Bacteriobot Drug-Liposome Carriers: An Optimization of Cancer-Drug Delivery to the Colon by Manipulating the Gut Microbiome

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ABSTRACT

Cancer is a horrendous disease. The toxicity and the lack of effective chemotherapeutics that can reach and penetrate tumors plague the effectiveness of cancer treatment. Cancer is treatable when it is quickly diagnosed. Colon cancer occurs with the gradual accumulation of oncogenes that transform into tumors and metastatic disease. However, most drugs for cancer therapy in clinical trials for 2010 were found to be ineffective and failed. Oral administered drugs face degradation by gastric acids of the stomach, the bile salts of the liver, and by the acids of the small intestines. The drugs accumulate within the small intestines as a result and never reach the large intestines, the colon. Bacteria in the large intestines interact with drugs taken by oral administration. Bacteria in the gut breakdown and metabolize oral drugs, help to dispense, and distribute the drugs into lymphatic and blood circulation even into the gut-brain axis.

Therefore, manipulating the gut microbiome by combining bacteria with colon cancer drugs may increase their effective delivery into the colon to treat colon cancer. The bacteria *S. typhimurium* can sense hypoxia or a lack of oxygen, which is displayed deep inside of tumors and help penetrate the tumors. If bacteria are added to liposomes that carry cancer drugs to the colon, the *S. typhimurium* can use its motility and its senses of hypoxia to deliver liposomes filled with drugs to its tumor-target site, releasing the therapeutics. It is proposed that the small molecule of cancer inhibitors can be loaded into liposomes and carried by attenuated *S. typhimurium* bacteria. The bacteriobot drug-Liposome carriers can increase the effectiveness and delivery of the drug into the colon for colon cancer therapy.

INTRODUCTION

The genes that cause cancer consist of multiple mutations. These mutations are single nucleotide, multiple-nucleotide, insertions, deletions, or complex variations of DNA changes. Additional copies of a gene, including inversions, additions, and translocations, can develop into oncogenes. The oncogenes called KRAS, NRAS, BRAF, and EGFR carry many loci with frequent mutations [1]. Cancer is genetically caused by overexpressed and deregulated genes that mutated within malignant cells and tissues. Antibodies used as molecular

biomarkers for diagnosing and targeting cancerous cells with therapy have increased our understanding of molecular processes, diagnosis, and potential treatments. However, antibodies are profusely broad and cannot invade deep into tumors [2].

Cancer develops from the gradual collection of gene mutations overtime. Environmental factors for cancer development can be prevented with diet and other adaptations of beneficial behaviors. Hereditary causes for cancer can help with identifying at-risk and predisposed genotypes and disorders. However, R mutations or random mistakes made during DNA replication give us an understanding of the cancers that exist in some tissues and not others [3]. A vast majority of mortalities are attributed to cancer.

Treatment clinics do not provide total remission for patients because there is a lack of understanding of the molecular processes of the disease [4]. Tumor cells that spread to other tissues in the body are termed metastatic. These metastatic tumor cells consist of the original and the mutated cell duplicates. Cells within tumors and the tumor cells that are transported to other tissues are a significant contributor to cancer cell metastasis and renewal. Due to technology advancements as genomics and molecular procedures, drug discovery that is based on one target has immensely increased. Over the past 20 years, the drug discovery industry has experienced much growth; however, the effectiveness of the drugs discovered are characterized by reduced efficacy [5].

The most carcinogenic mutations reside within the *RAS* genes. Pancreatic cancer is so deadly due to the *KRAS* gene mutation that results in the tumor becoming dangerously metastatic. In 90 to 95 percent of patient cases, the *KRAS* gene has a significant role in the development of pancreatic cancer [6]. Pancreatic cancer has a terminal prognosis and outcome with a survival rate of five years or less. A mutation in the *KRAS* gene induces the initial development of pancreatic cancer. The three primary *RAS* genes in humans are KRAS, NRAS, and HRAS. The splicing of the *KRAS* gene produces two variants or types that are termed KRAS4A and KRAS4B. Most mutations for KRAS exist in the codons called G12, G13, and Q21 [6]. A *KRAS* mutation in the alleles for KRAS 4A and KRAS4B produces a mutation in the G12 codon [6]. A mutation in the G12 codon alters the glycine to aspartic acid that then permanently confirms the KRAS protein into a persistent active and oncogenic conformation. The *KRAS* oncogene activates and induces downstream cascades of the RAF-mitogen activated protein kinase termed

MAPK and the phosphoinositide-3-kinase or the PI3K pathway that augment the proliferation, survival, and the mobility of cancer cells [6].

Phosphorylation of targeted activities by proteins in signaling pathways and networks contribute to the development and pathogenesis of many cancers. Kinases that add phosphates produce most tumor cases from patients. Kinase signaling pathways contribute to increased cell growth, mobility into metastasis, survival, and cancer cell metabolism, and innate immune anticancer responses [7]. Disrupting the phosphorylation of proteins has become the focus and aim of cancer research and pharmacology. For example, a drug termed imatinib inhibits the protein BCR-ABL1 that results in chronic myelogenous leukemia and acute lymphoblastic leukemia [7]. Kinase inhibitors can suppress the oncogenic activities that contribute to a malignant cancerous condition.

The symbiotic relationship between commensal bacteria in the GI tract and the human condition currently lacks much understanding. The microflora in the gut produces and releases low weight organic molecules that regulate signaling networks and pathways. These organic molecules alter epigenetics, changes chromatin structure, upregulates apoptosis, monitors the differentiation of stem cells, and eliminates inflammation [8]. Because the small organic molecules released by bacterial microflora, cancer may be prevented and treated with alternative therapies.

Fewer gut bacteria results in humans lead to the increased amounts of fecal excrement containing estrogens with more double bonded phenyl groups and a lack of estrogens in the urine. Gut bacteria that can modulate the output of estrogen and other metabolites may provide therapeutic targets to combat cancer [8]. Therefore, the metabolic processes of gut bacteria can affect the delivery and absorption of drugs that change the pharmacologic expression of those drugs. The extent to which pharmaceutical drugs can reach further pass the proximal regions of the GI tract can affect can influence the metabolism of commensal gut bacteria and the different populations of gut bacterial groups. Many drugs lack optimal and productive interactions with gut bacteria due to the degradation of drugs in the stomach and the upper GI tract. The composition of the gut bacteria can activate some drugs, inactivate others, or alter the pharmacologic components into toxic substances. The pharmacological activity of drugs relies on the stability and the widespread uptake of pharmaceutical drugs [9]. For example, commensal bacteria produce

azoreductases that metabolize the edible and oral drug called sulfasalazine into a metabolite that relieves the inflammation of ulcerative colitis [9].

Nanoparticles are ideal carriers for drug delivery; however, capable nanocarriers require a design with optimal timed-release and be able to load specific drug concentrations for advancing its circulation. For example, the uptake of drugs by tumors are characterized by diameters that are less than 100 nm [10]. However, the cancerous fibroblasts within tumors can block the advancement and penetration of a nanocarrier within cancerous tumors that proliferates angiogenesis and metastatic activities. The cells within these malignant fibroblasts impede blood circulation within a tumor, blocking the effectiveness of chemotherapeutic agents. The inhibition of a deeper penetration of therapeutics lowers the efficacy of their beneficial pharmacological effects. Adding a peptide type of ligand called PEG to the surface of nanocarriers increases the preservation of its structure and leads to its extended timed release of the pharmacologic. The addition of PEG can improve the targeting of selected organs [10].

Research Question: How can we optimize the delivery of cancer therapeutics?

Justification

However, novel drug discovery and formulation requires much monetary expense and effort. Drug discovery and the formulation of novel drugs, on average, takes 10 to 15 years. The research for one specific novel drug cost 1.8 billion USD (Prada-Garcia, 2016). For these reasons, a considerable gap in drug design and discovery remains partially filled. Also, cancer drug research is expensive and requires many years of effort. Computer technological tools can now create a 3D conformation of a molecule for collecting data of the genomics of protein structures to formulate its small molecule inhibitor that is specific for the target protein binding site (Prada-Garcia, 2016). A 3D image of a protein structure can result in a tighter bind of a target protein with its small molecule inhibitor due to computer-aided drug design.

The ingesting of drugs is the most challenging strategy of delivery. Ingested drugs can pass through the epithelial lining for blood circulation. If a drug has a specific solubility, is stable, is permeable across a lipid bilayer, and can be catabolized by gut microbiota, then the drug can effectively be delivered to its target site [9]. However, the metabolic interaction between a drug and the gut microbiota lacks specific and detailed study. The metabolism of drugs by gut bacteria alters drugs into phytochemicals that do not usually reside in the living organism. Gut bacteria release enzymes that metabolize drugs before the drug uptake from the GI tract into blood circulation. Unlike the liver, the gut microbiota produces hydrophobic metabolites through the redox-oxidative process of reduction and hydrolysis [9].

Hydrophilic types of oral drugs are not degraded by the gastric acids of the stomach and the pancreas, so these types of hydrophilic drugs reach the small intestines into the large intestines in which many gut commensal and microbiota inhabit. The bacterial gut microbiota alters the hydrophilic drugs into hydrophobic compounds [9]. The absorption of the hydrophobic compounds leads to the expression and the exertion of its pharmacological effects. Another factor includes the half-life of a drug that can lead to its therapeutic effectiveness. The half-life of many cancer drugs is minute due to their being vastly hydrophobic, highly degradable, and low in molecular weight. Therefore, the cancer drug is excreted from the body quickly, lessening the effectiveness of therapy in the tumor. The use of nanoparticles as drug carriers designed into spheres of micelles, attached to PEGs that are hydrophilic, optimizes the timed-release of the cancer drugs into the blood circulation [10]. Because most cancer drugs are designed into spheres of nanocarriers, there is a gap in the study of the effects of nanoparticle shape. Nanoparticle shape affects the absorption and its uptake by cells.

Nanocarriers shaped like worms have the worst active uptake by macrophages due to its increased degradability by hydrolysis. Researchers studied the uptake of sphere-like versus cylinder-like nanocarriers and found that the cylindrical nanoparticles had less frequent uptake by CHO cells compared to the spherical nanocarriers [10].

A more effective drug-delivery system was found using nanoparticles and nanotechnology. Nanoparticles are submicron particles that are 100 to 1000 nm in size with different physical and chemical components [11]. The use of nanoparticles is highly favorable in cancer drug research since nanoparticles can lead to an extended timed release of anticancer drug components, lessening toxic side effects. The use of liposomes in drug design is beneficial since it reduces the adverse side effects of chemotherapy as it optimizes the effectiveness of its anticancer cargo that is carried. Liposomes more specifically deliver its anticancer cargo to its target sites within cells and tumors through its active and passive

delivery. More liposomes in the cancerous tumor augment the anticancer effects as the toxicity, and the adverse side effects of chemotherapy that's administered is reduced.

Most chemotherapeutic agents are less effective because they are quickly excreted from the body as a result of their low concentration. However, if encapsulated in liposomes, the quick and frequent elimination of the chemotherapeutic agent is blocked, extending the time for its release into blood circulation where it is readily available for absorption by a tumor. The encapsulation of an anticancer drug with liposomes can passively diffuse across a cell's plasma membrane due to the high hydrophobicity of its surface interacting with the hydrophobic tail ends of the bilayer. Designing an aqueous region inside of a liposome can readily package hydrophilic chemotherapeutic drugs in its interior. The European Medicines Agency and the US Food and Drug Administration has approved many hydrophilic chemotherapeutic agents carried by liposomes duet to many successful prognoses of clinical studies [11].

The design of liposomes as nanocarriers of anticancer therapeutics has produced many positive results and outcomes where currently, liposomes are formulated to express pH- sensitivity, temperature-sensitivity, and sensitivity to magnetic fields [11]. There are also new novel liposome designs with an emphasis upon lipid nanoparticles, lipid vehicles, and lipid polymer nanoparticles that can remedy the issues for effective liposomal delivery [11].

According to the NCI updated report or National Cancer Institute Budget Proposal for 2010, the list of significant cancer funded studies included the following:

1. The FDA approved 12 new drugs or drugs use (protocol)

2. 348 phase III oncology trials are ongoing

3. 861 cancer drugs are in some form of the trial process

4. 2000-plus clinical trials are accepting children and young adults

5. 200-plus prevention trials are continuing, and 100-plus screening trials are open.

However, most of the above clinical trials of therapeutics have failed [12]. Methods may be approved using liposomes and by manipulating the gut microbiome. There are many gaps in the literature about the interaction between cancer, drug delivery, and the gut microbiome. The benefits of liposomes and manipulating the gut microbiome rely on more affordability, less toxicity, and more safety to the environment. Since

the microbiome of the gut co-developed with the host, the microbiota plays an essential role in the metabolism of drugs. The microbiota can alter the physiological effects and the level of toxicity of drugs. The microbiota of the gut can influence the regulation of cancer drugs by inducing a chemotherapeutic effect. Accumulated exposure to environmental toxins accompanies the aging process. The accumulation of toxins over more extended periods reduces the variability of the ecological community of the gut microbiota. Therefore, the gut microbiota over more extended periods loses the ability to combat the development of cancerous cells. The treatment administered as chemotherapies and surgery can lead to a state of dysbiosis within the gut microbiome that elevates the level of toxicity.

The Microbiota of the GI Tract and Cancer-Drug Delivery

The chemotherapies can worsen the state of drug toxicity by not improving the state of dysbiosis. Therefore, the vital role of microbiota should be considered when designing novel cancer drug therapies. Antibiotics lessen the potency of gram-positive bacteria to assist T helper cells with accumulating an uptake of the cyclophosphamide required for eradicating mastocytomas. The medicine called vancomycin limited the anti-cancer effects of a molecule, cyclophosphamide, that is released by bacterial microbiota in tumorigenic mice. The addition of a probiotic called *E. hirae* induced anti-tumor effects in mice that had a sharp reduction in the microbiota.

Administering antibiotics caused a lessened capacity for tumor degradation. Cell death and the activation of immune cells did not generate this antibiotic effect, but the lack of cytotoxic inhibition brought about the antibiotic effect of oxaliplatin after only two days of therapy [13]. Administering probiotics as *Bifidobacterium* regulated the development of tumors by increasing the immune response of specific T cells in the surrounding environmental matrix of tumors. By disabling bacteria through applying heat, the CD8+ T cell response becomes incapacitated [13]. Instead, signals from Bifidobacterium are carried by and relayed through dendritic cells into the immune lymphoid tissues and organs.

Through an investigation of dietary fiber, the result of toxicity did not derive from dysbiosis. However, the availability of the short-chain fatty acid called Butyrate regulates the intensity of dysbiosis. Consuming dietary fiber provides enough measures of glucose that provides energy for microbiota and to produce SCFAs as butyrate. Eradicating pathogenic bacteria as Firmicutes and Actinobacteria allowed the

restoration of proteobacteria. Bacterial microbiota needed for its anti-inflammatory effects immensely decrease after the administration of chemotherapy [13]. The focus of current therapeutic designs reduces toxicity but does not manipulate the gut microbiota to restore the effectiveness of chemotherapeutics.

The probiotic yogurt called Yakult was given to children. Doctors administered chemotherapy to those children and gave them Yakult consisting of B. breve probiotics [13]. The children displayed a reduction in fever, and fewer amounts of antibiotics were required. However, current clinical and cancer research lacks the study of the beneficial properties of probiotics and a balanced gut microbiota upon the improve prognosis of cancer patients. Reengineering the ecological state of the microbiome can provide the expected and favorable function of the microbiota. The steps required to reset the microbiota as a cancer therapy is still not well-understood. A researcher can engineer bacteria to become carriers and vehicles of drug therapies into target tissues and organs. After re-engineering the bacterial feedback loops, the transgenic bacteria lysed as it reached a specific population size, releasing its contents of drugs. Dr. Din gave mice an oral form of the lysis bacterial strain with 5-FU [13]. The mice exhibited a reduction in tumor development. Mice that harbored a well-balanced gut microbiota responded more intensely to the alkylating agent called cyclophosphamide or CTX.

A well-balanced gut microbiota initiated an effective chemotherapeutic response in a murine model of cancer in the lymphatic system. Researchers discovered a decrease in DNA degradation and enhanced the genes necessary for reactive-oxygen species or ROS. Mice labeled as Cybb-/- lacked NADPH oxidase that generates the ROS needed for restoring myeloid cells.

The Cybb-/- mice without NADPH oxidase expression reacted weakly to the chemotherapeutic drug called oxaliplatin. However, a well-balanced microbiota optimizes the antitumor effect of oxaliplatin. The healthy gut microbiota prepares the myeloid cells to discharge ROS, lessen inflammation through inhibiting cytokine assembly, and effectively treat tumors. The CpG oligodeoxynucleotides, CpG OGN, is a synthetic form of bacterial DNA. The CpG OGN, in mice, binds to the TLR9 receptor and initiates an immune reaction. The CpG OGN is less effective in germ-free and in mice that were given antibiotics due to increased inflammation fewer immune responses to

produce an anti-cancer effect.

A healthy gut microbiota combined with artificial monoclonal antibodies allowed mice to regulate their MCA205 sarcomas by inhibiting the cytotoxic T-lymphocyte-associated protein 4 [14]. Mice treated with antibiotics did not react to the inhibition of CTLA-4.

The microflora called *Bacteroides fragilis* restored the anticancer effects of the drug called ipilimumab in germ-free mice. T-cells with an affinity for *B. fragilis* increased an antitumor reaction to CTLA-4. Mice from two different farms called Taconic Farms, and the Jackson Laboratory displayed two dissimilar developments of tumors. The Taconic Farm developed more tumors than the Jackson laboratory. By exchanging fecal matter from Jax to the Tac, lessened the progression of tumor formation. The mice from Jax harbored more of *Bifidobacteria* in the gut microbiota. *Bifidobacterium* connects with T-cells to produce an anti-cancer effect. The exchange of the *Bifidobacterium* from Jax to Tac lowered tumor activity and increased the anticancer effects from the drug called anti-PD-L1.

Different characteristics of microbiota in the gut of *C. elegan* worms affected the metabolism of the colorectal cancer drug called fluoropyrimidines. Researchers gave an anticancer treatment to the *C. elegans* that consisted of camptothecin, 5-fluorouracil or 5-FU, and fluoro-2′-deoxyuridine (FUDR). The *C. elegans* were given *E. coli* and *Comamonas* bacteria for oral ingestion. Bacteria produce catabolite end products that enhance the effects of 5-FU and degrades oncogenes [14]. However, there is a lack of a clear and profound understanding of the metabolism that occurs in the gut microbiota. This deficient level of knowledge for the metabolic processes of the gut microbiota can be dangerous. Patients in Japan were given an antiviral and 5-FU that caused a toxic concentration of the 5-FU. Sixteen patients died due to sorivudine metabolite called E-5-(2-bromovinyl) uracil or BVU deactivating the dihydropyrimidine dehydrogenase, which deactivates 5-FU. Enzymes from bacteria generate BVU from the antiviral called sorivudine. Antibiotics given lessened the levels of BVU by the dwindling of the gut microflora.

Pillai et al. altered the genetics of *E. coli* to release human bone morphogenetic protein-2 or BMP-2. This *E. coli* caused apoptosis of cancerous colonocytes in the adenocarcinoma cell line. Din et al. rewired the feedback loops used for quorum sensing in E. coli to split and release Haemolysin E that helps to eradicate tumors. The release of the Haemolysin E initiates

the delivery of factors that control the capacity of bacterial populations. The re-engineered E. coli with the combination of 5-FU, increases the survival rate of mice, with metastatic colon cancer, by 50% [14]. Zitvogel et al. and Sivan et al. used a murine model that exhibited a gut microbiome with dysbiosis. They found that the germ-free murine model did not respond adequately to anti-PD-1 therapy for cancer.

After transferring fecal matter with the needed gut microflora, augmented the activity of chemotherapeutics. The germ-free mice were given oral probiotics that replenished the response to the PD-1. Patients with melanoma retained a high level of bacteria from the Rumnococcaceae family, and the patients contained a higher capacity for antitumor activity. Gajewski et al. found increased populations of *Bifidobacterium longum*, *Collinsella aerofaciens* and *Enterococcus faecium in the stool of* patients who responded well to anticancer treatments. The fecal matter from these patients was transferred to mice, and the mice showed antitumor effects when given the PD-1 inhibitor.

The manipulation of the microbiota that resides in the gut can improve the effectiveness of chemotherapeutic drugs. The combination of antibiotics with probiotics lowered the rate of irinotecan, a cancer drug, forming mucositis in mice. A lack of diversity and variations of bacterial populations reduced the survival rate by 31% [14]. Researchers studied 857 cancer patients with allo-HSCT, and they found that the use of antibiotics propagated pathogenic bacteria as *Akkermansia muciniphila*, leading to the failure of graft- versus-host disease. The GVHD yielded a mortality rate of 5 years. The *Akkermansia muciniphila* proliferated after antibiotic use, and epithelial lining of the colon deteriorated as the GVHD spread. Transferring healthy gut microbiota to 4 patients experiencing GVHD gave promising results [14].

Issues include 1) inability to target tumors, 2) unable to intrude tissues, and 3) a need for less toxicity to cancer cells [15]. These issues of drug delivery will negatively affect cancer treatments, resulting in higher rates of mortality. However, manipulating the microbiome through the bacteria termed *S. typhimurium* can tackle these issues. The benefits provided by *S. typhimurium* include increased sensitivity to target tumors, can favorably grow in the environment of cancer, can penetrate, less toxicity reduced immune responses, and are easy to re-engineer. The bacterium Salmonellae is an excellent choice for targeting cancer cells and tissues because it can be manufactured as a therapeutic in conjunction with chemotherapies.

Sensing the tumor microenvironment

Hypoxia or a reduction in the availability of oxygen is common for cancer pathogenicity, impeding the effectiveness of chemoradiation therapies. Tumors consist of sites that exhibit many states of hypoxia due to the narrowing of arteries, blood vessels, and the endothelial lining of cells. Because of this narrowing of vessels, the velocity of blood circulation is reduced, and hypoxia occurs. The use of *S. typhimurium* that is a facultative anaerobe can be engineered to express a hypoxia-inducible promoter-1 or HIP-1. The expression of HIP-1 allows the facile delivery of chemotherapeutics by *S. typhimurium,* causing a reduction in toxicity and more penetration of drugs with greater targeting success [15]. Re-engineering the *S. typhimurium* can allow this bacterial strain to become more sensitive to small organic and nutrient molecules through expressing properties of chemotaxis.

Tumor penetration and proliferation

Bacteria as Salmonellae can more deeply penetrate tumors with chemotherapeutics due to the high rate and velocity of their motility properties. Because of their ability to become mobile, they can navigate around cumbersome blood vessels; these bacteria can populate the total span of a tumor. Salmonellae were engineered to carry microbeads throughout a tumor. The bacterial strain of *S. typhimurium fill*s tumors at a higher rate than healthy organs at approximately 1,000- fold higher with 1010 CFU/g of tissue [15]. Bacteria that grow and populate in tumors starve the tumors of nutrients, initiate immune responses, and cause apoptosis.

Immune stimulation

Tumors innately inhibit the development of immune responses and escape most immune responses. *S. typhimurium* induce immune responses that produce suppression of tumor growth.

S. typhimurium has flagellin, LPS, and CpG sites that bind to toll-like receptors or TLRs that identify antigens. Activating the TLRs generate innate and adaptive immunity [15].

Programmability

Clinical research in phase I for the study of VNP200009 proves the ability of chemotherapeutics to invade and be delivered into tumors is a severe issue for advancing cancer research [15]. Therefore, the delivery of therapeutics through

bacteria- delivery systems should be investigated. Two options for optimizing tumor-targeting are using tumor-amplified protein expression therapy or TAPET for delivering chemotherapeutics and re- engineering the surface ligands of bacterial cells [15]. Also, Park et al. produced research for their findings in the "New paradigm for tumor theranostic methodology using bacteria-based microrobot" that presents a method of *In-vitro* and *in-vivo* tests that confirmed their constructed bacteriobots displayed chemotactic motility and tumor targeting capabilities [17]. They concluded that "The new bacteriobots act as microactuators and microsensors to deliver microstructures to tumors" [17].

Possible Outcomes

The importance of the research outcomes

We will be able to test various molecular weights of the liposome carrier. The weight of 40kDa is above the renal threshold and allows a feasible lymphatic clearance of nanoparticles [12]. The molecular weight of 40 kDa can enable the delivery of small- molecule inhibitor into tumors through tight vascular junctions. It allows for efficient delivery of drug at the tumor site through up-regulated membrane transporter systems.

The research outcomes will provide more specific methods for optimizing the oral delivery of chemotherapeutics by manipulating the microbial make-up of the lower GI tract. Bacteria of the GIT affect the delivery of cancer drugs and maximizing the delivery of medications via bacterial vehicles can lengthen the release and increase the efficacy of the cancer drug. The research will specifically target cancerous colonocytes. The goal is to amplify the uptake of the drug into the cancer colonocytes by manipulating the GI tract microbial.

The importance of the research will determine and confirm the measure of pharmacokinetics needed to affect the pharmacodynamic profile of GIT microbial. The study will test the drug release of small molecule inhibitors via engineered and attenuated bacteria. The outcomes also include results that show an increase in the drug's specificity toward colon cancerous tumors and cause a higher penetration of the cancer drug into tumors.

Theoretical outcome

In theory, the use of liposomes to carry small cancer inhibitors will enhance the delivery.

Liposomes are phospholipid vesicles that consist of one or more lipid bilayers enclosing an aqueous space. Hydrophobic compounds are inserted into the bilayer membrane as the hydrophilic components of the drug are entrapped in the aqueous center. Liposomes can transport DNA, proteins and imaging agents. Adding cholesterol molecules into the membrane lowers exchange with other lipids, red blood cells, and lipoproteins. Cholesterol into a small 100nm causes an electrostatic reaction to the neutral liposomes, which prolongs the circulation time for several hours.

The EPR effect allows liposomes to accumulate by passive targeting. Adding a surface charge of nano-delivery systems can influence the electrostatic interaction of the nanocarriers with the components in the GI tract following oral administration and improved selectivity of the drug to the diseased tissue. Cationic-nano delivery can adhere to GIT tissue. Positively charged nanocarriers can bind to the negatively charged intestinal mucosal walls. The design of a nanocarrier for small molecule inhibitors will manipulate the microbial make-up of the GI tract for delivering oral chemotherapeutics to cancerous melanocytes within the GI tract. The design will include a liposome, PEGylation, Biotinylation, avidin, and an attenuated *S. typhimurium*.

The liposome will be 200nm in diameter, carry at a slightly positive charge, and be PEGylated. An example of this design is the cancer drug called Doxil. The proposed model can allow the accumulation of cancer drugs in colon cancer tumors, lessen issues with opsonization and elongate its circulation time.

Limitations of the research

Liposomes protect and prevent a drug from early inactivation, degradation, and dilution in circulation. Since the liver has the most substantial value of drug-uptake, it has a 10-fold higher uptake of liposomes than the other organs. The pore diameter of liver capillaries ranges from 100 to 800nm and can remove liposomes at 50-100nm in size. Liposomes can be cleared by phagocytosis of macrophages in the RES or the organs that include the liver, spleen, kidney, and lymph nodes. Oversaturation of liposomes can induce an immune response of suppression and cause infection. Reduced clearance of liposomes can lead to disease. Giant unmodified liposomes are more easily cleared than small, neutral, positively charged liposomes. Large and charged liposomes are quickly cleared within an hour by the spleen. Cationic nanoparticles can raise toxicity levels, cause cell shrinking, reduce events of cytosis

and cell division, and degrade the cytoplasm. The limitations include the EPR effect, delivery of nanoparticles effectively, obstacles of solid tumors with high pressure at the center, and opsonization.

Possible Methods

The *S. typhimurium* cancer therapy and delivery of therapeutic agents are triggered by external L-arabinose-inducible pBAD promoter, tetracycline- or by the pTet promoter [15]. Secondly, *S. typhimurium* can sense hypoxia formation induced by fumarate and nitrates and can interact with the quorum-sensing signals of commensal bacteria in the GI tract. The *S. typhimurium* can detect the QS signals and then activates gene expression in alignment with other bacterial cells internal of tumor tissues. The *S. typhimurium* cells were engineered to target tumors more specifically. The *S. typhimurium* cells displayed antibodies on its surface to combat tumor-associated antigens called CD20 that infected lymphoma cells expressing CD20. The non- infectious ppGpp strain of *S. typhimurium* was engineered for expressing a surface RGD peptide, and its eradicated xenograft cancer cells.

Colon cancer cells were suppressed by the ΔppGpp strain of *S. typhimurium* that activated the inflammation process for inhibiting tumors with cytokines. The ΔppGpp strain can also act as a targeted tumor delivery of chemotherapeutics. Nguyen et al. found that after treating mice with attenuated strains of *S. typhimurium,* the size of the tumors was reduced [16]; however, the tumors regrow. Nguyen et al. concluded that only treating the tumors with *S. typhimurium* bacteria alone could not completely eradicate the cancerous tumors [16]. In the method described, *S. typhimurium* bacterial cells will be combined and co-cultured with liposomes for drug delivery of the small molecule inhibitors to cancer colonocytes. Materials include: Animal model of Five-to six-week-old male BALB/c and BALB/c athymic nu−/nu−mice (20–30 g body weight) were purchased from the Orient Company (Korea), bacterial strains: ΔppGpp S. typhimurium, SHJ2037, and cell lines: CT-26 colon carcinoma cells and Hep3B2.1-7 human hepatocellular carcinoma cells and tumors.

Methods may include the following: Organic Phase:

PLGA is dissolved in acetone at a 5mg/mL concentration. The small molecule inhibitors are added.

Aqueous Phase

Lecithin, DSPE-PEG-COOH, and DSPE-PEG-Biotin were dissolved in 4% aqueous ethanol solution with a concentration of 0.6mg/mL, 0.02 mg/mL, and 5mg/mL, respectively making up 30 mL.

Water Phase

The water phase of 70mL should be preheated to 65 degrees Celsius.

1. Add 0.3 g of the small molecule drug inhibitor with 30g PLGA to 9mL of acetone.

2. Drip the organic phase of 9mL at 1mL/min into the 65 Celsius heated water with medium magnetic stirring for 2 hours.

3. The aqueous phase was prepared with 4% ethanol, Soybean lecithin phosphatidylcholine, and with DSPE-PEG2000-Biotin. The soybean lecithin to DSPE-PEG-Biotin molar ratio should be between 7:3 to 8.5:1.5.

4. Acetonitrile can be added to PLGA (Durect Corporation, Pelham, AL) with the small drug molecule. The drug should weigh between 10-30% of the polymer weight for the drugs to be entirely encapsulated by the polymer. The lipid to polymer weight ratio should be between 15% to 20%. The aqueous solution is preheated at 65 degrees Celsius with medium stirring for 3 to 5 minutes.

5. Then add drops of the organic solution (1mL/minute) to the aqueous solution while using magnetic stirring and then strongly vortex solution for 3 min.

6. After adding 9mL of the organic solution, return to medium magnetic stirring and allow the LPNs to self-assemble at room temperature for 2 hours.

7. Wash the new LPNS three times in Amicon Ultra-4 centrifugal filters from Millipore, Billerica, MA. Wash 10,000 Da of the LPNs per filter and then resuspended in water or buffer solution at an ending desired concentration.

8. The LPNs should be stored at 4 degrees Celsius overnight, freeze-dried, or lyophilized for more extended storage at -80 degrees Celsius.

Addition of *S. typhimurium* to Liposomes

1. Incubate 500mg of biotin with 3.3 x 10^8 cells per mL of culture medium for 1 hour.

2. Microbeads   containing   Rhodamine-Containing

fluorescent carboxylated PS at 1.3 x 10^8 per milliliter were covalently bound to streptavidin-PerCp-Cy5.5 of 500 mg. Biotin expressing *S. typhimurium* and streptavidin-PerCP-Cy5.5-coated PS microbeads were cultured and incubated for 30 minutes at 37 degrees Celsius. The images of the *S. typhimurium* attached PS microbeads can be observed and examined with a confocal laser scanning microscope. Culture newly assembled liposomes with *S. typhimurium* and streptavidin-PerCP-Cy5.5-coated.

Analysis of Liposomes delivered by *S. typhimurium*

The bacterial strains of ΔppGpp *S. typhimurium*, SHJ2037 (relA::cat, spoT::kan), forming units were given to mice at 4.5 x 10^7 colonies per mouse. The mice carry the CT-26 colon carcinoma cells and Hep3B2.1-7 human hepatocellular carcinoma cells and tumors. The cell lines can be grown in high-glucose DMEM containing 10% fetal bovine serum and 1% penicillin-streptomycin. The tumors will then be measured in mm^3 every four days after oral administration of small molecule inhibitor through liposomes and delivered by attenuated *S. typhimurium*. The cells and tumors can be imaged and analyzed with confocal microscopy and with live-cell imaging after fluorescent staining.

CONCLUSION

In the method described, the *S. typhimurium* bacterial cells can be combined and co- cultured with liposomes for drug delivery of the cancer drug to colonocytes. Liposomes deliver drugs and small molecules to specific target sites. Liposomes consist of lipid nanoparticles that can bind and fuse with the phospholipid bilayers of cells. The liposomes fuse with the cell, and then the drug or small molecule is released into the cell through the process of diffusion.

Liposomes are a better way to deliver chemotherapeutics because the liposomes can fuse with a cell without acting as a ligand and can carry a broader and more extensive range of small molecule drugs and inhibitors. However, liposomes can be degraded by gastric juices of the stomach, bile salts of the liver, and are quickly removed from circulation via the small intestines. The liposome drug carriers accumulated within the small intestines and do not entirely enter the colon for treating colon cancer. Designing liposomes with a longer drug release rate and time can prevent the accumulation and clearance in the small intestines. The *S. typhimurium* cells combined with liposomes carrying a cancer drug work through increase motility. The *S. typhimurium* assists with

penetrating drugs deeper into tumors by their detection of hypoxia or a lack of oxygen. Inside of tumors, there is an abundance of angiogenesis that reduces the circulation of oxygen. The *S. typhimurium* carriers can penetrate and deliver cancer drugs to these sites of hypoxia. Also, the *S. typhimurium* can sense and taxis towards the presence of other pathogenic bacteria in a tumor and then release the chemotherapeutics.

The PEGylation and Biotinylation of liposomes can lengthen the rate of drug released. However, the liposome, after adding PEGs and Biotin, can remain susceptible to bile salts and the acidity of the GI tract. Therefore, the following method serves to protect and prevent the damage of liposomes by the GI tract's acidity, and further the delivery of liposomes carrying the drug inhibitor into the colon, treating the carcinoma colonocytes. The method will produce chitosan-coated liposomes for algae-based encapsulation. The chitosan-coated liposomes will carry the drug inhibitor and will be distributed into capsules produced from algal cells. Algae- based capsules cannot be degraded by stomach gastric juices, bile salts, and cannot accumulate within the small intestines. The cell walls of algal cells are like the cells' walls of plant cells that consist of cellulose. Gastric and acidic juices in the stomach and from the small intestines cannot degrade cellulose. The commensal bacteria of the large intestines can breakdown and metabolize the cellulose in the cell walls of the algal cells. The gut bacteria break down the cellulose of the algal capsule, releasing the liposomes into the colon. Therefore, future research can consist of applying many different methods of pharmacokinetics to liposomal formation for enhanced and targeted drug delivery.

REFERENCES

1. Lai Z, Markovets A, Ahdesmaki M, Chapman B, Hofmann O, McEwen R, et al. (2016) VarDict: a novel and versatile variant caller for next-generation sequencing in cancer research. Nucleic Acid Res 44(11): e108.

2. Ma H, Liu J, Ali MM, Mahmood MA, Labanieh L, Lu M, et al. (2015) Nucleic acid aptamers in cancer research, diagnosis and therapy. Chem Soc Rev 44(5): 1240-1256.

3. Tomasetti C, Li L, Vogelstein B (2017) Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention Science. 355(6331): 1330-1334.

4. Zhang X, Marjani S L, Hu Z, Weissman S M, Pan X, Wu S (2016) Single-cell sequencing for precise cancer research: progress and prospects. Cancer Res 76(6): 1305- 1312.

5. Bolognesi M L, Cavalli A (2016) Multitarget drug discovery and polypharmacology. ChemMedChem 11(12): 1190-1192.

6. Kent OA (2018) Increased mutant KRAS gene dosage drives pancreatic cancer progression: evidence for wild-type KRAS as a tumor suppressor? Hepatobiliary Surg Nutr 7(5): 403-405.

7. Gross S, Rahal R, Stransky N, Lengauer C, Hoeflich KP (2015) Targeting cancer with kinase inhibitors. J Clin Invest 125(5): 1780-1789.

8. Paul B, Barnes S, Demark-Wahnefried W, Morrow C, Salvador C, Skibola C, et al. (2015) Influences of diet and the gut microbiome on epigenetic modulation in cancer and other diseases. Clin Epigenetics 7(1): 112.

9. Kim DH (2015). Gut microbiota-mediated drug-antibiotic interactions. Drug Metab Dispos 43(10): 1581-1589.

10. Truong NP, Whittaker MR, Mak CW, Davis TP (2015) The importance of nanoparticle shape in cancer drug delivery. Expert Opin Drug Deliv 12(1): 129-142.

11. Yingchoncharoen P, Kalinowski DS, Richardson DR (2016) Lipid-based drug delivery systems in cancer therapy: what is available and what is yet to come. Pharmacol Rev 68(3): 701-787.

12. Maeda H, Khatami M (2018) Analyses of repeated failures in cancer therapy for solid tumors: poor tumor-selective drug delivery, low therapeutic efficacy, and unsustainable costs. Clin Transl Med 7(1): 11.

13. Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, Kinross JM (2017) Gut microbiota modulation of chemotherapy efficacy and toxicity. Nat Rev Gastroenterol Hepatol 14(6): 356-365.

14. Pouncey AL, Scott AJ, Alexander JL, Marchesi J, Kinross JM (2018) Gut microbiota, chemotherapy and the host: the influence of the gut microbiota on cancer treatment. ecancer 12: 868.

15. Zheng JH, Min JJ (2016) Targeted cancer therapy using engineered Salmonella typhimurium. Chonnam Medical J 52(3): 173-184.

16. Nguyen VH, Kim HS, Ha JM, Hong Y, Choy HE, Min JJ (2010). Genetically engineered Salmonella typhimurium as an imageable therapeutic probe for cancer. Cancer Res 70(1): 18-23.

17. Park SJ, Park SH, Cho S, Kim DM, Lee Y, Ko SY (2013) New paradigm for tumor theranostic methodology using bacteria-based microrobot. Sci Rep 3: 3394.