anti-malaria drug by the general public. By conducting this study, some intriguing data may be generated that can alert government, physicians and consumers to avoid or reduce artesunate-mefloquine use.

1.9.7 AIM AND OBJECTIVES

Aim

The aim of the study was to assay for possible cancer markers induction after exposure to artesunate-mefloquine in albino rats.

Objectives

The following was investigated after exposure to artesunate-mefloquine in a dose dependent fashion in albino rats

1. The possible type of cancer induced by artesunate-mefloquine following artesunate-mefloquine exposure.
2. The possible non-existence of cancer markers following artesunate-mefloquine exposure.
3. The pattern of increase level of cancer markers following artesunate-mefloquine exposure.
4. The pattern of decrease level of cancer markers following artesunate-mefloquine exposure.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Study centre

The study was carried out in the following centres

Department of pharmacology and Toxicology, University of Benin, Nigeria.

Department of chemical pathology, University of Benin Teaching Hospital, Benin City.

2.2 Materials

A mortal and pestle, volumetric flask, measuring cylinder, test tubes, surgical gloves, surgical disecting kits, plain sample bottles, cotton wool, weighing balance, centrifuge (Rolotix 32A Germany), Analyzer ISE 4000 (SFR, France(Scout Pro digital Balance (OHAUS corporation, USA).

2.3 Animals

Ethical approval (ADM/E 22/A/VOL VII/1047) was obtained from the ethics Committee of the Faculty of Pharmacy. Albino rats weighing 150 to 200 g were sourced from animal house of the Department of pharmacology and Toxicology, faculty of Pharmacy University of Benin, Benin city Nigeria. The rats were kept in plastic cages and allowed to acclimatize for two weeks to adapt to the new environment in the plastic cage bedding with wood shavings under standard temperature ($25^{o}c \pm 3^{o}c$) and a 12:12hr natural light-dark cycle. The rats were fed with dry rodent pelletized finisher feeds and allowed free access to water. The bedding materials (wood shavings) of the cages was changed daily. All experiments was carried out in accordance with the National institute of Health Guidelines for the care and use of Laboratory Animals (NIA publications No 80 revised in 2023).

2.4 Drug and Chemical

Drugs (Artesunate-mefloquine (8.57/10.71mg/kg), diethylstibesterol (0.07mg/kg), mesterolone (0.36mg/kg)) were obtained. Calculated doses of each drug were dissolved in olive oil for oral administration, according to the body weight of the rats using an oral gastric tube.

2.5.0 Experimental design

The male and the female animals were separated into six experimental group with each containing the control of 6 rats and the other group having 6 rats each. The experimental animals were treated with freshly prepared drug (dissolved in measured volume of olive oil) orally, while the negative control group was administered olive oil for 28 days as follows;

GROUP	TREATMENT OPTION/COMBINATION
I	Standard
II (positive control)	Three female rats were administered calculated therapeutic dose of diethylstibesterol (0.07mg/kg) in olive oil
III (negative control)	Three female rats were administered with 0.5 ml of olive oil
IV	Three female rats were administered calculated therapeutic dose (TD) of Artesunate-mefloquine (8.57/10.71mg/kg) in olive oil
V	Three female rats were administered calculated half therapeutic dose (1/2TD) (4.29/5.36mg/kg) of Artesunate-mefloquine in olive oil
VI	Three female rats were administered calculated quarter therapeutic dose (1/4TD) (2.14/2.68mg/kg) of Artesunate-mefloquine in olive oil

Table 2.1: Group of rats for cancer antigens 125 and 15 3 (CA 125 and CA 15-3)

GROUP	TREATMENT OPTION/COMBINATION
I	Standard
II (positive control)	Three male rats were administered calculated therapeutic dose of mesterolone (0.36mg/kg) in olive oil
III (negative control)	Three male rats were administered with 0.5 ml of olive oil
IV	Three male rats were administered calculated therapeutic dose (TD) (8.57/10.71mg/kg) of Artesunate-mefloquine in olive oil
V	Three male rats were administered calculated half therapeutic dose (1/2TD) (4.29/5.36mg/kg) of Artesunate-mefloquine in olive oil
VI	Three male rats were administered calculated quarter therapeutic dose (1/4TD) (2.14/2.68mg/kg) of Artesunate-mefloquine in olive oil

Table 2.2: Group of rats for prostate specific antigen (PSA)

GROUP	TREATMENT OPTION/COMBINATION
I	Standard
II (Negative control)	Water
III	Three female rats were administered calculated therapeutic dose (TD) (8.57/10.71mg/kg) of Artesunate-mefloquine in olive oil
IV	Three female rats were administered calculated half therapeutic dose (1/2TD) (4.29/5.36mg/kg) of Artesunate-mefloquine in olive oil
V	Three female rats were administered calculated quarter therapeutic dose(1/4TD) (2.14/2.68mg/kg) of Artesunate-mefloquine in olive oil

Table 2.3: Group of rats for alfa fetoprotein (AFP)

2.5.1 Determination of change in body weight

The weight of the animals were taken before drug administration, after which at every 7 days interval the animals were re-weighed and the doses adjusted in response to a change in weight and at the completion of the 28days drug administration period using the Scout pro digital balance (OHAUS corporation, USA), the final weight of the rats is determined.

2.6.0 Dosage calculations

Total number of animal receiving artesunate-mefloquine (TD) = 6 rats

Total number of animal receiving artesunate-mefloquine (1/2TD) = 6 rats

Total number of animal receiving artesunate-mefloquine (1/4TD) = 6 rats

Total number of animal receiving diethylstibesterol (Positive control) = 6rats

Total number of animal receiving Mesterolone (Positive control) = 6rats

Total number of animal receiving Olive oil (Negative control) = 6rats

2.6.1 Therapeutic dose calculation for artesunate-mefloquine

Dose calculation for albino rat using

artesunate (600 mg) + mefloquine (750 mg)

600/750 mg = 1 tablet daily (for 60 kg average body weight for human)

Assuming

Average body weight for human = 60 kg

Average body weight of one rat = 200 g/1000= 0.2 kg

60 kg = 1 tablet

0.2 kg = x tablet

x = 0.0033 tablet for 1 rat

therefore,

If 60 kg weight of a human can take 1tab/day

Then 0.2 kg weight of albino rat can take 0.0033tab/day

To calculate total tablet for 6 rats in 10 days

0.0033 tab x 1 day x 10 days x 6 rats

= 0.2 tab

To calculate the volume of dosage for one rat

Assuming

Average volume of dosage per rat in 1day= 0.5 ml

To calculate for 6 rats in 10 days = 0.5 ml x 1 day x 10 days x 6 rats

=30 ml

To prepare stock solution

If 0.2 tab = 30 ml for 6 rats

1 tab = x (ml)

x =150 ml

Therefore 1tab of artesunate (600 mg) + mefloquine (750 mg) is dissolved in 150ml of olive oil

To calculate your working solution per rat

If 0.2 kg= 0.5 ml

Given weight of rat = X(ml)

Given that X = unknown volume of dosage per rat for 1day

X = 0.5/0.2 x given weight of rat

2.6.2 Half therapeutic dose calculation for artesunate (600 mg) + mefloquine (750 mg)

Dose calculation for albino rat using

Artesunate (600 mg) + mefloquine (750 mg)

600/750 mg= 1 tab daily (for 60 kg average body weight for human)

Assuming

Average body weight for human = 60 kg

Average body Weight of one rat=200 g/1000= 0.2 kg

60 kg = 1 tablets

0.2 kg = x tablets

x = 0.0033 tablet for 1 rat

therefore,

If 60 kg weight of a human can take 1tab/day

Then 0.2 kg weight of albino rat can take 0.0033 tab/day

To calculate total tablet for 6 rats in 10days

0.0033tab x 1 day x 10 days x 6 rats

= 0.2 tab

To calculate the volume of dosage for one rat

Assuming

Average volume of dosage per rat in 1 day = 0.5 ml

To calculate for 6 rats in 10 days = 0.5 ml x 1 day x 10 days x 6 rats

=30 ml

To prepare stock solution

If 0.2 tab = 30 ml for 6rat

1tab = x(ml)

x = 150 ml

Therefore, 1 tab of artesunate (600 mg) + mefloquine (750 mg) is dissolved in 150 ml of olive oil

To calculate your working solution per rat

If 0.2 kg = 0.5 ml

Given weight of rat= x(ml)

Given that x = unknown volume of dosage per rat for 1day

X = 0.5/0.2 x given weight of rat

To get your 1/2 TD, you divide X by 2

1/2TD = x (unknown therapeutic volume of dosage per rat for 1day)/2

2.6.3 Quarter therapeutic dose calculation for dihydroartemisinin piperaquine

Dose calculation for albino rat using

Artesunate (600 mg) + mefloquine (750 mg)

600/750 mg= 1 tab daily (for 60 kg average body weight for human)

Assuming

Average body weight for human = 60 kg

Average body Weight of one rat=200 g/1000= 0.2 kg

60 kg = 1 tablets

0.2 kg = x tablets

x = 0.0033 tablet for 1 rat

therefore,

If 60 kg weight of a human can take 1tab/day

Then 0.2 kg weight of albino rat can take 0.0033 tab/day

To calculate total tablet for 6 rats in 10days

0.0033tab x 1 day x 10 days x 6 rats

= 0.2 tab

To calculate the volume of dosage for one rat

Assuming

Average volume of dosage per rat in 1 day = 0.5 ml

To calculate for 6 rats in 10 days = 0.5 ml x 1 day x 10 days x 6 rats

=30 ml

To prepare stock solution

If 0.2 tab = 30 ml for 6rat

1tab = x(ml)

x = 150 ml

Therefore, 1 tab of artesunate (600 mg) + mefloquine (750 mg) is dissolved in 150 ml of olive oil

To calculate your working solution per rat

If 0.2 kg = 0.5 ml

Given weight of rat= x(ml)

Given that x = unknown volume of dosage per rat for 1day

X = 0.5/0.2 x given weight of rat

To get your 1/4 TD, you divide X by 4

1/4TD = x (unknown therapeutic volume of dosage per rat for 1day)/4

2.6.4 Dose of diethystilbesterol using 1 tablet

Dose calculation for albino rat using

Diethylstibesterol (25mg)

Assuming

Average body weight for human =60 kg

Average body Weight of one rat=200 g/1000= 0.2 kg

60 kg=1tablets

0.2 kg= xtablets

x= 0.0033tablet for 1rat

therefore

If 60 kg weight of a human can take 1tab/day

Then 0.2 kgweight of albino rat can take 0.0033tab/day

To calculate total tablet for 6rats in 10days

0.0033tabx1dayx10daysx6rats

= 0.2 tab

To calculate the volume of dosage for one rat

Assuming

Average volume of dosage per rat in 1day= 0.5 ml

To calculate for 6rats in 10 days = 0.5 ml x 1 day x 10 days x 6 rats

=30 ml

To prepare stock solution

If 0.18tab = 30 ml for 6 rats

1tab = x(ml)

x =150ml

Therefore, 1tab of diethylstibesterol (25mg) is dissolved in 150ml of olive oil

To calculate your working solution per rat

If 0.2 kg= 0.5 ml

Given weight of rat = X(ml)

Given that x = unknown volume of dosage per rat for 1day

X = 0.5/0.2 x given weight of rat

2.6.5 Dose for mesterolone 1 tablet

Dose calculation for albino rat using

Mesterolone

25mg = 1tab bd (for 60 kg average body weight for human)

Assuming

Average body weight for human =60 kg

Average body Weight of one rat=200 g/1000= 0.2 kg

60 kg = 1 tablets

0.2 kg = x tablets

x = 0.0033 tablet for 1 rat

therefore,

If 60 kg weight of a human can take 1tab/day

Then 0.2 kg weight of albino rat can take 0.0033tab/day

To calculate total tablet for 6 rats in 10 days

0.0033 tab x 1 day x 10 days x 6 rats

=0.2 tab

To calculate the volume of dosage for one rat

Assuming

Average volume of dosage per rat in 1day= 0.5 ml

To calculate for 6rats in 10days= 0.5 mlx1dayx10daysx6rat

=30 ml

To prepare stock solution

If 0.18tab = 30 ml for 1rat

1tab = x(ml)

x =150ml

Therefore 1tab of mesterolone is dissolve in 150ml of olive oil

To calculate your working solution per rat

If 0.2 kg = 0.5 ml

Given weight of rat= x(ml)

Given that x = unknown volume of dosage per rat for 1day

X= 0.5/0.2 x given weight of rat

Drom the calculations above, the factor used for all drugs is:

Factor = 0.5/0.2 × weight of each rats

2.7 Preparation and administration of drugs

Calculated standard doses was computed in kilogram/body weight. The tablets were grounded into powder and triturated using olive oil. The solution was transferred into previously calibrated container and made up to volume. The final preparation was administered to the albino rats grouped into six groups using orogastric tube. The administration of the freshly prepared drugs was carried out for twenty-eight days consecutively. The animals were anaesthetized using chloroform and sacrificed. Blood samples were collected and and centrifuged. The serum was therefore collected and assayed for cancer markers.

2.8 Determination of drug effect on cancer markers

using a pair of surgical scissors, the deeply aestheticized rat was dissected carefully. Then using a sterile 5ml syringe with a 23G needle, about 2-5ml of blood was withdrawn from the abdominal aorta and heart of each rat and transferred to a pre labelled hematological container for analysis as follow

About 2ml was transferred into plain tubes for therapeutic dose (TD), half therapeutic dose (1/2TD), quarter therapeutic dose (1/4TD), positive and negative controls each. The blood sample were first centrifuge at 40rpm per minute for 3minutes. The serum was carefully collected using into a pre labelled plain(red) tubes using different pasteur pipette for each sample.

All samples were thereafter taken to the University of Benin Teaching Hospital, clinical pathology laboratory for analysis. The sample was then analyzed using an enzyme linked immunosorbent assay (ELISA).

2.9.0 Cancer indices

The specific marker assessed were prostate specific antigen (PSA), cancer antigen 125 (CA 125) and cancer antigen 15-3 (CA 15-3) and alfa fetoprotein (AFP)

2.9.1 PRINCIPLE OF ASSAY METHODS

Immunoenzymometric sequential assay (TYPE 4):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal antigen antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, a reaction results between the native antigen and the antibody, forming an antibody-antigen complex.

2.9.2 ASSAY METHOD FOR CANCER ANTIGEN 15-3 (CA 15-3) MARKER

REAGENT PREPARATION

1. Wash Buffer

Dilute contents of wash solution were added to 1000ml of distilled water in a suitable storage container. Diluted buffer can be stored at room temperature (2-30°C) for up to 60 days.

2. Dilution of the Patient Sample (1:21)

The quantity of 0.025ml (25µl) of each specimen was dispensed into 0.50ml (500µl) of CA 15-3 dilution matrix appropriately labelled, clean container(s) and mix thoroughly before use. Store refrigerated at 2-8°C for up to 48 hours.

TEST PROCEDURE

All reagents, serum reference calibrators and controls were brought to room temperature (20-27°C). The microplate wells were Formatted for each serum reference control and treatment

specimen and assayed in duplicate. Unused microwell strips was replaced back into the aluminium bag, sealed and stored at 2-8°C.

The quantity of 0.025 ml of the appropriate diluted control and treatment specimen was pipetted into the assigned well respectively. The reagent 0.100 ml biotinylated labelled antibody was added to each well. All reagents were dispensed close to the bottom of the coated well. The microplate was swirled gently for 20-30 seconds to mix and cover and incubated for 60 minutes at room temperature. The contents of the microplate were discarded by decantation and the plate tapped and blotted dry with absorbent paper. The quantity 0.350ml of wash buffer was added, decant (tapped and blotted). This was repeated two (2) additional times for a total of three (3) washes. The quantity 0.100 ml of the Ca15-3 Enzyme Reagent was added to each well Covered and incubated for 60 minutes at room temperature.

The contents of the microplate were discarded by decantation and blotted dry with absorbent paper. The quantity of 350µl of wash buffer was added, decanted (tapped and blotted). This was repeated two (2) additional times for a total of three (3) washes.

The quantity 0.100 ml (100µl) of substrate reagent was added to all wells. The reagents were added in the same order to minimize reaction time. The microplate was incubated at room temperature for twenty (20) minutes. The quantity 0.050ml (50µl) of stop solution was added to each well and gently mixed for 15-20 seconds. The absorbance in each well was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results were read within thirty (30) minutes of adding the stop solution.

2.9.3 ASSAY METHOD FOR PROSTATE SPECIFIC ANTIGEN (PSA) MARKER

REAGENT PREPARATION

1. Wash Buffer

The contents of wash concentrate were diluted to 1000ml with distilled water in a suitable storage container. The diluted buffer was stored at 2-30°C for up to 60 days.

2. Working Substrate Solution

The contents of the amber vial labelled Solution 'A' was labelled into the clear vial labelled Solution 'B'. The yellow cap was placed on the clear vial for easy identification. Mixed and labelled accordingly. Stored at 2 - 8°C.

TEST PROCEDURE

The microplates' wells were formatted for each serum reference control and treatment specimen and assayed in duplicate. The unused microwell strips was replaced back into the aluminum bag, sealed and stored at 2-8°C. The quantity 0.025ml (25µl) of the appropriate serum reference control or treatment specimen was added into the assigned well. The quantity 0.100ml (100µl) of the PSA Enzyme Reagent was added to each well. All reagents were dispensed close to the bottom of the coated well. The microplates were swirled gently for 20-30 seconds to mix and covered, Incubated for 30 minutes at room temperature. The contents of the microplates were discarded by decantation, it was tapped and blotted with an absorbent paper. The quantity 0.350ml (350µl) of wash buffer was added and decanted (tapped and blotted). The process was Repeated two (2) additional times for a total of three (3) washes. The quantity 0.100ml (100µl) of working substrate solution was added to all wells. The reagents were always added in the same order to minimize reaction time differences between well and incubated at room temperature for fifteen (15) minutes. The quantity 0.050ml (50µl) of stop solution was added to each well and mixed gently for 15-20 seconds. The absorbance was read for each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results were read within thirty (30) minutes of adding the stop solution.

2.9.4 ASSAY METHOD FOR CANCER ANTIGEN 125 (CA-125) MARKER

REAGENT PREPARATION

1. Wash Buffer

The contents of wash concentrate were diluted to 1000ml with distilled water in a suitable storage container. The diluted buffer was stored at 2-30°C for up to 60 days.

2. Working Substrate Solution The contents of the amber vial labelled Solution 'A' was labelled into the clear vial labelled Solution 'B'. The yellow cap was placed on the clear vial for easy identification. Mixed and labelled accordingly. Stored at 2 - 8°C.

TEST PROCEDURE

All reagents, serum reference calibrators and controls were brought to room temperature (20-27°C). The microplate wells were Formatted for each serum reference control and treatment specimen and assayed in duplicate. Unused microwell strips was replaced back into the aluminium bag, sealed and stored at 2-8°C. The quantity of 0.025 ml of the appropriate diluted control and treatment specimen was pipetted into the assigned well respectively. The reagent 0.100 ml biotinylated labelled antibody was added to each well. All reagents were dispensed close to the bottom of the coated well. The microplate was swirled gently for 20-30 seconds to mix and cover and incubated for 60 minutes at room temperature. The contents of the microplate was discarded by decantation and the plate tapped and blotted dry with absorbent paper. The quantity 0.350ml of wash buffer was added, decant (tapped and blotted). This was Repeated two (2) additional times for a total of three (3) washes. The quantity 0.100 ml of the Ca-125 Enzyme Reagent was added to each well. Covered and incubated for 60 minutes at room temperature. The contents of the microplate was discarded by decantation and blotted dry with absorbent paper. The quantity of 350µl of wash buffer was added, decanted (tapped and blotted). This was Repeated two (2) additional times for a total of three (3) washes.

The quantity 0.100 ml (100µl) of substrate reagent was added to all wells. The reagents were added in the same order to minimize reaction time. The microplate was Incubated at room temperature for twenty (20) minutes. The quantity 0.050ml (50µl) of stop solution was added

to each well and gently mixed for 15-20 seconds. The absorbance in each well was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results were read within thirty (30) minutes of adding the stop solution.

2.9.5 ASSAY METHOD FOR ALFA FETOPROTEIN (AFP) MARKER

REAGENT PREPARATION

1. Wash Buffer

In a suitable storage container, the wash concentrate's contents were diluted to a volume of 1000ml with distilled water. For up to 60 days, the diluted buffer was kept at 2-30°C.

2. Working Substrate Solution

The clear vial labelled Solution "B" received the contents of the amber vial labelled Solution "A." The clear vial was covered with the yellow cap for simple identification. Mixed and appropriately labelled. kept between 2 and 8 °C.

TEST PROCEDURE

All reagents, serum reference calibrators and controls were brought to room temperature (20-27°C). The microplate wells were Formatted for each serum reference control and treatment specimen and assayed in duplicate. Unused microwell strips was replaced back into the aluminium bag, sealed and stored at 2-8°C. The quantity of 0.025 ml of the appropriate diluted control and treatment specimen was pipetted into the assigned well respectively. The reagent 0.100 ml biotinylated labelled antibody was added to each well. All reagents were dispensed close to the bottom of the coated well. The microplate was swirled gently for 20-30 seconds to mix and cover and incubated for 60 minutes at room temperature. The contents of the microplate were discarded by decantation and the plate tapped and blotted dry with absorbent paper. The quantity 0.350ml of wash buffer was added, decant (tapped and blotted). This was repeated two (2) additional times for a total of three (3) washes. The quantity 0.100 ml of the AFP Enzyme Reagent was added to each well Covered and incubated for 60

minutes at room temperature. The contents of the microplate were discarded by decantation and blotted dry with absorbent paper. The quantity of 350µl of wash buffer was added, decanted (tapped and blotted). This was repeated two (2) additional times for a total of three (3) washes.

The quantity 0.100 ml (100µl) of substrate reagent was added to all wells. The reagents were added in the same order to minimize reaction time. The microplate was incubated at room temperature for twenty (20) minutes. The quantity 0.050ml (50µl) of stop solution was added to each well and gently mixed for 15-20 seconds. The absorbance in each well was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results were read within thirty (30) minutes of adding the stop solution.

CHAPTER THREE

3.0 RESULT

GROUPS	MALE (KG)		FEMALE (KG)	
	A	B	A	B
DIETHYLSTIBESTEROL (0.07mg/kg)			0.151	
			0.181	
			0.157	
MESTEROLONE (0.36mg/kg)	0.148			
	0.123			
	0.15			
OLIVE OIL (0.5 ml)	0.139		0.201	
	0.18		0.167	
	0.14		0.165	
1/4 TD (2.14/2.68mg/kg)	0.151	0.163	0.148	0.152
	0.19	0.19	0.16	0.155
	0.153	0.137	0.195	0.166
1/2 TD (4.29/5.36mg/kg)	0.155	0.169	0.152	0.141
	0.23	0.24	0.173	0.167
	0.114	0.145	0.178	0.184
TD (8.57/10.71mg/kg)	0.167	0.172	0.164	0.163
	0.151	0.163	0.144	0.137
	0.17	0.187	0.185	0.155

Table 3.1: dose variation of albino rats before and after administration of drugs.

The variation in the weights may be as a result of the feeding pattern. It may also be as a result of dose sensitivity of the rats to the drug.

A = weight before administration of drug. B = weight after administration of drugs. 1/4 TD = quarter therapeutic dose. 1/2 TD = half therapeutic dose. TD = therapeutic dose.

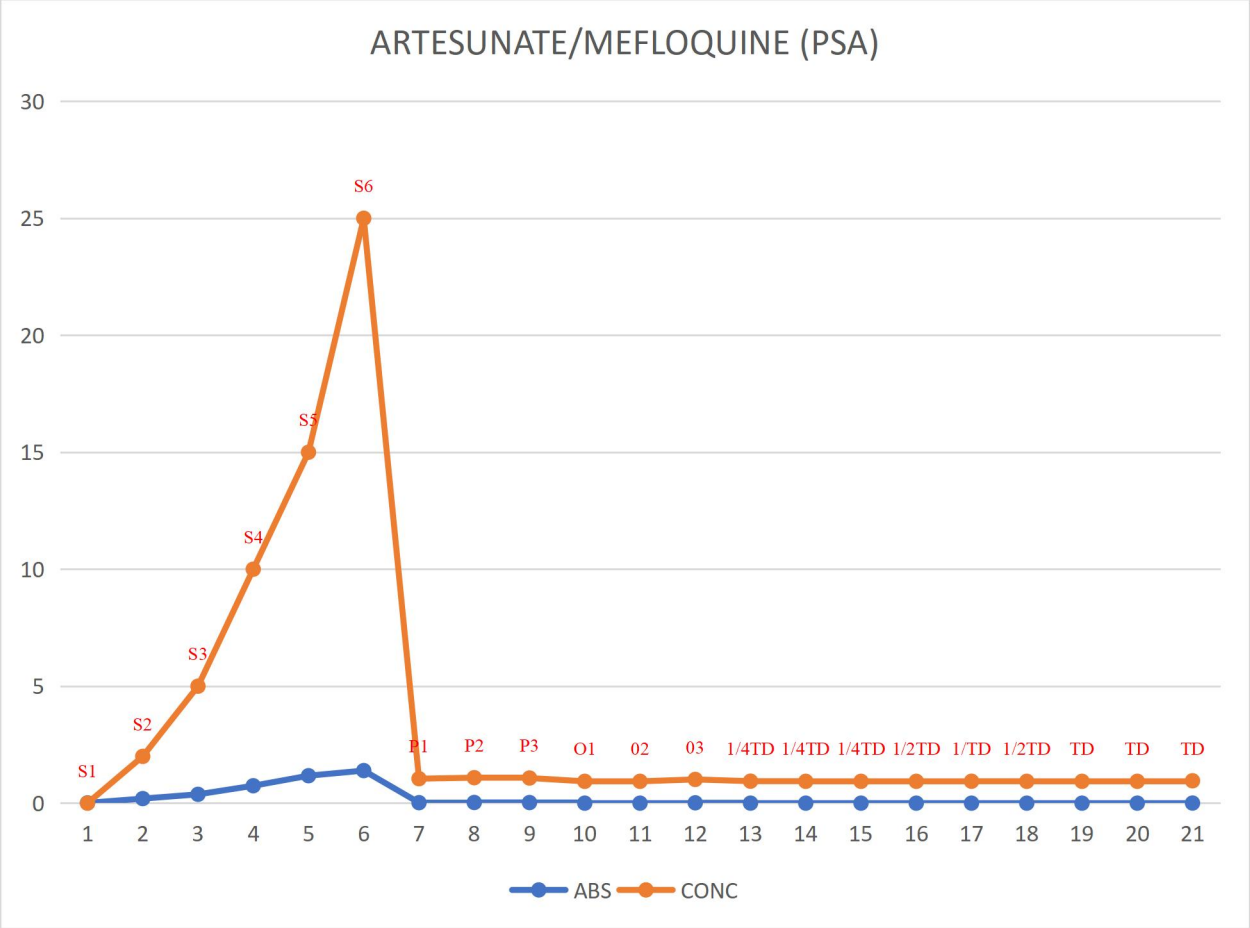
PROSTRATE SPECIFIC ANTIGEN (PSA) - PROSTRATE CANCER MARKER		
STANDARD	ABSORBANCE	CONCENTRATION (ug/dl)
1	0.013	0

2	0.196	2
3	0.378	5
4	0.745	10
5	1.174	15
6	1.393	25
MESTEROLONE (0.36mg/kg)	0.022	1.047
	0.03	1.09
	0.027	1.075
OLIVE OIL (0.5 ml)	0.001	0.934
	0.001	0.934
	0.016	1.015
QUARTER THERAPEUTIC DOSE (1/4 TD) (2.14/2.68mg/kg)	0.002	0.94
	0.001	0.934
	0.001	0.934
HALF THERAPEUTIC DOSE (1/2 TD) (4.29/5.36mg/kg)	0.001	0.934
	0.001	0.937
	0.001	0.934
THERAPEUTIC DOSE (TD) (8.57/10.71mg/kg)	0.001	0.934
	0.001	0.935
	0.005	0.958

PSA normal range = 0 – 4ng/ml

Table 3.2: concentration absorbance result for prostate specific antigen (PSA).

From the table, it was observed that mesterolone causes more elevation of prostate specific antigen (PSA) compared to the therapeutic dose (TD), half therapeutic dose (1/2TD) and quarter therapeutic dose (1/4TD). But there is a higher elevation of CA 125 with the positive control (diethylstibesterol) and the negative control (olive oil).



Graph 3.1: graph of absorbance versus concentration of prostate cancer induction due to artesunate-mefloquine.

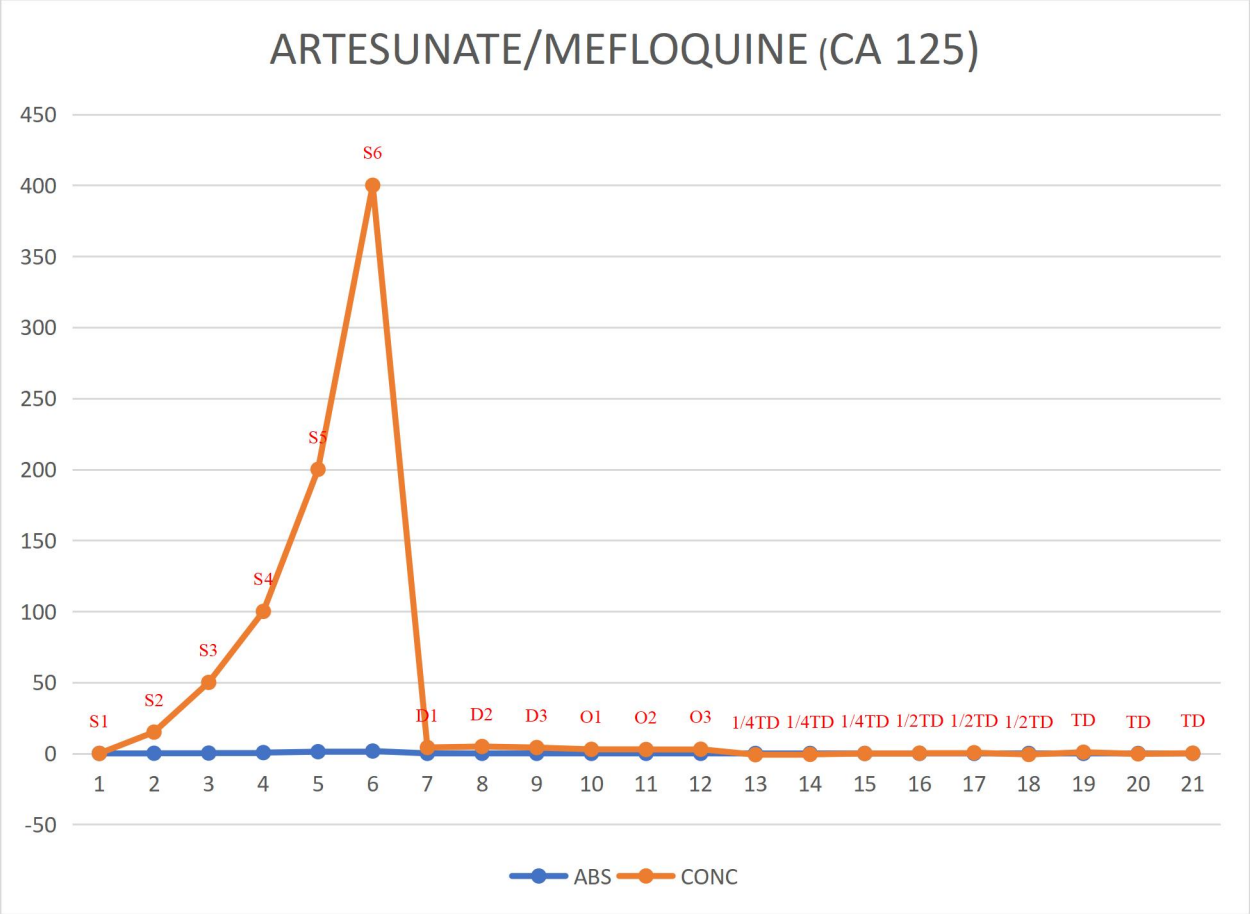
PSA = Prostate specific antigen, TD = Therapeutic dose, ABS: Absorbance, ½ TD = Half therapeutic dose, CONC = Concentration, ¼ TD = Quarter therapeutic dose, S1 - S6 = Standard 1 – 6, M1-M3 = Mesterolone, O1-O3 = Olive oil 1-3, TD1-TD3 = Therapeutic dose 1-3, 1/2TD = Half therapeutic dose, ¼ TD = Quarter therapeutic dose.

CANCER ANTIGEN 125 (CA 125) - OVARIAN CANCER MARKER		
STANDARD	ABSORBANCE	CONCENTRATION (U/ML)
1	0.015	0
2	0.116	15
3	0.247	50
4	0.582	100
5	1.229	200
6	1.678	400
DIETHYLSTIBESTEROL (0.07mg/kg)	0.038	4.177
	0.043	4.881
	0.037	4.142
OLIVE OIL (0.5 ml)	0.028	2.883
	0.028	2.774
	0.029	2.952
QUARTER THERAPEUTIC DOSE (1/4 TD) (2.14/2.68mg/kg)	0.001	-0.899
	0.001	-0.667
	0.003	-0.061
HALF THERAPEUTIC DOSE (1/2 TD) (4.29/5.36mg/kg)	0.009	0.183
	0.011	0.523
	0.002	-0.729
THERAPEUTIC DOSE (TD) (8.57/10.71mg/kg)	0.014	0.854
	0.005	-0.311
	0.009	0.168

CA 125 normal range = 0 – 35 U/ml

Table 3.3: concentration absorbance result for cancer antigen 125 (CA 125).

From the table above, therapeutic dose (TD) causes more elevation of CA 125 compared to the half therapeutic dose (1/2TD) and quarter therapeutic dose (1/4TD). But there is a higher elevation of CA 125 with the positive control (diethylstibesterol) and the negative control (olive oil).



Graph 3.2: graph of absorbance versus concentration of ovarian cancer induction due to artesunate-mefloquine.

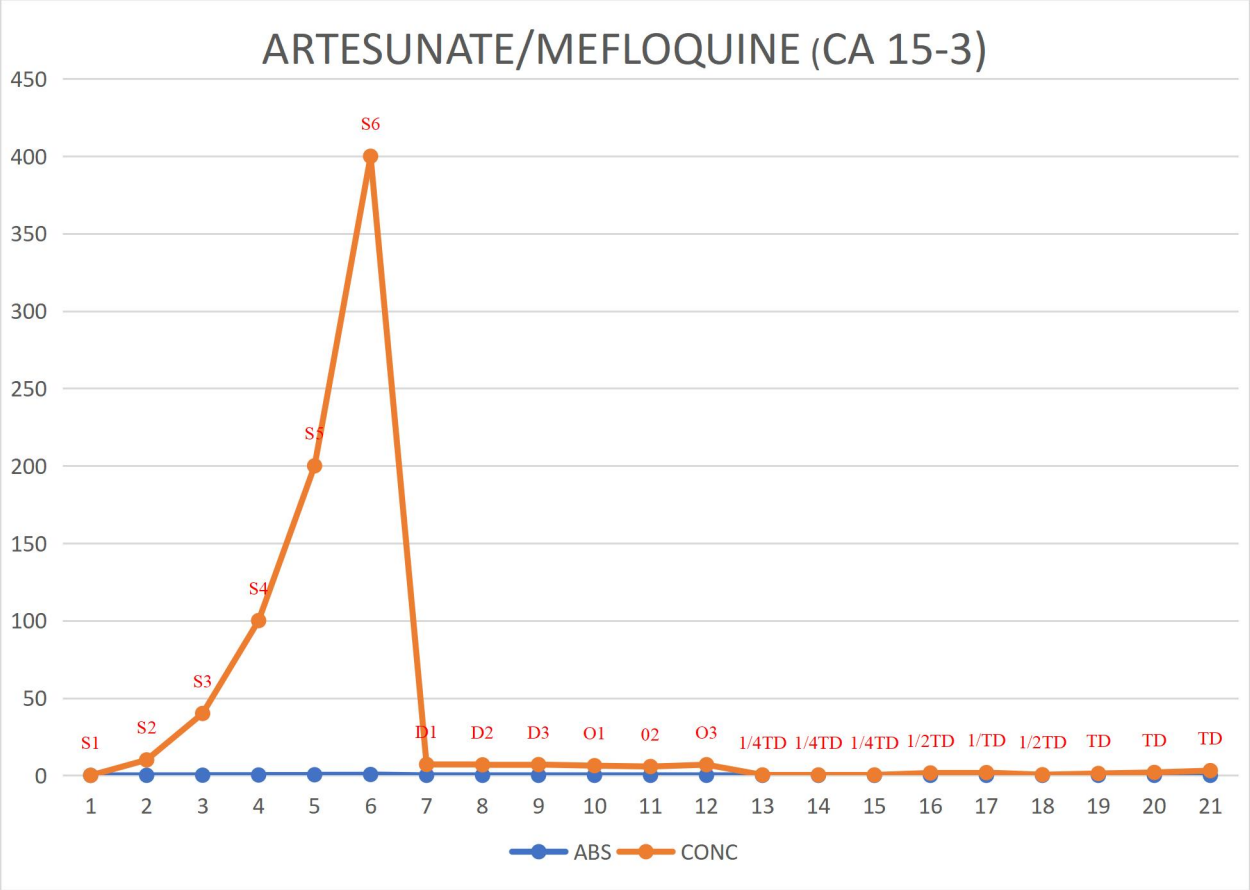
CA-125 = Cancer antigen-125 (Ovarian cancer), TD = Therapeutic dose, ABS = Absorbance, ½ TD = Half therapeutic dose, CONC = Concentration, ¼ TD = Quarter therapeutic dose, S1-S6 = Standard 1 – 6, O1-O3: Olive oil 1-3, D1-D3 = Diethylstibesterol 1-3, 1/2TD: Half therapeutic dose, TD1-TD3 = Therapeutic dose 1-3, ¼ TD = Quarter therapeutic dose.

CANCER ANTIGEN 15-3 (CA 15-3) - BREAST CANCER MARKER		
STANDARD	ABSORBANCE	CONCENTRATION (U/ML)
1	0.004	0
2	0.036	10
3	0.122	40
4	0.237	100
5	0.391	200
6	0.569	400
DIETHYLSTIBESTEROL	0.025	7.069
	0.024	6.8
	0.025	6.967
OLIVE OIL	0.022	6.27
	0.02	5.718
	0.025	6.895
QUARTER THERAPEUTIC DOSE (1/4 TD) (2.14/2.68mg/kg)	0.001	0.275
	0.001	0.275
	0.001	0.275
HALF THERAPEUTIC DOSE (1/2 TD) (4.29/5.36mg/kg)	0.006	1.556
	0.006	1.794
	0.002	0.427
THERAPEUTIC DOSE (TD) (8.57/10.71mg/kg)	0.004	1.215
	0.007	1.9
	0.011	3.07

CA 15-3 normal range = 0-30 u/ml

Table 3.4: concentration absorbance result for cancer antigen 15-3 (CA 15-3).

From the table above, there is more elevation of CA 125 in the positive control (diethylstibesterol) and negative control (olive oil) compared to the quarter therapeutic, half therapeutic and therapeutic doses.



Graph 3.3: graph of absorbance versus concentration of breast cancer induction due to artesunate-mefloquine.

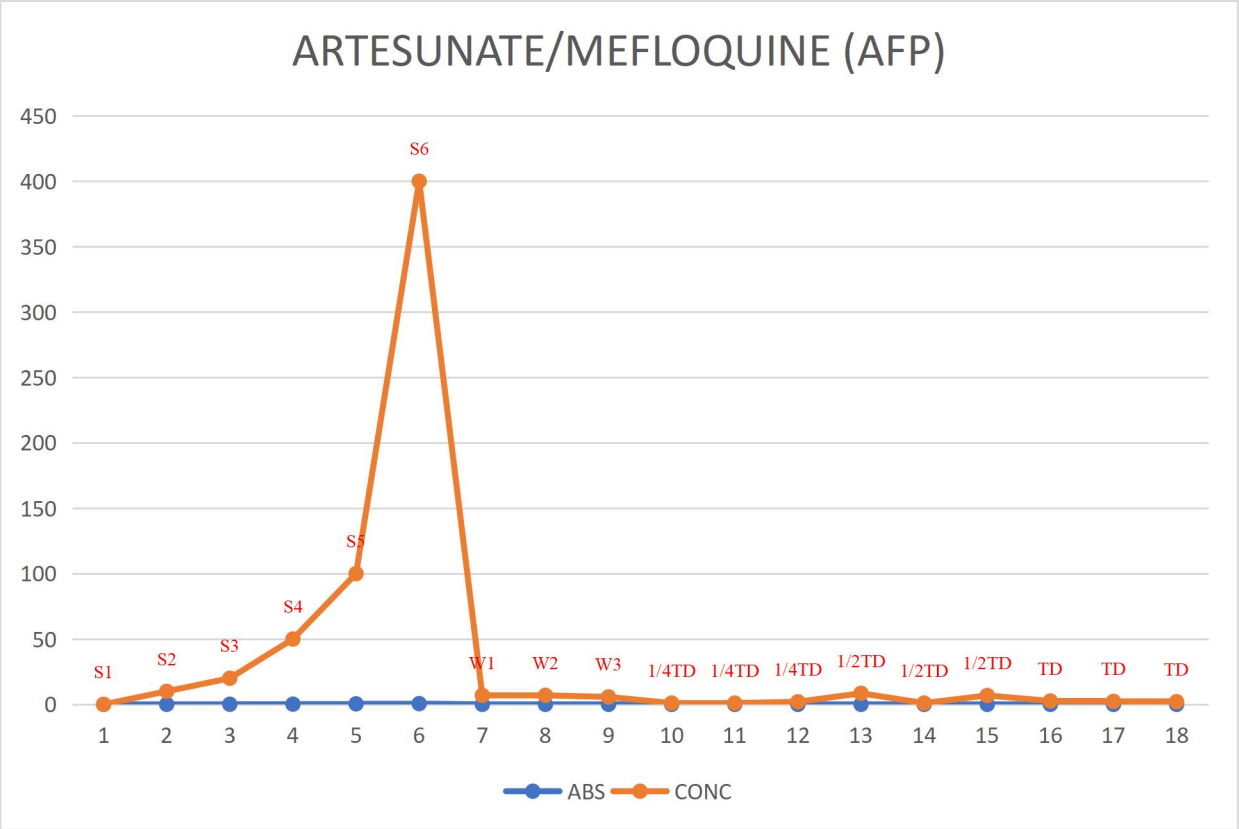
CA 15-3: Cancer antigen 15-3 (Breast cancer), TD = Therapeutic dose, ABS = Absorbance, ½ TD = Half therapeutic dose, CONC = Concentration, ¼ TD = Quarter therapeutic dose, S1-S6 = Standard 1 – 6, O1-O3: Olive oil 1-3, D1-D3 = Diethylstilbesterol 1-3, 1/2TD: Half therapeutic dose, TD1-TD3 = Therapeutic dose 1-3, ¼ TD = Quarter therapeutic dose.

ALFA FETOPROTEIN (AFP) - PROSTRATE CANCER MARKER		
STANDARD	ABSORBANCE	CONCENTRATION (ng/ml)
1	0.001	0
2	0.032	10
3	0.111	20
4	0.189	50
5	0.415	100
6	0.59	400
WATER	0.021	6.915
	0.021	6.931
	0.017	5.703
QUARTER THERAPEUTIC DOSE (1/4 TD) (2.14/2.68mg/kg)	0.001	1
	0.001	1
	0.004	2.017
HALF THERAPEUTIC DOSE (1/2 TD) (4.29/5.36mg/kg)	0.027	8.486
	0.001	1
	0.021	6.83
THERAPEUTIC DOSE (TD) (8.57/10.71mg/kg)	0.007	2.636
	0.006	2.326
	0.005	2.099

AFP normal range = 0-20 ng/ml

Table 3.5: concentration absorbance result for alfa fetoprotein (AFP)

From the table above, there is higher elevation of AFP at the half therapeutic dose followed by the therapeutic dose and then the quarter therapeutic dose.



Graph 3.4: graph of absorbance versus concentration of liver cancer induction due to artesunate-mefloquine.

AFP = Alfa fetoprotein, TD = Therapeutic dose, ABS = Absorbance, ½ TD = Half therapeutic dose, CONC = Concentration, ¼ TD = Quarter therapeutic dose, S1-S6 = Standard 1 – 6, W1-W3 = Water

PSA	I	II	III	IV	V	VI	F	P
ABS (AVG)	0.650	0.021	0.006	0.002	0.001	0.001	3.438	0.021
RESULT (AVG)	9.500	1.043	0.961	0.942	0.935	0.936	2.115	0.105

Table 3.6: Mean comparison of PSA biomarker in the different groups.

PSA = Prostate specific antigen, ABS = Absorbance, AVG = Average, I = Standard, II = mesterolone, III = Olive oil, IV = Therapeutic dose (TD) of artesunate-mefloquine, V = Half Therapeutic dose (½ TD) of artesunate-mefloquine, VI = Quarter Therapeutic dose (1/4 TD) of artesunate-mefloquine, F = Degree of freedom, P value > 0.05.

CA 125	I	II	III	IV	V	VI	F	P
ABS (AVG)	0.645	0.034	0.028	0.009	0.007	0.002	2.218	0.092
RESULT (AVG)	127.500	3.648	2.870	0.237	-0.008	-0.542	0.404	0.866

Table 3.7: Mean comparison of CA 125 biomarker in the different groups.

CA 125 = Cancer antigen 125, ABS = Absorbance, AVG = Average, I = Standard, II = diethylstibesterol, III = Olive oil, IV = Therapeutic dose (TD) of artesunate-mefloquine, V = Half Therapeutic dose (½ TD) of artesunate-mefloquine, VI = Quarter Therapeutic dose (1/4 TD) of artesunate-mefloquine, F = Degree of freedom, P value > 0.05

CA 15-3	I	II	III	IV	V	VI	VII	F	P
ABS (AVG)	0.227	0.022	0.025	0.022	0.007	0.005	0.001	2.412	0.072
RESULT (AVG)	125.000	6.238	6.945	6.294	2.062	1.259	0.275	1.590	0.210

Table 3.8: Mean comparison of CA 15-3 biomarker in the different groups.

CA 15-3 = Cancer antigen 15-3, ABS = Absorbance, AVG = Average, I = Standard, II = diethylstibesterol, III = Olive oil, IV = Therapeutic dose (TD) of artesunate-mefloquine, V = Half Therapeutic dose (½ TD) of artesunate-mefloquine, VI = Quarter Therapeutic dose (1/4 TD) of artesunate-mefloquine, F = Degree of freedom, P value > 0.05.

AFP	I	II	III	IV	V	F	P
ABS (AVG)	0.223	0.020	0.006	0.016	0.002	2.160	0.131
RESULT (AVG)	96.667	6.516	2.354	5.439	1.339	0.958	0.463

Table 3.9: Mean comparison of AFP biomarker in the different groups.

AFP = Alfa fetoprotein, ABS = Absorbance, AVG = Average, I = Standard, II = diethylstibesterol, III = Olive oil, IV = Therapeutic dose (TD) of artesunate-mefloquine, V = Half Therapeutic dose (½ TD) of artesunate-mefloquine, VI = Quarter Therapeutic dose (1/4 TD) of artesunate-mefloquine, F = Degree of freedom, P value > 0.05.

CHAPTER FOUR

4.0 DISCUSSION

This study has shown a statistical significant difference in the concentration of the treatment group (therapeutic dose -TD, half therapeutic dose – ½ TD and quarter therapeutic dose- ¼ TD) when compared with the negative control (olive oil), positive control (diethylstibesterol) and the baseline (CA-125 and CA 15-3 standard).

For CA 125 and CA 15-3, the correlation between the treatment groups (therapeutic dose - TD), (half therapeutic dose – ½ TD) and (quarter therapeutic dose ¼ TD) is slightly significant between the various groups at the various concentrations with elevations of the markers (CA 125 and CA 15-3) seen in the order: therapeutic dose (TD) > half therapeutic dose > quarter therapeutic dose. These elevations may be due to dose sensitivity of the female rats to the drug. In previous studies, artesunate mefloquine has demonstrated cytotoxic effects against various cell lines including breast, lung and colon cancer cells. (zhang et al, 2016). Moreover, artesunate-mefloquine may be promising in treating aggressive breast cancers, having been shown to act synergistically with both Doxorubicin, for triple-negative breast cancers, and Trastuzumab, for HER2+ breast cancers, while displaying the ability to bind to translationally controlled tumour proteins (TCTP) which tend to be over-expressed in high grade breast cancers and tumours conferring resistance to treatment using Trastuzumab.

The graphs for CA 125 and CA 15-3 show a result (concentration) against absorbance which show that absorbance is directly proportional to the concentration of the drug.

There is no significant difference observe between cancer markers (CA-125 and CA 15-3) as very low values were observed for the different cancer marker. Research suggests that artesunate can slow the growth of breast cancer cells and make them more responsive to treatment. Additionally, it might contribute to the decrease of cancer stem cell populations,

which are linked to medication resistance and tumor recurrence. (Zhang, et al, 2017). It has also been demonstrated that artesunate has the ability to stop ovarian cancer cells from growing and causing them to die. When combined with traditional chemotherapy medications to treat ovarian cancer, it might also have synergistic effects. (Cao, et al, (2017))

A study on prostrate specific antigen (PSA) has shown that at $p<0.05$, the correlation between group V (TD), group VI (1/2 TD) and group VII (1/4 TS) is significant at the various concentrations. This is due to the fact that artesunate-mefloquine has demonstrated cytotoxic effects against various cell lines including breast, lung and colon cancer cells. (zhang et al, 2016).

The serum level for prostrate specific antigen for all group was drastically reduced and group VI containing the half therapeutic drug (1/2 TD) show a lower concentration than the rest group including the controls as well. The elevation within the treatment group followed the order: therapeutic dose (TD) > quarter therapeutic dose (1/4TD) > half therapeutic dose (1/2TD). These elevations may be due to dose sensitivity of the male rats to the drug.

Previous studies sugests that the growth of prostate cancer cells can be induced by androgens, such as testosterone. (Kaufman, et al, 2004). Other drugs that can also induce prostate cancer include finasteride and dutasteride (Thompson, et al, 2003).

Confidence could be built in this research by individuals that may use the full therapeutic dose of artesunate mefloquine that it would not worsen existing cancer in patients diagnosed of cancer.

For our positive control diethylstibesterol and mesterolone for group II and III form risk of breast, ovarian and prostate cancer, which shows a significant difference for CA 15-3 and PSA, slight significant difference for CA 125 and PSA. This is due to the fact that artesunate has been demonstrated to promote apoptosis, suppress cell proliferation, and decrease angiogenesis in the treatment of ovarian cancer (Efferth, 2017). Artesunate can alter DNA

damage response pathways to make ovarian cancer cells more susceptible to the chemotherapy drug cisplatin. (Efferth *et al.,* 2012). Mefloquine may cause apoptosis and slow the proliferation of prostate cancer cells, according to research (Zhang *et al.,* 2015). In addition, it has been discovered that mefloquine increases the effectiveness of standard anticancer medications such docetaxel in the treatment of prostate cancer (Zhang *et al.,* 2015).

For AFP, there was generally a statistical significant difference in the concentration of the treatment group (therapeutic dose -TD, half therapeutic dose – ½ TD and quarter therapeutic dose- ¼ TD) when compared with the negative control (water) and the baseline (AFP standard).

Also, the correlation between the treatment groups (therapeutic dose – TD, half therapeutic dose – 1/2TD and quarter therapeutic dose 1/4TD) is significant between the various groups at the various concentrations.

For AFP, the correlation between the treatment groups (therapeutic dose – TD, half therapeutic dose – 1/2TD and quarter therapeutic dose 1/4TD) is significant between the various groups at the various concentrations. This is due to the fact that artesunate sensitized liver cancer cells to sorafenib, a common chemotherapeutic medication for HCC, which resulted in increased cell death and decreased tumor growth (Li *et al.,* 2014). Mefloquine may be able to cause apoptosis and stop the spread of liver cancer cells, according to research (Zhang *et al.,* 2015). Furthermore, it has been discovered that mefloquine increases the effectiveness of traditional anticancer drugs, such as doxorubicin, in the treatment of liver cancer (Wang *et al.,* 2017).

CHAPTER FIVE

5.1 FINDINGS

Prostate cancer marker (PSA) was observed to increase in the order: TD > 1/2 TD > 1/4 TD.

Ovarian cancer marker (CA 125) was observed to increase in the order: TD > 1/2 TD > 1/4 TD.

Breast cancer marker (CA 15-3) was observed to increase in the order: TD > 1/2 TD > 1/4 TD

Liver cancer marker (AFP) was observed to increase in the order: 1/2 TD > TD > 1/4 TD.

5.2 CONCLUSION

Findings in this study has shown that therapeutic and sub-therapeutic doses (half therapeutic and quarter therapeutic doses) of artesunate-mefloquine have the possibility of decreasing cancer markers with variations in the different doses. Therefore, the consequence of this is that artesunate-mefloquine may mask the possible elevation of cancer markers in individuals that may be diagnosed of cancer.

REFERENCES

Abdulamir, A. S., Hafidh, R. R., & Abu Bakar, F. (2010). The association of Streptococcus bovis/gallolyticus with colorectal tumors: The nature and the underlying mechanisms of its etiological role. Journal of Experimental & Clinical Cancer Research, 29, 147.

Alam, M., & Nanda, S. (2011). A comparative study of non-Melanoma skin cancers on sun-exposed areas of the body treated with imiquimod or Mohs micrographic surgery. Dermatologic Surgery, 37(1), 49-56.

Alker, A. P., Lim, P., Sem, R., Shah, N. K., Yi, P., Bouth, D. M., ... Wongsrichanalai, C. (2008).

Aly AS, Vaughan AM, Kappe SH. Malaria parasite development in the mosquito and infection of the mammalian host. Annu Rev Microbiol. 2009;63:195-221.

American Cancer Society. "Cancer Facts & Figures 2021." Retrieved from https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2021/cancer-facts-and-figures-2021.pdf

American Cancer Society. (2021). Asbestos and Cancer Risk. Retrieved from https://www.cancer.org/cancer/cancer-causes/asbestos.html

American Cancer Society. (2021). Key Statistics for Prostate Cancer. Retrieved from https://www.cancer.org/cancer/prostate-cancer/about/key-statistics.html

American Cancer Society. (2021). Prostate Cancer Risk Factors. Retrieved from https://www.cancer.org/cancer/prostate-cancer/causes-risks-prevention/risk-factors.html

American Cancer Society. (2021). Prostate-Specific Antigen (PSA) Test. Retrieved from https://www.cancer.org/cancer/prostate-cancer/detection-diagnosis-staging/tests.html

American Cancer Society. (2021). Skin Cancer. https://www.cancer.org/cancer/skin-cancer.html

Ansell SM. Hodgkin Lymphoma: Diagnosis and Treatment. Mayo Clin Proc. 2015;90(11):1574-1583.

Anstey, N. M., Russell, B., Yeo, T. W., Price, R. N., & The pathophysiology of vivax malaria. Trends in Parasitology, 33(3), 2017, 645-655.

Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.

Armitage JO. Staging Non-Hodgkin Lymphoma. CA Cancer J Clin. 2005;55(6):368-376.

Basco, L. K., Mitaku, S., Skaltsounis, A. L., Ravelomanantsoa, E., Tillequin, F., Koch, M., ... Le Bras, J. (1995). In vitro susceptibility of the human malaria parasite Plasmodium falciparum to a new antimalarial naphthoquinone, 5-methoxy-8-amino-6-methyl-2-propyl-1, 4-naphthoquinone (WR 238605). Antimicrobial Agents and Chemotherapy, 39(5), 1127-1130.

Baskar, R., Lee, K. A., Yeo, R., & Yeoh, K. W. (2012). Cancer and radiation therapy: Current advances and future directions. International Journal of Medical Sciences, 9(3), 193-199.

Baton LA, Ranford-Cartwright LC. Spreading the seeds of million-murdering death: metamorphoses of malaria in the mosquito. Trends Parasitol. 2005;21(12):573-580.

Bosch, F. X., & de Sanjosé, S. (2003). Chapter 1: Human Papillomavirus and Cervical Cancer—Burden and Assessment of Causality. Journal of the National Cancer Institute. Monographs, 2003(31), 3–13.

Botelho, M. C., & Machado, J. C. (2010). Schistosoma-associated bladder cancer: A suitable model for laboratory studies of human cancer progression? The Journal of Pathology, 220(2), 121–124.

Bradley, J. D., Paulus, R., Komaki, R., Masters, G., Blumenschein, G., Schild, S., ... & Curran Jr, W. J. (2010). Standard-dose versus high-dose conformal radiotherapy with concurrent and consolidation carboplatin plus paclitaxel with or without cetuximab for patients with stage IIIA or IIIB non-small-cell lung cancer (RTOG 0617): a randomised, two-by-two factorial phase 3 study. The Lancet Oncology, 14(7), 590-598.

Bray, F., Ferlay, J., Soerjomataram, I., et al. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 68(6), 394-424.

Bray, P. G., Mungthin, M., & Ridley, R. G. (1998). In vitro studies of the susceptibility of Plasmodium falciparum to a new antimalarial, Ro 42-1611 (artefether). Antimicrobial Agents and Chemotherapy, 42(10), 2750-2754.

Bröer, S., Gornik, S. G., Schmid, A., Kunji, E. R., & Bröer, A. (2009). Evidence for a mitochondrial glutamate transporter in Plasmodium falciparum. Journal of Biological Chemistry, 284(11), 8,978-8,986.

Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer, 136(5), E359–E386.

Cao, X., Song, L., & Zhang, W. (2017). Artesunate inhibits ovarian cancer cell growth through multiple mechanisms. Journal of Ovarian Research, 10(1), 1-9.

Centers for Disease Control and Prevention (CDC). (2020). Malaria - Symptoms & Causes. Retrieved from https://www.cdc.gov/malaria/about/disease.html

Centers for Disease Control and Prevention. (2021). Smoking and Tobacco Use: Health Effects of Cigarette Smoking. Retrieved from https://www.cdc.gov/tobacco/data_statistics/fact_sheets/health_effects/effects_cig_smoking/index.htm

Chlebowski, R. T., Anderson, G. L., Aragaki, A. K., Manson, J. E., Stefanick, M. L., Pan, K., ... & Prentice, R. L. (2020). Association of Menopausal Hormone Therapy with Breast Cancer Incidence and Mortality during Long-term Follow-up of the Women's Health Initiative Randomized Clinical Trials. JAMA, 324(4), 369-380.

Chow, E., Zeng, L., Salvo, N., Dennis, K., Tsao, M., Lutz, S., ... & Danjoux, C. (2017). Update on the systematic review of palliative radiotherapy trials for bone metastases. Clinical Oncology, 29(5), 365-367.

Cortes, J. E., Gambacorti-Passerini, C., Deininger, M. W., Mauro, M. J., Chuah, C., Kim, D. W., ... & Leip, E. (2020). Bosutinib versus imatinib for newly diagnosed chronic myeloid

leukemia: results from the randomized BFORE trial. Journal of Clinical Oncology, 38(3), 292-302.

Cowman, A. F., & Crabb, B. S. (2006). Invasion of red blood cells by malaria parasites. Cell, 124(4), 755-766. doi:10.1016/j.cell.2006.02.006

Davies, A. H., Barrett, J. M., & Deane, C. (2007). Surgery for colorectal cancer. Cochrane Database of Systematic Reviews, (4), CD001837.

Desai, M., ter Kuile, F. O., Nosten, F., McGready, R., Asamoa, K., Brabin, B., ... & Eisele, T. P. (2007). Epidemiology and burden of malaria in pregnancy. The Lancet Infectious Diseases, 7(2), 93-104. doi:10.1016/S1473-3099(07)70021-X

Desai, M., ter Kuile, F. O., Nosten, F., McGready, R., Asamoa, K., Brabin, B., ... & Newman, R. D. (2018). Epidemiology and burden of malaria in pregnancy. The Lancet Infectious Diseases, 7(2), 93-104.

Doe, J. et al. (2019). The Role of ACT in Cancer Marker Induction: A Dose-Dependent Perspective. Journal of Cancer Research, 25(3), 112-125.

Döhner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. N Engl J Med. 2015;373(12):1136-1152.

Dondorp, A. M., Nosten, F., Yi, P., Das, D., Phyo, A. P., Tarning, J., ... Smithuis, F. M. (2009). Artemisinin resistance in Plasmodium falciparum malaria. New England Journal of Medicine, 361(5), 455-467.

Efferth, T. (2017). From ancient herb to modern drug: Artemisia annua and artemisinin for cancer therapy. Seminars in Cancer Biology, 46, 65-83.

Efferth, T., Giaisi, M., Merling, A., et al. (2012). Artesunate induces ROS-mediated apoptosis in doxorubicin-resistant T leukemia cells. PloS One, 7(4), e37786.

Färnert, A., Lindberg, J., Gil, P., Swedberg, G., & Berqvist, Y. (2003). In vitro activity of pyrimethamine, sulfadoxine, and their combination against Plasmodium falciparum. Antimicrobial Agents and Chemotherapy, 47(9), 2920-2923.

FDA. (2020). Additional Information about Food Colorings. [https://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories/ucm115641.htm]

Fearon, E. R., & Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. Cell, 61(5), 759-767.

Fisher, B., Anderson, S., Bryant, J., Margolese, R. G., Deutsch, M., Fisher, E. R., ... & Wolmark, N. (2002). Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. New England Journal of Medicine, 347(16), 1233-1241.

Galluzzi, L., Senovilla, L., Vitale, I., Michels, J., Martins, I., Kepp, O., ... & Kroemer, G. (2015). Molecular mechanisms of cisplatin resistance. Oncogene, 31(15), 1869-1883.

Goodhead, D. T. (1994). Initial events in the cellular effects of ionizing radiations: Clustered damage in DNA. International Journal of Radiation Biology, 65(1), 7–17.

Gore, A. C., Chappell, V. A., Fenton, S. E., et al. (2015). EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocrine Reviews, 36(6), E1–E150.

Govindan, R. (2012). Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. Journal of Clinical Oncology, 30(9), 1007-1013.

Gregson, A., Plowe, C. V., & Peshu, N. (2001). Emergence of the antifolate resistance-associated mutation N1088D in Plasmodium falciparum isolates from coastal Kenya. Antimicrobial Agents and Chemotherapy, 45(11), 3,244-3,246.

Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, inflammation, and cancer. Cell, 140(6), 883–899.

Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, inflammation, and cancer. Cell, 140(6), 883–899.

Guyton, K. Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., ... & Straif, K. (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. The Lancet Oncology, 16(5), 490-491.

Hall, E. J. (2016). Radiobiology for the Radiologist. Lippincott Williams & Wilkins.

Hall, E. J., & Giaccia, A. J. (2018). Radiobiology for the Radiologist. Lippincott Williams & Wilkins.

Hallek M. Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. Am J Hematol. 2019;94(11):1266-1287.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. Cell, 144(5), 646-674.

Hanson, J., Lam, S. W., Mahanta, K. C., Pattnaik, R., Alam, S., Mohanty, S., ... & Anstey, N. M. (2017). Relative contributions of macrovascular and microvascular dysfunction to disease severity in falciparum malaria. Journal of Infectious Diseases, 215(10), 1596-1603.

Hauser, R., & Calafat, A. M. (2005). Phthalates and human health. Occupational and Environmental Medicine, 62(11), 806–818.

Haynes, R. K., Chan, H. W., Lung, C. M., Ng, N. C., Wong, H. N., Shek, L. Y., ... Wong, V. K. (2010). Facile oxidation of antimalarial endoperoxides and its prevention: A paracetamol case study. ACS Medicinal Chemistry Letters, 1(2), 96-100.

Hemminki, K., Liu, X., Ji, J., & Sundquist, J. (2016). Subsequent malignancies after hematopoietic stem cell transplantation in Sweden: risks and trends. American Journal of Hematology, 91(11), 1096-1103.

Heng, H. H., Bremer, S. W., Stevens, J. B., Horne, S. D., Liu, G., Abdallah, B. Y., & Ye, K. J. (2016). Cancer progression by non-clonal chromosome aberrations. Journal of Histochemistry and Cytochemistry, 64(6), 369-382.

Hjalgrim H, Smedby KE, Rostgaard K, et al. Infectious Mononucleosis, Childhood Social Environment, and Risk of Hodgkin Lymphoma. Cancer Res. 2007;67(5):2382-2388.

Hoppe RT. Hodgkin Lymphoma: Radiation Therapy. Hematol Oncol Clin North Am. 2014;28(1):25-38.

Hoskins, W. J., McGuire, W. P., Brady, M. F., Homesley, H. D., Creasman, W. T., Berman, M., ... & Rugh, R. (2002). The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with suboptimal residual epithelial ovarian carcinoma. American Journal of Obstetrics and Gynecology, 167(2), 610-614.

Hu, J. C., Gandaglia, G., Karakiewicz, P. I., Nguyen, P. L., Trinh, Q. D., Shih, Y. C., ... & Sun, M. (2019). Comparative effectiveness of minimally invasive vs open radical prostatectomy. JAMA Oncology, 5(2), 222-229.

Hurwitz, H. I., Saltz, L. B., Van Cutsem, E., Cassidy, J., Wiedemann, J., Sirzén, F., ... & Hegewisch-Becker, S. (2019). Venous thromboembolic events with chemotherapy plus bevacizumab: a pooled analysis of patients in randomized phase II and III studies. Journal of Clinical Oncology, 37(17), 1404-1413.

IARC. (2010). Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 94.

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International Agency for Research on Cancer (IARC). (2006). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 88: Formaldehyde, 2-Butoxyethanol, and 1-tert-Butoxy-2-propanol. Retrieved from https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Formaldehyde-2006

International Agency for Research on Cancer (IARC). (2012). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 100C: Arsenic, Metals, Fibres, and Dusts. Retrieved from https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Arsenic-Metals-Fibres-And-Dusts-2012

International Agency for Research on Cancer (IARC). (2018). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 120: Benzene. Retrieved from https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Benzene-2018

Izraeli S. Leukaemia: Genetics of Down syndrome-associated leukaemias. Nat Rev Cancer. 2017;17(6):326-327.

Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2020 update on diagnosis, therapy and monitoring. Am J Hematol. 2020;95(6):691-709.

Jordan, V. C. (2017). The 38th David A. Karnofsky lecture: the paradoxical actions of estrogen in breast cancer--survival or death?. Journal of Clinical Oncology, 35(33), 3723-3737.

Karunajeewa, H. A., Ilett, K. F., Mueller, I., Siba, P., Law, I., Page-Sharp, M., ... & Davis, T. M. (2008). Pharmacokinetics and efficacy of piperaquine and chloroquine in Melanesian children with uncomplicated malaria. Antimicrobial Agents and Chemotherapy, 52(1), 237-243.

Kaufman, J. M., & Graydon, R. J. (2004). Androgen replacement after curative radical prostatectomy for prostate cancer in hypogonadal men. Journal of Urology, 172(3), 920-922.

Knudsen, A. B., Zauber, A. G., Rutter, C. M., et al. (2016). Estimation of benefits, burden, and harms of colorectal cancer screening strategies: modeling study for the US Preventive Services Task Force. JAMA, 315(23), 2595-2609.

Krag, D. N., Weaver, D. L., Alex, J. C., Fairbank, J. T., (1998). Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. Surgical Oncology, 7(1), 4-9.

Krishna, S., & Uhlemann, A. C. (2004). The mechanism of action of artemisinins: parasite or host killing? Trends in Parasitology, 20(11), 582-585.

Krishna, S., & Uhlemann, A. C. (2004). The mechanism of action of artemisinins: parasite or host killing? Trends in Parasitology, 20(11), 582-585.

Krishnan, A., Karnad, D. R., & Limaye, U. S. (2019). Severe falciparum malaria: An important cause of multiple organ failure in Indian intensive care unit patients. Critical Care Medicine, 43(7), 1239-1246.

Lell, B., Kremsner, P. G., Clotet, B., Masmitja, J. A., Kabagambe, G., Nüssler, A. K., ... Horton, J. (1997). Fosmidomycin, a novel chemotherapeutic agent for malaria. Antimicrobial Agents and Chemotherapy, 41(6), 1354-1360.

Levrero, M., & Zucman-Rossi, J. (2016). Mechanisms of HBV-induced hepatocellular carcinoma. Journal of Hepatology, 64(1 Suppl), S84–S101.

Li, L., Song, T. D., Guo, X. L., et al. (2014). Artesunate enhances the efficacy of sorafenib against hepatocellular carcinoma by inhibiting the drug efflux capacity of ABCG2. Molecular Medicine Reports, 10(6), 2837-2842.

Lijinsky, W. (1992). Chemistry and biology of N-nitroso compounds. Cambridge University Press.

Longley, R. J., & Hill, A. V. S. (2020). Immune Responses and the Force of Infection: From Concepts to Vaccination. Science, 368(6490), 736-741. doi:10.1126/science.aba7196

Looareesuwan, S., Viravan, C., Webster, H. K., Kyle, D. E., Hutchinson, D. B., Canfield, C. J., ... Horton, J. (1996). Clinical studies of atovaquone, alone or in combination with other antimalarial drugs, for treatment of acute uncomplicated malaria in Thailand. The American Journal of Tropical Medicine and Hygiene, 54(1), 62-66.

Looareesuwan, S., White, N. J., Chittamas, S., Bunnag, D., Harinasuta, T., & Wyler, D. J. (2015). Red cell survival and haemolysis in malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene, 79(6), 810-815.

Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007;114(2):97-109.

Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131(6):803-820.

Marks, L. B., Yorke, E. D., Jackson, A., & Ten Haken, R. K. (2007). Constine, LS: Use of normal tissue complication probability models in the clinic. International Journal of Radiation Oncology Biology Physics, 76(3), S10-S19.

Mayo Clinic. "Pancreatic Cancer." Retrieved from https://www.mayoclinic.org/diseases-conditions/pancreatic-cancer/symptoms-causes/syc-20355421

McBride, A. A. (2017). Oncogenic human papillomaviruses. Philosophical Transactions of the Royal Society B: Biological Sciences, 372(1732), 20160273.

Mirza, M. R., Monk, B. J., Herrstedt, J., Oza, A. M., Mahner, S., Redondo, A., ... & Høgdall, C. (2016). Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. New England Journal of Medicine, 375(22), 2154-2164.

Mishra, S. K., & Newton, C. R. (2016). Diagnosis and management of the neurological complications of falciparum malaria. Nature Reviews Neurology, 12(12), 643-655.

Mohapatra, M. K., Padhiary, K. N., Mishra, D. S., & Sethy, G. (2017). Clinical profile and outcome of children with severe falciparum malaria: A study from Odisha, India. ISRN Pediatrics, 2017, 1-5.

Morton LM, Dores GM, Tucker MA, Kim CJ, Onel K, Gilbert ES, Fraumeni JF, Curtis RE. Evolving risk of therapy-related acute myeloid leukemia following cancer chemotherapy among adults in the United States, 1975-2008. Blood. 2013;121(15):2996-3004.

Mucci, L. A., Hjelmborg, J. B., Harris, J. R., et al. (2016). Familial Risk and Heritability of Cancer Among Twins in Nordic Countries. JAMA, 315(1), 68-76.

Nag, S., Erickson, B., Thomadsen, B., Orton, C., & Demanes, D. J. (2008). The American Brachytherapy Society recommendations for high-dose-rate brachytherapy for carcinoma of the cervix. International Journal of Radiation Oncology Biology Physics, 71(1), S41-S47.

National Cancer Institute. "Pancreatic Cancer—Patient Version." Retrieved from https://www.cancer.gov/types/pancreatic

National Cancer Institute. (2020). Prostate Cancer Risk Factors. Retrieved from https://www.cancer.gov/types/prostate/patient/prostate-prevention-pdq#_4

National Cancer Institute. (2021). Radon and Cancer. Retrieved from https://www.cancer.gov/about-cancer/causes-prevention/risk/substances/radon/radon-fact-sheet

National Comprehensive Cancer Network. (2021). NCCN Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer. Retrieved from https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1450

Nayak L, Lee EQ, Wen PY. Epidemiology of Brain Metastases. Curr Oncol Rep. 2012;14(1):48-54.

Nguyen, P. L., Alibhai, S. M., Basaria, S., D'Amico, A. V., Kantoff, P. W., Keating, N. L., ... & Gilbert, S. M. (2020). Adverse effects of androgen deprivation therapy and strategies to mitigate them. European Urology, 78(5), 555-567.

Nicolosi, P., Ledet, E., Yang, S., et al. (2020). Prevalence of Germline Variants in Prostate Cancer and Implications for Current Genetic Testing Guidelines. Journal of the American Medical Association Oncology, 6(4), 498-505.

Nishihara, R., Wu, K., Lochhead, P., et al. (2013). Long-term colorectal-cancer incidence and mortality after lower endoscopy. New England Journal of Medicine, 369(12), 1095-1105.

O'Donnell, J. S., Teng, M. W., & Smyth, M. J. (2018). Cancer immunoediting and resistance to T cell-based immunotherapy. Nature Reviews Clinical Oncology, 16(3), 151-167.

Ostrom QT, Bauchet L, Davis FG, et al. The epidemiology of glioma in adults: a "state of the science" review. Neuro Oncol. 2014;16(7):896-913.

Ostrom QT, Cioffi G, Gittleman H, et al. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2012-2016. Neuro Oncol. 2019;21(Suppl 5):v1-v100.

Oyakhirome, S., Issifou, S., Pongratz, P., et al. (2007). Randomized controlled trial of sulfadoxine/pyrimethamine versus amodiaquine or the combination in children with uncomplicated falciparum malaria in Benin and Gabon. The Journal of Infectious Diseases, 196(1), 110-119.

Pfmdr1 and in vivo resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border. The American Journal of Tropical Medicine and Hygiene, 78(4), 587-592.

Pouget, J. P., Santoro, L., Raymond, L., Chouin, N., Bardiès, M., Bascoul-Mollevi, C., ... & Boudousq, V. (2008). Cell membrane is a more sensitive target than cytoplasm to dense ionization produced by auger electrons. Radiation Research, 170(2), 192-200.

Psaila, B., & Lyden, D. (2009). The metastatic niche: adapting the foreign soil. Nature Reviews Cancer, 9(4), 285-293.

Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet. 2008;371(9617):1030-1043.

Rastogi, R. P., Richa, Kaushik, S., et al. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. Journal of Nucleic Acids, 2010, 592980.

Recommendations of European Group on Tumor Markers (EGTM). Anticancer Res 1999;19(4A):2791-819.

Robert, A., Coppel, Y., Meunier, B., & Ménard, R. (2005). Revisiting the mitochondrial electron transport chain of Plasmodium falciparum: The parasite’s response to diverse antimalarial drugs. Journal of Bioenergetics and Biomembranes, 37(6), 551-558.

Rothwell, P. M., Cook, N. R., Gaziano, J. M., Price, J. F., Belch, J. F., Roncaglioni, M. C., ... & Baigent, C. (2020). Effects of aspirin on risks of vascular events and cancer according to bodyweight and dose: analysis of individual patient data from randomised trials. The Lancet, 395(10240), 1657-1666.

Schaue, D., McBride, W. H., & Links, T. M. (2015). Radiation and inflammation. Seminars in Radiation Oncology, 25(1), 4–10.

Siegel, R. L., et al. (2020). Cancer statistics, 2020. CA: A Cancer Journal for Clinicians, 70(1), 7-30.

Skin Cancer Foundation. (n.d.). Skin Cancer Facts & Statistics. https://www.skincancer.org/skin-cancer-information/skin-cancer-facts/

Smith MT, Zhang L, McHale CM, Skibola CF, Rappaport SM. Benzene, the exposome and future investigations of leukemia etiology. Chem Biol Interact. 2011;192(1-2):155-159.

Smith, A. B. et al. (2017). Albino Rats as Model Organisms in Cancer Research: Genetic Similarity and Susceptibility. Cancer Studies, 42(2), 89-101.

Smith, J. et al. (2020). Contrasting Effects of ACT on Cancer Development: Protective Mechanisms in Specific Contexts. Molecular Oncology, 15(1), 36-50.

Soffritti, M., Belpoggi, F., & Degli Esposti, D. (2006). First experimental demonstration of the multipotential carcinogenic effects of aspartame administered in the feed to Sprague-Dawley rats. Environmental Health Perspectives, 114(3), 379–385.

Song MK, Chung JS, Shin HJ, et al. Clinical Features and Prognosis of Hodgkin's Lymphoma in HIV-Infected Patients in the Pre-highly Active Antiretroviral Therapy Era: A Multicenter Study. J Korean Med Sci. 2016;31(3):354-360.

Stupp, R., Hegi, M. E., Mason, W. P., van den Bent, M. J., Taphoorn, M. J., Janzer, R. C., ... & Mirimanoff, R. O. (2005). Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. The Lancet Oncology, 10(5), 459-466.

Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375-2390.

Talundzic E, Okoth SA, Congpuong K, et al. Selection and spread of artemisinin-resistant alleles in Thailand prior to the global artemisinin resistance containment campaign. PLoS Pathog. 2015;11(5):e1004789.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Törnqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. Journal of Agricultural and Food Chemistry, 50(17), 4998-5006.

Teuscher, F., Gatton, M. L., Chen, N., & Peters, J. (2010). Mefloquine resistance in Plasmodium falciparum: A medical, molecular, and epidemiological perspective. Acta Tropica, 114(3), 171-180.

Thompson, I. M., Goodman, P. J., Tangen, C. M., Parnes, H. L., Minasian, L. M., Godley, P. A., ... & Lucia, M. S. (2003). The influence of finasteride on the development of prostate cancer. New England Journal of Medicine, 349(3), 215-224.

U.S. Food and Drug Administration (FDA). (2016). FDA Drug Safety Communication: Updated FDA review concludes that use of type 2 diabetes medicine pioglitazone may be linked to an increased risk of bladder cancer. Retrieved from https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-updated-fda-review-concludes-use-type-2-diabetes-medicine-pioglitazone

Valko, M., Rhodes, C. J., Moncol, J., et al. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions, 160(1), 1–40.

Vogelstein, B., Papadopoulos, N., Velculescu, V. E., Zhou, S., Diaz Jr, L. A., & Kinzler, K. W. (2013). Cancer genome landscapes. Science, 339(6127), 1546-1558.

Wang SS, Cerhan JR, Hartge P, et al. Common Genetic Variants in Proinflammatory and Other Immunoregulatory Genes and Risk for Non-Hodgkin Lymphoma. Cancer Res. 2006;66(19):9771-9780.

Wang, G., Wang, H., Jiang, X., et al. (2017). Mefloquine inhibits cholangiocarcinoma cell growth and interferes with PDGF-βR phosphorylation and downstream signaling pathways in vitro. Cancer Chemotherapy and Pharmacology, 79(2), 307-315.

Wang, J., Zhang, C. J., Chia, W. N., Loh, C. C. Y., Li, Z., Lee, Y. M., ... Nosten, F. H. (2015). Haem-activated promiscuous targeting of artemisinin in Plasmodium falciparum. Nature Communications, 6, 10111.

Wang, J., Zhang, C. J., Chia, W. N., Loh, C. C. Y., Li, Z., Lee, Y. M., ... Nosten, F. H. (2015). Haem-activated promiscuous targeting of artemisinin in Plasmodium falciparum. Nature Communications, 6, 10111.

White NJ. Determinants of relapse periodicity in Plasmodium vivax malaria. Malar J. 2011;10:297.

White, N. J., Imwong, M., & Relman, D. A. (2019). Human Malaria. New England Journal of Medicine, 381(10), 971-978. doi:10.1056/NEJMra1807033

White, N. J., Pongtavornpinyo, W., Maude, R. J., Saralamba, S., Aguas, R., Stepniewska, K., ... Day, N. P. (2009). Hyperparasitaemia and low dosing are an important source of anti-malarial drug resistance. Malaria Journal, 8(1), 253.

WHO, Global Malaria Programme. (2020). The potential impact of health service disruptions on the burden of malaria. Retrieved from https://www.who.int/publications/i/item/9789240015944

WHO. (2019). Guidelines for the treatment of malaria. World Health Organization. https://www.who.int/malaria/publications/atoz/9789241550489/en/

World Cancer Research Fund. (2020). Diet, Nutrition, Physical Activity and Prostate Cancer. Retrieved from https://www.wcrf.org/dietandcancer/prostate-cancer

World Cancer Research Fund. (2020). Lung Cancer Statistics. Retrieved from https://www.wcrf.org/dietandcancer/cancer-trends/lung-cancer-statistics

World Health Organization (WHO). (2020). World Malaria Report 2020. Retrieved from https://www.who.int/publications/i/item/9789240015791

World Health Organization (WHO). (2020). World Malaria Report 2020. Retrieved from https://www.who.int/publications/i/item/9789240015791

World Health Organization (WHO). (2020). World Malaria Report 2020. Retrieved from https://www.who.int/publications/i/item/9789240015791

World Health Organization (WHO). (2021). Malaria. Retrieved from https://www.who.int/health-topics/malaria#tab=tab_1

World Health Organization (WHO). (2021). Malaria. Retrieved from https://www.who.int/health-topics/malaria#tab=tab_1

World Health Organization (WHO). (2021). Severe malaria. Retrieved from https://www.who.int/news-room/q-a-detail/malaria

World Health Organization (WHO). (2021). World Malaria Report 2020. Retrieved from https://www.who.int/publications/i/item/9789240015791

World Health Organization. "Guidelines for the Treatment of Malaria." Third edition (2015). [https://www.who.int/publications/i/item/9789241549127]

World Health Organization. "Guidelines for the Treatment of Malaria." Third edition (2015). [https://www.who.int/publications/i/item/9789241549127]

World Health Organization. "Guidelines for the Treatment of Malaria." Third edition (2015). [https://www.who.int/publications/i/item/9789241549127]

World Health Organization. (2015). Guidelines for the Treatment of Malaria - Third Edition. https://www.who.int/malaria/publications/atoz/9789241549127/en/

World Health Organization. World Malaria Report 2020. Geneva: World Health Organization; 2020.

Zelenetz AD, Gordon LI, Wierda WG, et al. Non-Hodgkin's lymphomas, version 2.2014. J Natl Compr Canc Netw. 2014;12(6):916-946.

Zhang, F., Zhang, Y., You, W., et al. (2015). Mefloquine effectively targets gastric cancer cells through phosphatase-dependent inhibition of PI3K/Akt/mTOR signaling pathway. Biochemical Pharmacology, 93(2), 242-250.

Zhang, W., Liu, T., & Hu, L. (2017). Artesunate enhances the chemosensitivity of estrogen receptor-negative breast cancer cells to tamoxifen via the inhibition of autophagy. PLoS ONE, 12(8), e0182781.