DRUG REPURPOSING: A STUDY OF THE POTENTIAL ANTITUMORGENIC ACTIVITY OF NONCHEMOTHERAPEUTIC DRUGS

By

Katelyn Hoagland

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Thesis Director: Jeffry Fasick, Ph.D.

Associate Professor, College of Natural and Health Sciences

University of Tampa

Thesis Committee Member: Scott Burghart, Ph.D.

Associate Professor, College of Arts and Sciences

University of South Florida St. Petersburg

Thesis Committee Member: Thomas Smith, Ph. D

Associate Professor, College of Arts and Sciences

University of South Florida St. Petersburg

ABSTRACT

In this thesis, the topic and applications of drug repurposing are explained. Drug repurposing is the process of finding new biological targets for existing drugs which have already been approved for treatment of other diseases, or whose targets have already been discovered [1]. The fact that there are many drugs that interact with biological elements outside their targets is being continually reinforced as more and more drug repurposing success stories are revealed [2]. In this thesis, the process of drug development is outlined and the benefits and ethics of drug repurposing are discussed. Possible applications of drug discovery are outlined, namely malaria, and other infectious and neglected diseases in developing countries. Then, a brief history of chemotherapeutic drugs is outlined.

Following this discussion is a study analyzing previously obtained data of a drug library containing 1,639 diverse drugs that were run against colon tumor cells, pancreas tumor cells, and normal fibroblast cells. Data was collected based on how the drugs affected the cells regarding proliferation. The target drugs were the ones which decreased cell proliferation in tumor cells but had no or very little effect on normal cells. The top 12 drugs of this nature were selected for experiment duplication, and the data is analyzed. This paper outlines the top 12 drugs and what they were originally intended for, and how they might be useful in cancer treatment. Lastly, growth curves and colonogenic assays were performed using these drugs as an example of how drug repurposing might be studied in a laboratory setting.

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Chapter 1: The Ethics of Drug Repurposing: A Case Study of Anti-Malarial Drugs

An Introduction to Drug Repurposing

The process of drug development occurs in three stages: discovery, preclinical trials, and clinical development. In the discovery stage, a new target is validated or a new compound is proposed and is developed and screened. In preclinical trials, the proposed compound is tested *in vitro* and in animal models, where the compound's efficacy is analyzed and information is obtained on its toxicity. Finally, in clinical development, the compounds are tested in human beings in a series of trials which exposes its effects on humans. The costs of those processes, of taking a new drug from the lab bench to market, are cited as high as USD 2.6 billion, including losses incurred, according to Tufts center for the Study of Drug Development [3]. In addition, the time invested in the development of a drug can last 10-17 years depending on safety requirements and the drug's efficacy and quality. [4] Keeping these costs in mind, the complications increase when the fact that only 16% of all drugs in development will reach the market is considered. [2] The amount of novel drugs approved per billion US dollars for research and development has halved every 9 years since 1950. [5] This 'productivity crisis' makes the case that the paradigm of drug discovery could use some improvements. [2] This model is time-consuming, expensive, and financially high-risk.

An alternative model which seems to be increasingly attractive to researchers is drug repurposing. Drug repurposing is the process of finding new, safe targets for existing drugs which have already been approved for treatment of other diseases or whose targets have already been discovered [2]. The fact that there are many drugs that affect biological pathways outside their target molecule is being continually reinforced as more

and more drug repurposing success stories are revealed [2]. Understanding these off-target interactions is key to finding novel uses for old drugs [1].

There are many benefits to “exploring the existing pharmacopeia”, as it decreases the time and financial resources needed to bring a drug to market [2]. Drug repurposing is appealing because it exploits drugs which we already have information on regarding its efficacy and toxicity, which decreases the financial burden that researchers or companies have to take on. Finding additional uses for existing drugs would also add value to the pharmaceutical company’s portfolio, adding an extra incentive [2].

There are many avenues to explore when seeking drugs to repurpose. Researchers can study drugs that are currently approved by the FDA, the most cost-effective option, or drugs which made it to phase II or III clinical trials and failed, but do not raise specific safety concerns. [6] Also, drugs that have been discovered and analyzed in emerging markets but haven’t been investigated in developed countries, or drugs that have been studied minimally in academic institutions or public laboratories, but were not able to be completely evaluated because of lack of resources. [7] In addition to the financial resources that are saved when some portion of the drug discovery process has been completed, time is also gained. Working within the current model of drug discovery, it is difficult to make new pharmaceuticals quickly for rapidly emerging or re-emerging infectious diseases. [8] As with financial resources, time is saved when researchers already have a large amount of information on a drug when they start working with it. Many times, it becomes feasible to transition into phase II and III clinical trials quickly. [1]

The reliability of drug repurposing is enhanced with each of it's triumphs. The most well known instances is the compound sildenafil, also known as Viagra, made by Pfizer pharmaceuticals. Sildenafil was originally developed as a medication treating angina, a condition characterized by pain in the chest, as a result of inadequate blood supply to the heart. [9] However, the treatment produced an unexpected side effect of decreased erectile dysfunction in human volunteers during clinical trials, and has gone on to be one of the best-selling drugs of all time. [9] Another example is Thalidomide, a drug prescribed to prevent morning sickness in pregnant women, which was later banned in most countries because it was found to cause birth defects. [10] The same compound has now been repurposed to treat erythema nodosum leprosum, a type of leprosy, and multiple myeloma, a cancer that infects plasma cells. [11] Lastly, minoxidil, a drug whose target is the potassium ion channel which was targeted for treatment of hypertension, was approved by the Food and Drug Administration to be used as a treatment for pattern baldness in men. [2] Below is a table that describes some of the examples of successes in drug repurposing.

Drug	Original indication	New indication
Amantadine	Influenza	Parkinson's disease[9]
Amphotericin	Fungal infections	Leishmaniasis[10]
Aspirin	Inflammation, pain	Antiplatelet[11]
Atomoxetine	Antidepressant	Attention deficit hyperactivity disorder[12,13]
Bromocriptine	Parkinson's disease	Diabetes mellitus[14]
Bupropion	Depression	Smoking cessation[3]
Colchicine	Gout	Recurrent pericarditis[12]
Gabapentin	Epilepsy	Neuropathic pain[15]
Methotrexate	Cancer	Psoriasis, rheumatoid arthritis[16]
Miltefosine	Cancer	Visceral leishmaniasis[3]
Propranolol	Hypertension	Migraine prophylaxis[17]
Retinoic acid	Acne	Acute promyelocytic leukemia[18,19]
Ropinirole	Parkinson's disease	Restless leg syndrome[20]
Zidovudine	Cancer	HIV/AIDS[3]

Figure 1: Examples of Drug Repurposing Successes [1]

These examples are just a small testimony to the sentiment that drug repurposing works, and it is worth considering in order to find new treatments for a variety of diseases, including cancers.

A Case-Study of Anti-Malarial Drugs

One application in which drug repurposing may be especially relevant is in diseases of the developing world, where financial resources are especially low, but health needs are high. Many of these countries suffer from infectious and neglected diseases, that are not seen as profitable to pharmaceutical companies in developing countries, therefore are not significantly invested in. [1] Despite their virtually nonexistent financial market, these diseases are of high public health importance, leaving a huge unmet medical need. One of these diseases affecting major populations of developing countries is malaria.

Malaria is one of the leading causes of death worldwide, totaling 438,000 deaths in 2015 so far. [12] This disease is especially lethal in the developing world, and the main strategy to manage and control malaria relies on the availability of effective drugs. There are few antimalarials on the market initially, and the parasites that cause malaria are constantly adapting towards resistance to the drugs that are used against them, making drug discovery and development even more important. Because of this resistance, new antimalarials are needed constantly and quickly. [13]

Malaria is caused by the transmission of parasites by four different species, *Plasmodium falciuparum*, *Plasmodium vivax*, *Plasmodium knowlesi*, and *Plasmodium malariae* carried by a female mosquito of the genus *Anopheles*. *Plasmodium falciparum* is the most common in Africa, while *P. vivax* is prevalent in the Middle East, Asia, Oceania, and Central and South America. When a female of the *Anopheles* genus feeds on human blood, it injects the malaria-causing parasites, in the immature stage of sporozoites, into the bloodstream. The sporozoites then travel to and invade the liver, and

grow and mature into schizonts, which then rupture and release merozoites. In the case of the *P. vivax* and *P. ovale* parasites, the parasites can remain in an intermediate life stage called hypnozoites, dormant over periods of time, with the possibility of causing relapse by invading the blood months or years later. [14]

After the release of the merozoites, they enter the bloodstream and invade red blood cells, and some parasites differentiate into sexual gametocytes. The parasites' infection of the bloodstream is what causes the symptoms of malaria including fever, chills, headache, sweats, fatigue, nausea and vomiting or more severe symptoms including convulsions, severe anemia, low blood sugar, and fluid-filled lungs. The gametocytes that develop and circulate in the bloodstream is what infects another previously uninfected mosquito if it bites an infected human, ingesting the parasites. The gametocytes generate zygotes, which become mobile and elongated. The zygotes then burrow into the midgut wall of the mosquito and develop into oocytes. The oocytes grow until they rupture, releasing sporozoites, which travel to the salivary glands. These sporozoites infect the next human when the mosquito has its next blood meal. [14]

There has been a steady evolution of the drugs used to treat malaria. Quinine was the first effective treatment used against malaria which was extracted from the bark of the cinchona tree by chemists in 1820. Quinine's mechanism of action is not completely known, however it is thought to attack the parasite during its merozoite stage [15] Since then, chloroquine has also been used with great success, which works similarly but has less adverse side effects, but the mosquitos have become resistant to these treatments. Another strategy that has been used is to combine these drugs or

versions of these drugs with aartesunate, another antimalarial which inhibits a membrane glutathione S-transferase enzyme in the *P. falciparum* mosquito. [15]

Drug repurposing has also played a part in the development of antimalarials. Several classes of drugs have been investigated to determine their efficacy as a treatment of malaria. Sulfur-based antibacterial drugs that were first developed as industrial azo-dyes in the 1920s, later were found to have success treating bacterial infections. Derivatives were investigated to see if they could treat other diseases, because its target, the folate synthesis pathway, exists both in bacteria and in the malaria parasite. However, use of these drugs was abandoned soon after the beginning of its use because of its low efficacy and high toxicity. After more study into drug targets, combining the antifolates with the drug Fansidar, a Dihydrofolate reductase inhibitor, increased its efficacy and was used as a treatment, until widespread drug resistance led to its discontinuation. [13]

Another attempt at repurposing against malaria involved Co-trimoxazole, a combination of an antibacterial and sulfamethoxazole, which was shown to be efficacious in treating malaria infection. It was eventually discovered, however, that malarial parasites became resistant to this combination as well, and it was discontinued as a treatment against malaria. [13]

Anticancer antifolates like methotrexate have also been explored as a treatment for malaria because like cancer cells, malaria parasite cells lack cell cycle regulation. This may mean that the essential pathway that causes their cell division may be inhibited by the same compounds. In the 1970s, this drug target seemed efficacious when methotrexate inhibited a malaria parasite from growing *in vivo*. In the first stages of clin-

ical trials this drug was shown to be safe, but because of concerns over toxicity, the use of the drug did not go any further at that time. Today, methotrexate is used at much lower doses for treating other diseases such as rheumatic diseases, multiple sclerosis, inflammatory bowel disease, chromical cholestatic disorder, and lupus. Because of successes in these areas, methotrexate is currently being reconsidered and trials are being run to see if it still might have potential against malaria in appropriate doses. [13]

Antibiotics have also been looked into, specifically the ones that target the parasite's apicoplast, an organelle used for energy storage that is unique to apicomplexa parasites. The drug disrupts translation machinery within the apicoplast, leaving them non-functional. However, normal parasite growth occurs through the merozoite life stage, meaning these drugs are slow-acting. [13] This component of the drugs makes them not ideal as an antimalarial. Many other kinds of antibiotics have been evaluated in the treatment of malaria, but efficacy is generally low. To offset their downfalls, the slow-acting antibiotics have been combined with fast-acting antimalarials, and trials have been run that show promise, but these combinations have never made it to Phase III or IV clinical trials. This could be, once again, because of concerns with drug resistance. [13]

Rapid-acting antibiotics have been researched and are viewed as more favorable then slow-acting antibiotics. One target that was discovered is the non-mevalonic pathway, used to synthesize isoprene. This is a pathway that occurs in both bacteria and the malaria parasite. The discovery of this similarity led to the discovery of fosmidomycin, an inhibitor of an important enzyme in this pathway, the deoxy-xylulose 5-phosphatereductoisomerase, as a powerful antibacterial agent. Because of its fast-acting nature, it

can be combined with other slow-acting antimalarials, and these combinations have made it to Phase II and III clinical trials. More studies are needed to solidify information on its efficacy. [12]

Beginning in the early 1900s, drug repurposing has played a major role in the discovery of antimalarial drugs over the years. Drug resistance is one of the biggest hurdles to overcome in the treatment of this disease, and it will only be overcome with more time and resources dedicated to the task of developing antimalarials that are efficacious, safe, and long-lasting treatments.

Outside of the chemical obstacles that there are in the development of antimalarials, another major hurtle is that relatively few companies are investing their time and money in it. Malaria leaves 3.2 billion people, half of the world's population, at risk. In 2013, 430,000 African children died of malaria before their fifth birthdays. [12] While mortality rates have fallen in recent years because of prevention and control efforts, this is still a huge, unmet medical need. Malaria is highest among the poorest countries, and these populations need effective drugs. [12]

Ethical Concerns Regarding Forgotten or Neglected Diseases

Whose responsibility is it to put time and resources toward these forgotten and neglected diseases if the countries who are suffering financially cannot afford to do so? These countries many times cannot afford the medication they need, and they certainly do not have the resources to develop medications. Without discrediting the weight that lifestyle diseases have on peoples' lives, it seems that pharmaceutical companies display a much bigger interest in the development of these drugs, and are disinclined to look into infectious diseases from which profitability would be much less. How should this issue be addressed?

While the process of discovery is a financial hurdle, a potentially larger hurdle is manufacturing the discovered drug at a large scale. Some pharmaceutical companies, however, do have these resources. It's possible that since pharmaceutical companies do not have the incentives to spend the time finding cures for these infectious diseases, maybe other organizations would take on the responsibility. A new model that would be beneficial to all parties, including the sick, might be that smaller non-profits, academic institutions, or public sector labs could lend resources toward discovery, while Big Pharma could lend the resources for manufacturing.

Many universities, private companies, and non-profits have made their chemical compound libraries and data open to the general public, to take a more open-source and cooperative approach to affordable drug discovery. There are many resources available online such as databases that track clinical programs, as well as databases that allow free access to information on many drug profiles and their targets. Open Source Drug Discovery (OSDD) was developed in 2008 as a global initiative where re-

searchers across the globe can work cooperatively to solve the challenges that drug discovery faces, with the aim of providing affordable healthcare to developing countries. [1] In addition to these formal databases and collaborations, disease-oriented social networks might be another approach to bring together human resources and data to find solutions to these research problems. These online communities, PatientsLikeMe, My-DaughtersDNA, or Genetic Alliance, to name a few, have resulted in patients having an increased consumer activism, which may lead to gains in research and eventually new treatments. [16]

With all of this information available to researchers around the world, working together to find novel uses of previously approved drugs is possible. Since these resources are available, they can potentially be pulled together to find treatments for these neglected diseases that need attention. It is a desperate unmet medical need, and those who have the resources to meet it should be compelled and feel obligated to.

Chapter 2: A History of Chemotherapeutic Drugs

It's important to consider the past of cancer drug discovery to understand the origins of the techniques that scientists use today. The history of chemotherapy, using drugs to treat cancer, is marked by bursts of promising breakthroughs with periods of widespread skepticism in between, with decades in which each advancement that gave the impression of being a step-forward in the field of chemotherapeutics was shadowed by doubters offering their critiques. It is only through a century's worth of diligent scientists combatting the main problems facing drug discovery and collaborating with one another to bring chemotherapy to where it is today.

"Chemotherapy" was coined in the early 1900s by the chemist Paul Ehrlich, who sought to develop drugs to treat infectious diseases. While he was curious about using chemotherapy for cancer treatment, he wasn't convinced that it would be effective. In Ehrlich's time, the nature of the scientific community and technology was not ideal for beginning the effort to find drugs to treat cancer. [17] There were two main problems contributing to this: 1) an efficient procedure to narrow down the extensive collection of chemicals to the ones that might effectively target cancer in humans was still to be discovered; and 2) access to facilities in which to use this procedure were limited. The first breakthrough in these problems came in the early 1910s, when a system was developed that allowed tumors to be transplanted into rodents. [17] This would evolve over the decades into one of the main mechanisms for compound screening. The effort to improve and standardize this model led to an increased number of chemicals being tested, and efforts to find even better models drove chemotherapy research over the next few decades.

Interestingly, many of the early chemicals that were considered to treat cancer came from events surrounding and caused by World War II. During the war, gases weren't used as an agent of warfare on the battlefield, but vesicant war gases were being researched and developed [18]. An accidental spill of one of these gases, sulfur mustard, on a bombed ship led to the somewhat unfortunate discovery that mustard gas depletes bone marrow and lymph nodes to those that are exposed to it. [19] Propelled by this discovery, scientists started researching the possible chemotherapeutic uses of the chemicals. [20] In 1943, observations of tumor regressions in mice administered with nitrogen mustard were performed, and it was then tried on a human suffering from non-Hodgkin's lymphoma. [21] When marked regression was also observed in the human, the study was published and its implications were spreading rapidly around the country, with use of nitrogen mustard against lymphomas becoming somewhat common in response. [21] However, when remissions turned out to be incomplete and short-lived, an opinion of pessimism dominated intellectuals during the following decade of literature. [21]

As the decades went on, periodic chemotherapy successes were discovered. In 1948, nutritional research revealed that folic acid was important for bone marrow function, meaning that a deficiency could lead to effects similar to the effects of nitrogen mustard. [22] With this in mind, a series of folic acid analogues were developed, later to be tested in children with leukemia. Unquestionable remissions were the result, and with that, chemotherapy took another step forward. [23]

Towards the end of World War II, fermentation products were being tested in an attempt to produce antibiotics to treat wound infections. During testing, antitumor effects

were observed, yielding the antibiotic actinomycin D, which was used in treatment of pediatric tumors throughout the 1950s and 1960s. [24] This success pushed scientists to look for other antibiotics that could have antitumor activity, some of which are in common use today. [17] In 1948, another treatment of acute leukemia was discovered when a substance was isolated which inhibited that metabolism of adenine, a building block of DNA. [25] This biochemical pathway target proved to have broad-spectrum range against solid tumors, and remains the mechanism of many chemotherapy drugs, especially in the treatment of colorectal cancer. [26] This was the first example of "targeted therapy", which is now the major mechanism of cancer drug development today.

As more chemicals were discovered to be efficacious, the accessibility of more diverse tumors to test on grew, and drug screeners began searching for transplantable tumors that would best mimic human activity. As new screening systems were developed, organizations that sought to financially support cancer drug discovery were being formed, one of which was the Cancer Chemotherapy National Service Center in 1955. [27] The National Cancer Institute was also greatly involved, and more and more funding became available. Along with these huge steps forward also came advancements in pharmacology and toxicology testing that established a system in which drugs that were to be used on humans had to meet certain criteria before proceeding to production. [28] These organizations, along with the American Cancer Society, worked together throughout the late 1950s and 1960s to address the major issues which affected cancer drug development. [17] Despite minor successes, the 1950s ended much in the way that it began, with an air of skepticism surrounding the concept of chemotherapy. [17] In the 1960s, medical oncology was not considered a clinical specialty, and the majority of

doctors were not sure cancer drugs were of any use whatsoever, and many times “talk of curing cancer with drugs was not considered compatible with sanity”. [17]

Research continued into the 1960s and 1970s that focused on what dosage of drugs was most effective and that combinations of drugs were more successful than single agents. [17] As more compounds were explored, successes resulted in increased remission rates, with major breakthroughs with L1210, a type of childhood acute leukemia, and Hodgkin’s disease. [17] In 1964, a combination of drugs called “VAMP” (cincristine, amethopterin, 6-mercatopurine, and prednisone) was first used and brought acute lymphatic leukemia remission rates from near zero to 80%. [29] In 1970, Hodgkin’s disease became the “first advanced cancer of a major organ system in adults to be cured by chemotherapy” when a combination of drugs known as MOPP (Nitrogen mustard, Mustine, Mechlorethamine, and Hydrochloride) proved to be extremely effective. [30] By the end of the 1960s, chemotherapy had become more accepted as scientists were now convinced that anticancer drugs can potentially cure cancer. [17] In 1974, yet another solid tumor in adults was treated by combinatorial chemotherapy when metastatic testicular cancer went from being 10% growth inhibited to 60% through the use of cis-platium, cinblastine, and bleomycin. [31] This new concept of a cure for cancer saturated the 1970s and chemotherapy was being used even in earlier stages of cancer. [17]

Collaboration made the effort of cancer drug development more efficient, and helped by standardizing techniques and providing stable funds to research new approaches to drug discovery. During Richard Nixon’s presidency, The National Cancer Act of 1971 was passed to launch the nation’s “War on Cancer”. [32] By 1974, the Can-

cer Chemotherapy National Service Center and its partners totaled an annual budget of $68 million which allowed for about 3 million mice with transplantable tumors and over 40,000 compounds being screened a year. [17] Chemotherapy programs were being expanded and the number of new drugs that were being clinically tested was increasing. At this time, cancer drug development changed as the pharmaceutical industry began to pursue their interests in chemotherapy as they saw a market in it's success. [17]

The growing market of cancer drugs has motivated the industry to invest in new drugs, much of which was in the control of the major pharmaceutical companies. Because of this boom, many new types of anticancer drugs were released to clinicians since the 1980s. [17] The majority of these drugs have been discovered using the prominent technique of today: targeted therapy. [17] Relatively recent work has focused on identifying oncogenes, suppressor oncogenes, and pathways used for cell signaling in developmental biology. [33] These targets have resulted in the current focus of cancer drug development. Advances in molecular biology, including data from the Human Genome Project, has pointed out that the abnormal function of protein kinases are associated with abnormalities of cancer, which directed many scientists to examine kinase inhibitors as potential drug targets. [34] Several of these have been efficacious and have been FDA approved for use in the treatment of renal cell cancer, hepatocellular cancer, and gastrointestinal stream tumors, all of which were observed to be resistant to traditional chemotherapy. [17] The use of chemotherapy to treat solid tumors is expanding, with the greatest success in producing progression-free survival. [17]

The national incidence and mortality of cancer began to decline in 1990 and has continued to decline since, with the rate of decline doubling between 1990 and 2007.

Some of this decline is attributed to prevention and early diagnosis, while the other half is due to advancements in cancer treatment, many of which are supplemented with chemotherapy. [17]

Chapter 3: Data Analysis of a Drug Library

While targeted therapy is the dominant mechanism for cancer drug discovery by scientists, using drug repurposing as a means of drug discovery takes a slightly different approach in finding drugs that are efficacious against novel drug targets. Lab research regarding drug repurposing can begin in many ways, whether it be simply noticing an unintended health improvement in a drug trial or during a patient's treatment, or intentionally seeking out drugs to be repurposed against specific diseases, which would be considered targeted therapy. One main mechanism for drug discovery through drug repurposing is to test many possible compounds at once against a cancer model, to narrow down which drugs might have targets that can be used against that particular model. The following analysis, findings, and subsequent experiments display how drug repurposing may be used to investigate compounds to be used in the treatment of cancer.

In this study, a collection of diverse drugs were assayed against different kinds of human tumor cells with the intention of finding new targets for the drugs. The John Hopkins Clinical Compound Library version 1.3 was analyzed in this study. This library includes 1,524 drugs, 1,186 of which are FDA approved drugs, and 338 of which are Foreign Approved Drugs. The diverse collection of drugs was mostly defined by the library. Table 1 shows the most prevalent drug types in the library.

Antineoplastics	Antihelmintics	Antibacterials	Antiprotozoals
Antivirals	Antifungals	Antibiotics	Antiseptics
Analgesics	Anesthetics	Antispasmodics	Muscle relaxants
Neuromuscular blocking agents	Antidiabetics	Thyroid drugs	Antihistaminic
Antihypertensives	Diuretics	Astringents	Laxatives
Anticonvulsants	Antidepressants	Antipsychotic	Anti-inflammatory
Sedatives	Glucocoriticoids	Dermatoligic	Vitamins and nutrients

Table 1: Types of drugs in Chemical Compound Library

Each of these drugs was assayed against pancreatic tumor cells, colorectal tumor cells, and normal fibroblast cells. Data were collected based on cell proliferation (cell growth) after drug incubation. The drugs either facilitated or inhibited proliferation for each type of tissue cell, and then the amount of proliferation was quantified based on percentage.

This large data set was obtained and analyzed to find the top 10 drugs that reduced proliferation in tumor cell lines but had little to no effect on the proliferation of normal fibroblast cells. Each of the top drugs had a limit of 50% growth inhibition or higher against the tumor cell line but had +/- 9.5 % growth inhibition against the normal fibroblast cell line. Before the most effective drugs for each cancer line were obtained, the typical chemo-therapeutic drugs were eliminated. Also, the drugs that were in common between the two tumor cell lines decreased the number of overall drugs of interest. After these reductions were made, there were a total of 12 top drugs. Included in this study were hydrochloric acid and glucose, to be used as negative and positive controls, respectively. The results from this study and further experiments are outlined in the next

chapter. The remainder of this chapter is spent outlining the top 12 drugs used in the experiment, in order of descending ability to decrease proliferation of pancreatic and colorectal tumor cells. For each drug, their previously approved target and mechanism is outlined. Then, hypotheses are made concerning why these might be effective drugs against cancer cells.

Benzbromarone

Benzbromarone was originally approved for the treatment of gout, a disease characterized by attacks of inflammatory arthritis which is caused by too much uric acid in the blood. [35] Monosodium urate, or uric acid, crystals form in the patient's blood, making the goal of treatment to dissolve existing crystals and stop the formation of new ones by reducing plasma rate concentrations. [35] Benzbromarone is an inhibitor of a large enzyme called xanthine oxidase, which catalyzes hypoxanthine to xanthine, and xanthine to uric acid. [35] This inhibits postsecretory tubular resorption or uric acid. [35] The pathway described earlier is also involved in the catalyzation of purines, which are building blocks that help make up genetic code in DNA. [36]

There are several studies examining how this drug can be useful against cancer. One study examined older women with diverse types of cancer and found a correlation between cancer deaths and high uric acid levels. [37] It is possible that cancer causes some type of imbalance between uric acid and purines, leading to symptoms like progressive kidney damage, hypertension, or systemic inflammation. [37] In addition, if there is a problem with purine metabolism, there can be problems with DNA replication

or the formation of mRNA to make proteins. The failure to do this properly can cause mutations and problems with the cell cycle.

There are two enzymes involved in the synthesis and catalyzation of purines that are essential to be in balance: xanthine oxidase, which helps in breaking down purines into uric acid, and amidotransferase, which helps in the formation of purines. [38] If the ratio of their products gets shifted, causing an enzymatic imbalance, it can be an advantage to cancer cells causing malignancy. [38]

Nelfinavir

Nelfinavir is an antiretroviral drug used in treatment of HIV. It is a protease inhibitor, specifically inhibiting HIV protease which cuts viral protein molecules into smaller fragments after it infects a cell, so its DNA can be released and copied by the host cell machinery. [39] This breaking up into fragments is essential to the replication of viruses in a cell, and the release of mature viral DNA from an infected cell. Amide substituents of the drug interact with subsites of HIV protease, inhibiting it from its normal function. [39]

A side effect of nelfinavir, along with other antivirals, is stress on the endoplasmic reticulum of the affected cells. [40] Because the ER is the site of protein synthesis, this stress can lead to misfolded proteins, which can be fatal to cells. [40]

These misfolded proteins can cause proteotoxicity, toxicity caused by proteins, for cancer cells. [40] In a study of cervical cancer, it was shown that in low concentrations, nelfinavir promoted apoptosis and arrested the cell in G1 phase of the cell cycle, prohibiting it from replication. [41] Nelfinavir was also shown to downregulate phospho-

tidylinostotol 3-kinase pathway, which is normally activated in human malignancies. [42] Any or a combination of these things might lead to apoptosis of cancer cells.

Carbadox

Carbodox is a drug used to treat bacterial infection exclusively in pigs, that has since been banned in Canada and other countries as a livestock feed additive because it showed carcinogenic and birth defect-inducing properties. [43] The drug causes growth-promoting effects on young pigs, possibly by working in physiological processes such as their metabolism. [43] Carbadox also is used to improve the feed conversion efficiency in livestock. [44] It also controls swine dysentery and bacterial infections within the pigs' intestines. [43] Carbadox causes base pair mutations and frameshift mutations in DNA, that result in the intended effects described above. [44]

It is possible that carbadox causes mutations in the genome of cancer cells, changing the components that make cancer cells "immortal", like loss of control of their cell growth and self-death. There is not literature available on any other possible mechanisms of why carbadox may cause a decrease in cell proliferation of cancer cells.

Fendiline

Fendiline is used with other drugs to treat high blood pressure and coronary heart disease. [45] It is classified as a lipophilic calcium antagonist, meaning it can dissolve in fats and acts as a calcium channel blocker. [45] Fendiline binds to calcium channels and calmodulin, a calcium binding messenger protein. [45] This binding causes an inhibition of the calcium current that occurs throughout a membrane, a reduction of contraction of arteries in smooth muscle, and a reduction of the force of the contrac-

tion of the heart. [46] These effects, along with others, result in the lowering of blood pressure.

A study was done to assess the effects of fendiline as an inhibitor of K-Ras, a protein that is important in regulating cell growth, differentiation, and survival. [47] In many cancer cells, Ras proteins are mutant and overexpressed, K-Ras proteins being the most prevalent. [47] The same study also found that fendiline stopped the proliferation of many cancer cell lines possessing this K-Ras mutation, including pancreatic, colon, lung, and endometrial cancers. [47] These results provide strong evidence that fendiline might be a viable anticancer therapeutic.

Emetine

Emetine has traditionally been used for the treatment of amoebiasis, a gastrointestinal infection caused by an amoeba, after the parasite is taken in by mouth. [48] It interacts with the amoeba or protozoan's ribosomal small subunit E-site by binding and blocking mRNA/tRNA translocation, which blocks the translation of mRNA into proteins. [48] Essentially, it inhibits protein synthesis of the amoeba at its early life stage when it is known as a trophozoite. [48] This stops its growth and eventually leads to death.

Emetine could be efficacious in treatment of cancer cells in the same way it works against the amoeba. Cancer cells also need to synthesize proteins to continue to grow and divide rapidly. One of the trademarks of cancer cells is that they no longer undergo apoptosis, or cell death, like normal cells. However, studies have shown that blockages in protein synthesis can induce apoptosis in cancer cells and can decrease their ability to form colonies. [49] Although the mechanism of this effect is largely un-

known, an inability to synthesize proteins might lead to the activation of caspases, which lead to apoptosis. [50] Emetine might also down-regulate the expression of non-apoptotic proteins, aiding in the process of cell death. [50]

Tioxolone

Tioxolone was originally approved for use as a topical treatment for acne. [51] It has astringent properties which cause the contraction of body tissues, and keratolytic properties, meaning it treats skin lesions by initiating regrowth. [51] It aids in skin cell growth, and is also used as an antibacterial and antifungal drug. [51] Tioxolone inhibits the enzyme carbonic anhydrase, which catalyzes the conversion of carbon dioxide and water to bicarbonate ions and protons. This reversible reaction helps maintain the acid-base balance within the blood and tissues. [52]

One characteristic of the microenvironment surrounding a cancerous tumor is the existence of physiological gradients which cause the plasticity of tumor cells and the diversity of tumor tissue. One factor that generates an oxygen gradient is hypoxia, or an insufficient amount of oxygen. [53] The gradient that hypoxia induces can affect tumor cell expression and aid it in the resistance of treatment. [53] One way cancer cells survive in these conditions is to increase the expression of carbonic anhydrase which helps the cells control the pH in their environment by neutralizing excess acid. [53] This gives cancer cells an advantage and allows them to more effectively migrate, invade, and metastasize in hypoxic environments that may be lethal to normal tissue cells. [53] It's possible that tioxolone could inhibit this over expression of carbonic anhydrase, leading to the loss of the advantage of the cancer cell.

Desmethyl Astemizole

Desmethyl Astemizole is a metabolite of astemizole, which is an antihistamine, used to treat allergic reactions, edema, and itching. [54] It is a competitor of the receptor site of histamine H1-receptors in blood vessels, bronchial muscle, and the gastrointestinal tract, blocking the formation of edema and pruritus. [54] H1 receptor antagonists also show the ability to be K+ channel antagonists. [54]

Histamine has a critical effect on cancer cell proliferation, invasion, and migration. [55] Histamine also plays a role in eliciting immune-modulatory and pro-inflammatory cellular responses by interacting with G-protein coupled receptors. [56] It is possible that the antihistamine effects of desmethyl astemizole might have a negative effect on cancer cell proliferation, although evidence of this in the literature is scarce. However, there is evidence that histamine H1 receptors are expressed in endometroid adenocarcinoma cell lines, so suppressing certain H1 expression with antihistamine might also be efficacious for other types of cancer cell death. [55] There is also evidence that histamine and histamine signaling may be a potential drug target for treating pancreatitis and pancreatic cancer. [56] One study confirmed that cancer cells overexpress histamine H1 receptors, as well as H2 receptors. [56] This overexpression may be advantageous to these cells because the histamine can act as a growth factor, facilitating cell proliferation through its binding of histamine. Since desmethyl astemizole is a competitive inhibitor of this receptor, it might decrease this advantage, and therefore be a viable treatment option.

Miltefosine

Miltefosine has been used for many clinical applications, including parasites, fungi, bacteria, skin ulcers, and was even considered as an experimental cancer treatment but never was approved. [57] This may be considered an example of a drug that has properties that affect many diverse targets throughout the body. Miltefosine is an analog of phosphocholine, which is an intermediate of the synthesis of phosphatidylcholine, an abundant component of cell membranes, which is also involved in cell signaling. [57] Miltefosine acts as a competitive inhibitor of the enzyme that catalyzes the formation of phosphocholine. [57] If phosphatidylcholine is not made in the correct amount there can be changes in membrane fluidity and composition. [57] This can lead to changes in membrane function, like cell signaling.

These effects on cell membranes can also be disadvantageous for cancer cells. Cancer cells have many distinctive alterations including the ability to grow without growth factors, the ability to invade surrounding tissues, and the ability to evade apoptosis, which is normally a healthy mechanism that limits cell proliferation. [58] Each of these are partly due to alterations in their cell-signaling pathways. [58] Again, cell-signaling is largely determined by the structure and function of cell membranes. Its possible that miltefosine has some kind of disadvantageous effect on the cell membranes of cancer cells, “fixing” the abnormalities of the cancer cell’s cell signaling pathways. Maybe cancer cells have an influx of phosphatidylcholine and sphingomyelin in their membranes, and miltefosine inhibiting their biosynthesis leads to difficulty surviving or apoptosis. [59]

6-Mercaptopurine monohydrate:

6-Mercaptopurine monohydrate is used for diverse applications such as leukemia, inflammatory bowel disease, and other autoimmune disorders. [60] It is another drug that inhibits purine synthesis by incorporating thiopurine metabolites into DNA and RNA. [19] It decreases inflammation by incorporating metabolites of itself into DNA and into small GTPases, one of which is Rac1. One of the metabolites, 6-thio-GTP, is competitive against GTP in its binding site on Rac1, a small signaling G protein that regulates cell motility and cell growth. Its binding suppresses the action of Rac 1, inducing apoptosis. [60]

Through this pathway, 6-Mercaptopurine monohydrate has been efficacious against childhood acute lymphoblastic leukemia, so it might be efficacious for other cancers as well. Another possible mechanism in which 6-MP might be a viable cancer treatment is by taking advantage of the high copper levels present in cancer cells. [61] 6-MP has a pro-oxidant property when in the presence of Cu (II), redox cycling it into Cu (I). [61] There is evidence for 6-MP's DNA damage ability is increased during this process because of the production of reactive oxygen species, which may be able to induce apoptosis. [49]

Carbenicillin:

Carbenicillin is a semisynthetic penicillin, shown to be effective against urinary tract infections, *Escherichia coli,* and a wide range of gram-positive and gram-negative bacteria. [62] It is active against a wider range of bacteria than ampicillin is, and it af-

fected many other species that were resistant to penicillin. [62] Carbenicillin inhibits the synthesis of a component of the bacteria's cell wall, causing their death. [62]

Bacterial infection is common in patients with certain cancers, and can even be a cause of death. [63] The use of carbenicillin and other antibiotics might be efficacious to cure the patient of their infection to at least prolong their life. One study used carbenicillin and another antibiotic, gentamicin, to treat bacterial infection in patients with cancer and granulocytopenia, a disease which decreases the white blood cell count of a patient. [63] The majority of patients improved completely. [63] Its possible that taking advantage of carbenicillin's wide range of targets could make it useful in cancer treatment.

Tilorone dihydrochloride

Tiolorone dihydrochloride is an antiviral drug that treats influenza, hepatitis, herpes and some autoimmune diseases by activating the production of interferons, which cause nearby cells to heighten their anti-viral defenses. [64] The release of interferons involve the activation of signal transducers to alert other cells, and activators of transcription factors to stop translation so as to not continue to aid the virus' growth as well as induce the expression of gene products involved in immune defense. Tilorone dihydrochloride has also been shown to elicit other immune responses including the release of T lymphocytes. [22]

This immune response could be lethal to cancer cells, but only if they are recognized by the body as non-self cells. While future studies are needed to confirm the mechanism, tilorone dihydochloride has been shown to decrease cell growth of human

prostate cancer cells, which inactivates their cyclin-dependent kinase 5. [65] This enzyme is a potential target for prostate cancer treatment because it is essential for tumor growth and metastases formation. [65] It is possible that the cyclin-dependent kinase 5 target can be used in other cancers as well.

Trifluridine

Trifluridine is an antiviral drug commonly used topically on the eye to treat herpes. [66] The herpes virus that infects the eye causes the cornea and conjunctiva to become inflamed. [66] Although trifluridine's specific mechanism is unknown, it has shown the ability to inhibit enzymes involved in the DNA synthesis pathways of the herpes simplex virus type 1, possibly by inserting itself into the DNA to block it from successful replication. [66]

Studies have shown that using trifluridine in combination with other drugs to treat colorectal cancer has increased patient survival. [67] A new antitumor agent called TAS-102 composed of trifluridine and tipiricil hydrochloride has been shown to induce p53-sustained arrest in G2 phase in clinical trials. [67] This agent is approved for use in Japan. [68] Although this mechanism remains unclear, the drug seems promising.

Chapter 4: Drug Repurposing Laboratory Experiments

Introduction

After surveying data for the top 12 drugs that decreased proliferation in cancer cells and hypothesizing why they might be effective in the treatment of cancer, laboratory experiments were carried out to see if the original results could be duplicated. This involved ordering and gathering equipment to start cell cultures, to grow normal fibroblasts, pancreatic, and the colorectal tumor cell lines. The following data describes the experiments carried out using the top 12 drugs against the three cell cultures of interest, including data collected using a cell culture of an African Green Monkey kidney cell line as practice in cell culture procedures. Experiments were performed to analyze the effects of these drugs against the cancer cell lines, to determine if they would be adequate candidates as drugs repurposed for the treatment of cancer.

Growth Curves

Materials and Methods

African Green Monkey Cell Line (BGM)

A T-25 flask with a sealed lid of African Green Monkey kidney cell line was obtained from Dr. Shannon Ulrich (St. Petersburg College). The cell line was obtained to practice cell culture without using certain essential equipment such as a CO_2 incubator. Two growth curve experiments were performed to determine normal growth without any added compounds, to see if adding any of our drugs in question would have an effect

on cell proliferation. Again, the growth curves with the BGM cell line were to practice the procedures to be used on the cancer cell lines.

Before plating, 5 mL of the RPMI complete media using a disposable 10 mL serological pipette and the cells were counted so each 6 well plate would start with approximately 250,000 cells/well. First, the Airclean Systems AC600 Series cell culture hood, all materials used, and hands were cleaned using 70% ethanol. Then, the cells were observed in the T-25 flask under an inverted microscope to determine their confluence. 90-100% confluence means the flask is ready to be harvested. The RPMI media was purchased from Life technologies (Carlsbad, CA), and contained 10% Fetal Bovine Serum (FBS) and 5% Anti-Biotic/Anti-Mycotic purchased from Fisher Scientific (Waltham, MA). Excess media was decanted and 3 mL of Dulbecco's Phosphate Buffered Saline (PBS), purchased from Life technologies, was pipetted onto the cells to "wash" them using a disposable 5 mL serological pipettes. PBS was decanted and the step was repeated. 1 mL of Trypsin, purchased from Life Technologies, was pipetted onto the cells to hydrolyze the bonds making them adherent to the bottom of the flask. After 10-15 minutes, the remaining cells left on the bottom of the flask were lifted off using a 1 mL serological pipette in an electronic pipettor. 10 µL of the cells were mixed with an equal volume of Trypan Blue in a 1.5 microcentrifuge tube using a 2-20 µL pipette and counted. 10 µL of this solution was placed onto a hemocytometer under a cover slip. The viable cells were counted under the inverted microscope using a cell counter, and calculations were made to determine the number of cells/ml in the T-25 flask. 1 mL of complete RPMI media was added to deactivate the Trypsin in the flask. This was the procedure to count the original T-25 flask, and the same procedure was

used to count each well of the six-well plate each day of the growth curve. After counting, the excess media was discarded and the plate was placed in an incubator at 37 ºC, wrapped in plastic wrap. Because a CO_2 incubator was unavailable, we relied on the cells to generate CO_2.

Once the cells concentration was determined, a volume of 250,000 cells along with 3 mL of media were pipetted into each well of a six-well plate to begin the first growth curve using the BGM cells. When the second growth curve was run, the same procedures were used except instead of starting with roughly 250,000 cells/well each well started with roughly 125,000 cells. After these growth curves were completed, the BGM cell line was discarded, and the focus of the experiments was placed on the normal fibroblasts, the colorectal LS174T cell line, and the pancreatic Capan-2 cell line, which were purchased from American Type Culture Collection (Manassas, VA).

Capan-2 Cell Line

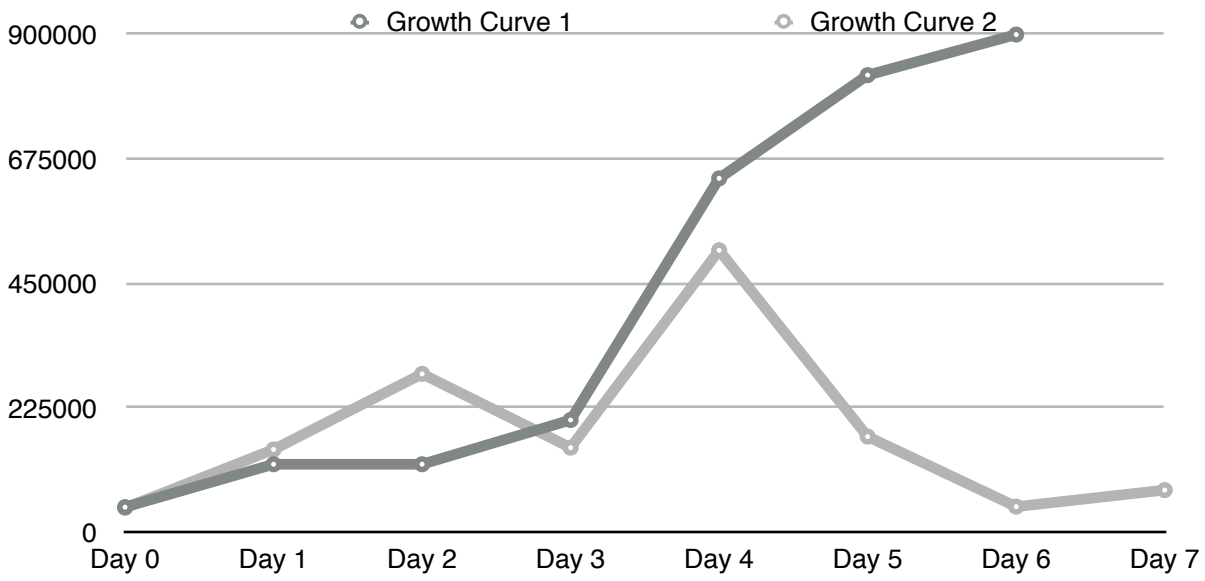
After the BGM growth curves, a growth curve was started in two 24-well plates to determine the effect of glucose (a positive control) on the growth of the Capan-2 cell line. A six-day growth curve (Capan-2 Growth Curve 1) in triplicate was set up in which six days were left untreated and six days were treated with a 10 mM glucose solution. Volumes were adjusted due to smaller wells, however, the same procedure was carried out using the Capan-2 cells. It is hypothesized that the wells treated with glucose will have an increased cell proliferation when compared to the untreated wells.

LS174T Cell Line

A seven day growth curve in duplicate was set up to determine the normal growth rate of the LS174T cell line. The growth curve was conducted in a 24-well plate, where are the wells were left untreated. Again, the same procedures were utilized.

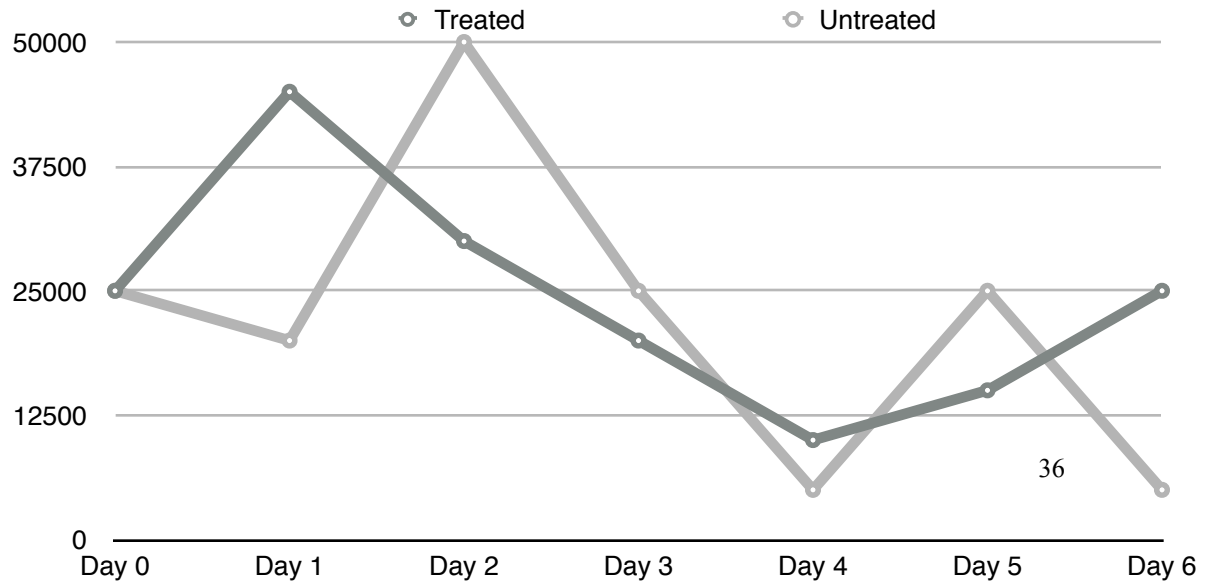
Results

BGM Growth Curves

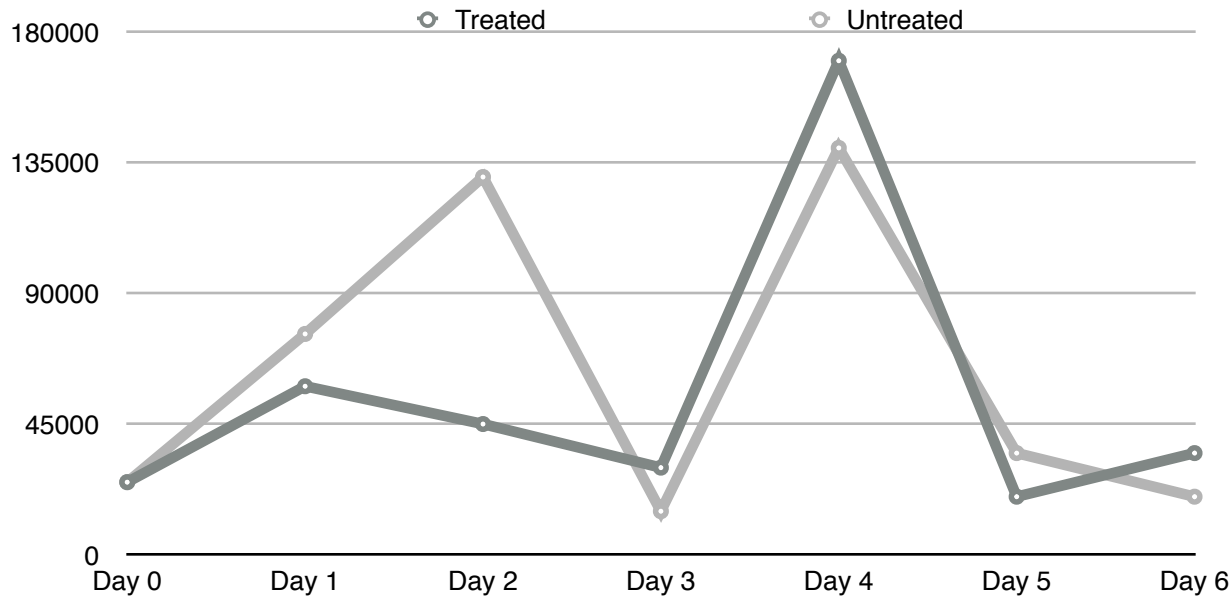


Capan-2 Growth Curves

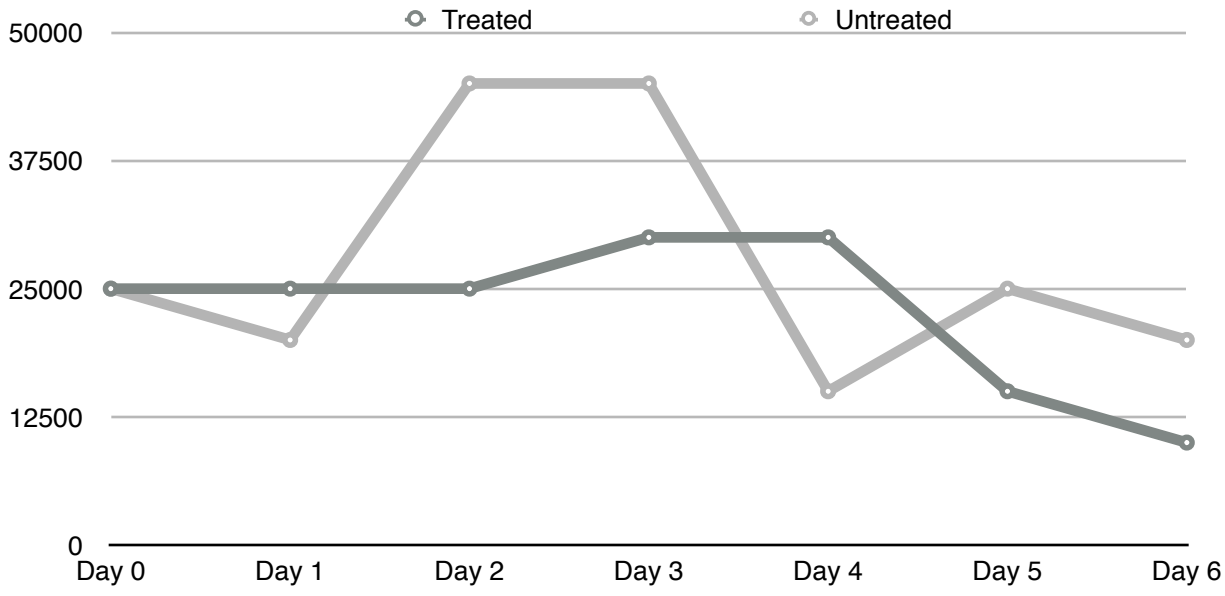
Trial 1



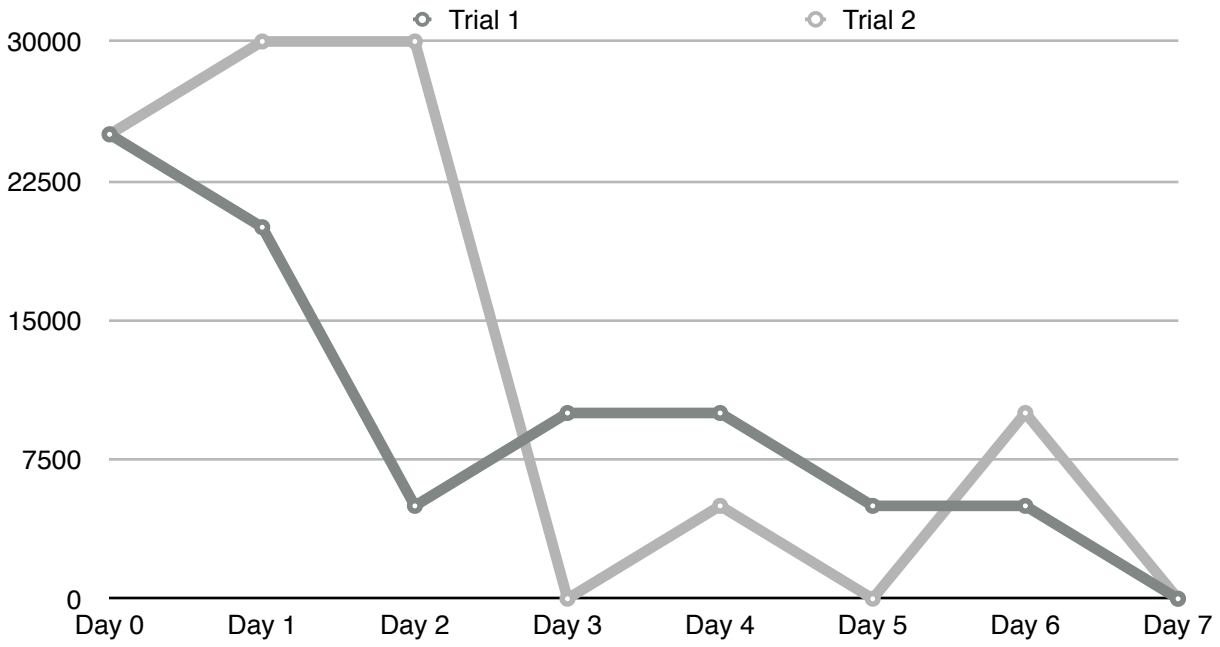
Trial 2



Trial 3



LS174T Growth Curve



Discussion

The first BGM growth curve yielded relatively expected results, with a curve that started with a lag phase, then entered an exponential phase, followed by a stationary phase. The second growth curve had slightly less normal results, with growth declining after Day 5. This could possibly due to lack of adequate equipment. One piece of equipment that is important to cell culture is a CO_2 incubator, which is used to not only limit contamination, but regulate the CO_2 exchange that occurs between the cell culture and its environment. If the cell culture is not allowed to build up CO_2, the pH change in the media can be lethal to the cells. This was a limitation that existed for the following growth curves as well.

The Capan-2 growth curve results were not as expected, most likely to the restrictions discussed above. The cell line hovered around the starting concentration of cells and even decreased as the cells died off, within only the first seven days. The problems that stem from performing cell culture without a CO_2 incubator make results somewhat invalid. When adequate equipment is obtained, this experiment will be performed again.

The LS174T growth curve yielded similar results. The cells hovered around the starting concentration and after Day 4 begin to die off completely. In the future, when the experiments are duplicated, the starting concentration will be increased to provide for a greater amount of CO_2 exchange.

Conclusion

The intent of the growth curve experiments is to determine how the tumor cell lines grow normally, and then observe any differences caused by the potentially chemotherapeutic drugs. Because of the lack of appropriate equipment, only some preliminary growth curves were able to be performed. In the future, growth curves should be performed adding the top drugs, glucose, and hydrochloric acid with a variety of combinations and dosage to determine the effect that the drugs have on proliferation.

Colonogenic Assays

Introduction

Colonogenic, or colony formation, assays are used to determine cell survival in vitro based on the capability of a single cell to develop into a colony of cells. [69] When testing cytotoxic agents, the colonogenic assay can determine the extent of cell reproduction death as only a fraction of the cells should be able to retain the ability to produce colonies.[69] When comparing the number of cells plated and the amount of colonies formed, one can generate a dose-survival curve for the treatment. [69]

Materials and Methods

A live culture of LS174T colorectal tumor cells was obtained and 1 mL of Trypsan was pipetted onto the cells to hydrolyze the bonds making them adherent to the flask. The flask was inverted to mix, and if sell clumps were still observed, a disposable 5 mL serological pipette was used to pipette the solution up and down gently to reduce clumping. 10 µL of the cell solution was pipetted into a clean micro centrifuge tube, and an equal volume of Trypan Blue was added and pipetted up and down to mix. A hemocytometer with a cover slip was assembled and 10 µL of the solution was pipetted onto the slide. The cells were counted and the cells/mL was determined. This was used to calculate the volume necessary to plate 1000 cells into 12 mL of media. 2 mL of the diluted cell culture was pipetted into each of the 6 wells on a 6 well plate, and the plates were placed in a 27 ℃ CO_2 incubator overnight.

Seven of the top twelve drugs were used for this colonogenic assay: Benzbromarone, Emetine, Tioxolone, 6-Mercaptopurine, Tilorone dihydrochloride, Fendiline, and Trifluidine. Glucose was also used as a positive control. All of the drugs were diluted to

a 10mM solution and dissolved in a universal solvent, Dimethyl sulfoxide (DMSO), purchased from Life Technologies. All of the drugs were purchased from Sigma Aldrich (St. Louis, MO). Two days after the initial incubation, the plates were removed from the incubator and 2 µL were pipetted into the three left wells on the 6-well plate, and 2 µL of DMSO were pipetted into the three right wells as a negative control. The plates were then placed back into the 27 ºC incubator for the following 5 days.

After 5 days, colonies were visible so the plates were removed from the incubator for counting. The excess media was pipetted out of each well, and the cells were washed carefully with PBS. The PBS was then decanted and 2 mL of a mixture of 6.0% glutaraldehyde and 0.5% crystal violet was added to stain the colonies. The plates were left to dry for several minutes, and the colonies were counted. The Plating Efficiency was calculated for the control wells and the Surviving Fraction was calculated for the experimental wells. Lastly, a chi-square analysis was calculated to determine the percent significance of the results.

Results

Drugs	Average Control	Average Experimental	Plating Efficiency (%)	Surviving Fraction (%)	x2 P-value
Tioxolone	150	5	15	3.3	<0.005
Fendiline	10	0	1	0	<0.005
6-Mercaptopurine	15	0	1.5	0	<0.005
Benzbromarone	59	51	5.9	86.4	0.30
Tiolorone	18.7	0	1.87	0	<0.005
Emetine	13	0	1.3	0	<0.005
Trifluridine	13	5	1.3	38.5	0.005-0.025
Glucose	117	117	11.7	100	0.995

Table 2: Results of colonogenic assay

The first column on Table 2 was the average number of colonies when DMSO added to the cells as a control, and the second column is the average number of colonies that were formed when the drug was added to the cells. Glucose was added to the cells to be used as a positive control. To analyze these results, the Plating Efficiency and Surviving Fraction were calculated. A chi-square analysis was also run to determine statistical significance. The plating efficiency is calculated based on the control wells and tells how well the cells were able to form colonies without adding anything to inhibit their growth. Each well had 1000 cells in it on Day 0 of the experiment. A 100% plating efficiency would mean 1000 colonies formed in the control wells when counted in week two. Because the plating efficiency ranged from 1% to 11%, there could have been some variables other than the drugs keeping colonies from forming. The most probable explanation for this is that LS174T cells do not plate well at low concentrations. The experiment should be repeated with efforts to raise the plating efficiency.

The surviving fraction is calculated based on the experimental wells, and tells how efficient the drugs were at inhibiting colony growth. The surviving fraction also takes the plating efficiency into account. A surviving fraction of 0% would mean that none of the colonies survived after the drugs being added, whereas a surviving fraction of 100% would mean every colony survived and the drug used is ineffective at inhibiting proliferation and colony formation as observed with glucose. Four of the drugs assayed in this experiment, Fendiline, 6-Mercaptopurine Monohydrate, Tilorone, and Emetine, had surviving fractions of 0%, suggesting that these are significant killers. Tioxolone had a surviving fraction under 5%, suggesting that it is also effective at limiting cell growth. When the chi-square analysis was run, p-values of less than .005 would suggest results

that are statistically significant. All the experimental drugs had values in this range except for Benzbromarone, which tells us that will 99.99% certainty these results did not occur by just random chance. The results suggest that most of the experimental drugs show promise as chemotherapeutics.

Discussion

Although plating efficiencies were relatively low, there are still important implications one can pull from these data. For most of the experimental drugs, the fraction of cells that survived after the drug was under 38%, leaving p-values for all of the experimental drugs, excluding Benzbromarone, to be less than 0.005. This suggests that the results are highly statistically significant. The drugs with these p-values were effective at limiting colony formation, suggesting they had a negative effect on cell proliferation in the LS174T colorectal tumor cells.

Each of the experimental drugs that had a negative effect on colony formation has relatively diverse mechanisms. Tilorone and Trifluridine have similar biological targets, as they are both antivirals, but Tilorone induces the production of interferons, while Trifluridine inhibits the virus' DNA synthesis. Like Trifluridine and Fendiline, 6-Mercaptopurine monohydrate's mechanism is likely to induce apoptosis. Besides these similarities, each drug's mechanism is different, which makes them possible candidates to be combined to form a "drug cocktail". The effect of each drug might be enhanced when they are combined with each other. For instance, combining the inhibition of DNA synthesis that 6-Mercaptopurine monohydrate induces with the apoptotic effects of Fendiline, with the inhibition of protein translation that Emetine causes might produce a combination of drugs that would inhibit colony formation completely. This combination could

be explored even more by running different kinds of assays and determine what dosage of each might work best.

Conclusion

To determine how significant these findings are in determining the efficacy of these drugs in cancer chemotherapy, the colonogenic assay should be performed on the Capan-2 pancreatic tumor cell line as well as the normal fibroblast cell line. If experiments continued to show that these drugs had negative effects on cell proliferation of the tumor cell line yet had no effect on the normal fibroblast cell line, they should be considered for further experiments such as additional growth curves, MTT assays, and apoptotic assays to investigate when the cells' proliferation is being inhibited, how their metabolic activity is being affected by the drugs, and whether or not significant apoptosis is taking place, respectively.

If the necessary equipment is available, these types of drug repurposing experiments can be relatively inexpensive. High throughput screening can be done quickly and in high volumes to identify the pre-approved drugs that show promise, and then more specific assays can be performed similar to those presented here to determine which drugs are effective. Certain assays can even lend information on the drug's mechanism. Experimenting with dosage and combinations of drugs can create "drug cocktails" which might be even better chemotherapeutics. Working with the drugs that are already available, and thus already approved for safety, saves years of development time, which saves hundreds of thousands of dollars. The potential treasures that drug repurposing holds should be exploited, not only for gains of the pharmaceutical industry, but for the health of the general public. When the companies that are discovering and

producing drugs are managing these costs better, those savings get passed down to the consumer, and unmet medical needs become met. The most important goal of the drug development process should be that patients who need medications have access to them. Drug repurposing may be a valuable resource to explore to make that happen.

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